

Editor's comments:

manuscript much more be useful if authors can make minor revision in material and method

Examples:

	NOW	REVISED
1	<p>Sample Collection The roots of <i>Cochlospermum tinctorium</i> were collected from the rock side in Dambu Gomo, Rafin Zuru District, Zuru Local Government Area of Kebbi State. The samples were packaged in sterile polythene bags and it was transported to the Department of Microbiology Laboratory of Usmanu Danfodiyo University, Sokoto.</p> <p>Sample Processing and Preparation <i>Cochlospermum tinctorium</i> 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.</p>	<p>Sample Collection, Processing and Preparation The roots of <i>Cochlospermum tinctorium</i> were collected from the rock side in Dambu Gomo, Rafin Zuru District, Zuru Local Government Area of Kebbi State. The samples were packaged in sterile polythene bags and it was transported to the Department of Microbiology Laboratory of Usmanu Danfodiyo University, Sokoto. <i>Cochlospermum tinctorium</i> 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.</p>
2	<p>Test Bacteria The test bacteria used in this research were obtained from an ongoing research . The organisms collected from Ten (10) food-borne isolates strains of <i>Staphylococcus aureus</i> and <i>Listeria monocytogene</i> isolated from onion, cabbage, lettuce and tomato</p>	<p>Test Bacteria Used for Antibacterial Screening The test bacteria used in this research were obtained from an ongoing research . The organisms collected from Ten (10) food-borne isolates strains of <i>Staphylococcus aureus</i> and <i>Listeria monocytogene</i> isolated from onion, cabbage, lettuce and tomato</p>
3	<p>Preparation of Extract Concentrations In different test tubes One (1 gram) of the extract was weighed and were dissolved in 5 mL of DiMethyl Sulphoxide (DMSO) to obtained concentration of 200 mg/mL. This was the initial concerntration of the extract used to check the antimicrobial activities of the plant. Mueller Hinton agar was used as the growth medium for antibacterial screening [16].</p>	<p>Preparation of Extract Concentrations One (1 gram) of the extract was weighed and dissolved in 5 mL of DiMethyl Sulphoxide (DMSO) to obtained concentration of 200 mg/mL. Subsequently, the following concentrations were prepared 10 mg/mL, 5mg/mL and 2.5 mg/mL and were used for antibacterial sensitivity testing [16].</p>
4	<p>Preparation of Inocula The stock cultures were sub-culture on</p>	<p>Standardization of Bacterial Culture The test bacteria cultures were sub-culture on</p>

<p>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 inocula suspension was adjusted and standardized to that of 0.5 McFarland standard.</p>	<p>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 inocula suspension was adjusted and standardized to that of 0.5 McFarland standard.</p>
<p>Antibacterial Sensitivity The antibacterial activity of methanol extracts of <i>Cochlospermum tinctorium</i> was determined using agar well diffusion method. Sterilized cotton swabs were dipped in the bacterial culture in nutrient broth and then swabbed on the Mueller Hinton plates. Wells of equal size (10.00 mm) were made with the aid of sterile cork borer and the plant extracts were added aseptically into the well. Then the plates were incubated at 37°C and observed for zones of growth inhibition after 24 hours.</p>	<p>Determination of Antibacterial Activity of Plant Extract The antibacterial activity of methanol extracts of <i>Cochlospermum tinctorium</i> 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°C for 24 hours.</p>
<p>Determination of minimum inhibitory concentration (MIC) of the extracts. The minimum inhibitory concentration of the extracts was determined using the broth dilution method in nutrient broth. Normal saline was used to make a turbid suspension of the microbes; the dilution of microorganisms was done continuously in normal saline until the turbidity matched that of the McFarland's standard by visual comparison. Five hundred micro-litres (500µL) of the test organism were aseptically inoculated in each of the four tubes containing the extract in order of increasing dilution (500, 250, 125 and 62.5 mg/mL). Thereafter, the test tubes were incubated at 37°C for 24 hours. After incubation, the test tube with the lowest</p>	<p>Determination of minimum inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extracts. The minimum inhibitory concentration 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 concentration of extracts without visible turbidity was taken to be the minimum inhibition concentration (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</p>

<p>concentration of extracts without visible turbidity was taken to be the minimum inhibition concentration (MIC) [16].</p> <p>Determination of Minimum Bactericidal Concentration (MBC) of the Extracts. 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].</p>	<p>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].</p>
<p>Thin-Layer Chromatography Analysis of <i>Cochlospermum tinctorium</i> Methanolic Extracts The TLC plate used for the separation was made with silica gel on aluminium (TLC silica gel 60₂₅₄ 20.0cm × 20.0cm). Thin layer chromatography was carried out using TLC pre-coated plate (TLC silica gel 60 F₂₅₄) by conventional one dimensional ascending technique. Spotting was done using capillary tube and developed chromatography tank at room temperature. TLC separations were conducted using 100% methanol as the solvent system. The positions of the different compounds were observed on TLC plates. They were placed under UV light which showed the presence of different spots on the chromatogram. The movement of the active compound was expressed by its retention factor (R_f), values were calculated for different samples.</p>	<p>Thin-Layer Chromatography Profile of <i>Cochlospermum tinctorium</i> Root Extracts Thin layer chromatography was run using TLC silica gel pre-coated plates in ascending manner. Capillary tube were 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 chamber at room temperature. TLC separation of the <i>Cochlospermum tinctorium</i> 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 <i>Cochlospermum tinctorium</i> root were calculated using the formula;</p> $R_f = \frac{\text{Distance travelled by the spot}}{\text{Distance travelled by solvent}}$
<p>Column chromatography (CC) analysis of <i>Cochlospermum tinctorium</i> methanolic extracts glass tube with a circle large inlet and a small outlet with a plug or tap known as column was cleaned and dried. Cotton pad was placed at the bottom of the column. The</p>	<p>Column chromatography analysis of <i>Cochlospermum tinctorium</i> 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</p>

<p>column was packed with 107 gram of column grade silica (60 grade, Mesh size was 70-230um). The silica was added to the column by;</p> <p>Elution: The elution was done using methanol, and ethyl acetate in different ratio as given below:</p> <p>All the fractions were collected separately and subjected to antimicrobial screening.</p>	<p>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 subjected to antimicrobial screening and GC/MS analysis.</p>
<p>Antibacterial Sensitivity of Active Fractions of <i>Cochlospermum tinctorium</i> root powder</p> <p>The antibacterial activity of active fractions of <i>Cochlospermum tinctorium</i> root powder was determined by well diffusion method. Sterilized cotton swabs were dipped in the bacterial culture in nutrient broth and then swabbed on the Mueller Hinton plates. Wells of 10.00 mm size were cut on Mueller Hinton agar and the extracts were added into it. Then the plates were incubated at 37°C and observed for zones of growth inhibition after 24 hours.</p>	<p>Determination of Antibacterial Activity of Active Fractions of <i>Cochlospermum tinctorium</i> Extract</p> <p>The antibacterial activity of active fractions of <i>Cochlospermum tinctorium</i> 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.</p>
<p>Gas Chromatography Mass Spectroscopy (GC-MS) analysis of the active fractions</p> <p>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</p>	<p>Gas Chromatography Mass Spectroscopy (GC-MS) analysis of the active fractions</p> <p>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</p>

<p>temperature 250⁰C; ion-source temperature 200⁰C. the oven temperature was programmed from 60.0 (for 0.00 minute) with an increase of 160⁰C (Isothermal for 2.00 minutes) ending with a 2.00 minutes isothermal at 280⁰C. 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].</p>	<p>programmed from 60.0 (for 0.00 minute) with an increase of 160⁰C (Isothermal for 2.00 minutes) ending with a 2.00 minutes isothermal at 280⁰C. 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].</p>
<p>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.</p>	<p>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.</p>

Author's feedback:

- The manuscript was revised and all corrections were effected