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PHYTOCHEMICAL AND ANTIBACTERIAL ACTIVITY OF *Cochlospermum tinctorium* ROOT POWDER AGAINST FOODBORN PATHOGENS

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ABSTRACT

The plant *Cochlospermum tinctorium* is a sub-shrub that belongs to the family *Cochlospermaceae*. The plant has been used in traditional medicine for the treatment of malaria, rickets, stomachache, diarrhea, gastric ulcer, parasitic infestations, liver diseases, fever, pain, inflammation, infectious diseases, epilepsy, snake bite, burns, orchitis, labour, menstrual problems, and many other diseases. The aim of study was to evaluate the phytochemical composition and antibacterial activity of *Cochlospermum tinctorium* against Staphylococcus aureus, Escherichia coli and Klebsiella procured from Microbiology Research Laboratory Sokoto State University Sokoto. The root powder of *Cochlospermum tinctorium* was collected from the market, Tambuwal Local Government Area of Sokoto State, Nigeria. Cooled extraction method was used for extraction using methanol and aqueous as solvent. The antibacterial activity of the plant was determined on Mueller Hinton agar using agar well diffusion method. Furthermore, the Minimum Inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC) of plant extract against the isolate were also investigated.

Keywords: *Cochlospermum tinctorium*, antibacterial activity, Staphylococcus, Escherichia coli

INTRODUCTION

Herbal medicines have gained patronage in developing and developed countries as a result of their effectiveness and safety (Unuofin et al., 2018). However, the safety of the medicinal plants could not be guaranteed despite their wide use (Unuofin et al., 2018). Many scientific studies have shown that many of the herbal plants used as food or medicines are potentially toxic, mutagenic, and carcinogenic (Unuofin et al., 2018). Therefore, it is essential to evaluate and document their safety to determine the consequences of their long use for drug development.

For decades, humans majorly rely on plants for food and management of diseases (Sen and Samanta, 2014). It is estimated that approximately 75% of the global population relies on herbal medicines for their basic health care needs. Indeed, many drugs that are currently in use in modern medicine are obtained from plants (Martins and Brijesh, 2018). Given the predominant uses of medicinal plants in traditional medicine, there is an upsurge in research to investigate the active medicinal compounds, efficacy, and safety of such plants (Tekuri et al., 2019).

The plant Cochlospermum tinctorium (Cochlospermaceae) has been used for years in traditional medicine for the treatment of various ailments in many African countries such as Ivory Coast, Ghana, Cameroon, Nigeria, Gambia, Guinea, Senegal, Burkina Faso, and many others (Ballin et al., 2002). The plant is predominantly available in the savannah and throughout the dried parts of West Africa. It is locally called Oja Ikoko or Sewutu (Yoruba), Obazi or Abanzi (Igbo), and Rawaya or Kyamba (Hausa) languages of Nigeria (Akinloye et al., 2012). Furthermore, alkaloids, tannins, cardiac glycosides and flavonoids were detected in the methanol extract of C. tinctorium rhizomes (Tijjani et al., 2009). In a related development, five compounds were isolated from the dichloromethane extracts of Cochlospermum tinctorium rhizome and phytochemical test showed that they are carotenoids and triterpenes (Traoré et al., 2006). However, the structures of these isolated compounds have not been fully elucidated.

C. tinctorium have been found to exhibit various kinds of biological activities, which include antiulcer, radical scavenging and immunomodulating activities of the polymers in the aqueous extracts (Nergard et al., 2005). Antihepatotoxic actions of the aqueous, ethanol and hydro-ethanol extract (Diallo et al., 1991). Antiplasmodial activity of the ethanol extracts of the leaves (Ballin et al., 2002; Traoré et al., 2006). Antimalarial activity of the water extracts of the leaves (Benoit et al., 1995), as well as antifungal and antibacterial activities of petroleum ether extract (Nikiani et al., 1990). 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 (Igoli et al., 2003). 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 (Chaibenjawong et al., 2011).

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 (Abdullahi et al., 2003).

MATERIAL AND METHOD

Sample Collection

A sample of Chochlospermum tinctorium root powder was obtained from local supplier within Sokoto metropolis and stored using sterile polythene bag. The sample was transfer to the Microbiology Laboratory, Sokoto State University, for analysis.

Extraction of the Sample

The solvents used for extraction are methanol and distilled water. Thirty gram (30g) of the Cochlospermum tinctorium root powder was weighted and transferred in each of the conical flask. 300mls of methanol solvent and distilled water was poured in to the conical flask separately and allowed the powder to soak for 24hour (Ann, 1985). The precipitate was discarded and supernatant was collected for evaporation. The color of the soaked sample after filtration;

- Aqueous filtrate of Cochlospermum tinctorium root powder were turned to coffee color
- Methanol filtrate of Cochlospermum tinctorium root powder was turned to light brown.

The filtrate were poured into crucible and placed on a steam bath at 100°C for evaporation to dryness (Douglas and Gomez-Almote, 2008)

Phytochemical screening of the sample

Qualitative tests

An aliquot (1.0mL) of each extract obtained from roots of C. tinctorium was subject to qualitative phytochemical analysis to ascertain the presence of secondary metabolites such as alkaloids, flavonoids, tannins, carbohydrates, sterols, triterpenes, and saponosides. The compound classes will be characterized using adequate techniques and specific reagents according to the respective methods (Karumi, 2004; Edeoga et al., 2005) described below.

Test for alkaloids

The aqueous and methanolic extracts was evaporate to dryness and the residues were heated with 2% hydrochloric acid on a boiling water bath. The extracts will be cool, filter and treated with the Mayer's reagent. The samples were then observed for the presence of yellow precipitation or turbidity.

Test for flavonoids

1.5 ml of 50% methanol was added to 4 ml of crude aqueous and methanolic extracts. The solution was warm and metal magnesium was added. Then 5 to 6 drops of concentrated hydrochloric acid was added to the solutions and observed for red coloration.

For tannins

To 0.5 ml of crude hydromethanolic solutions, 1 ml of distilled water and 1 to 2 drops of Ferric chloride solution was observed for blue or green black coloration.

Test for Phenolic compounds

Two (2.0ml) of methanol was added to the test solutions and few drops of ferric chloride solution was added and observed for coloration.

Test for Saponosides

Two 2.0 ml of distilled water was added to 2 ml of the test solutions and well shaken and observed for frothing.

Identification of sterols and triterpenes:

10mL of crude hydromethanolic solutions was placed in small beakers and evaporated to dryness. The residues were dissolved in acetic anhydride (0.5 mL) and methanol (0.5 mL). The solution was transferred into dry tests tubes and concentrated sulfuric acid (2 mL) was added. Brownish red or violet rings at the zone of the contact with the supernatant and green or violet coloration denoted the presence of sterols and triterpenes.

Confirmation of bacterial species

Gram staining

Gram staining and biochemical tests (catalase, urease, motility, indole, hydrogen sulphide etc) was used to confirm (characterized) the bacterial isolate procured from the Microbiology research laboratory of Sokoto State University to species level.

Catalase Test

A pinch of 24 hours old bacterial colony was transferred to a surface of clean, grease free slide using sterile wire loop, a drop of 3% H202 were placed on the slide and mixed (Igekele et al., 2012).

Coagulase Test

A pinch of 24 hours old bacterial colony were transferred to a surface of clean, grease free slide using sterile wire loop, a drop of serum was placed on the slide and mixed. A positive result is a coarse clumping of bacteria visible to the naked eye within 10 seconds. Negative result is the absence of clumping or any reaction taking more than 10 seconds to develop (Igekele et al., 2012).

Motility Test

A semi-solid agar medium were prepared in a test tube and inoculate with a straight sterile wire loop, making a single stab down the center of the tube to about half the depth of the medium. It was then being incubated at 37ºC, for 24 hours (Igekele et al., 2012).

Urease Test

This will be done as described by Singleton, (1997), A speck of isolates will inoculate into Christensen's urea agar and incubate at 370C for 24 hours. Liberation of red color indicates urease - positive test while initial yellow color indicates negative (Igekele et al., 2012).

Sugar Fermentation Test

A Pinch of 24hrs colony was inoculate on trifle sugar ion agar (TSI) by first stabbing through the center of the medium to the bottom of the tube and then streaked on the surface of the slant. The cap will be loosed and incubate at 35ºC for 18 to 24 hours (Igekele et al., 2012).

Citrate Test

Simmons citrate agars were inoculated lightly with a pinch of a 24hours old colony of the test organisms. It was then being incubated at 37ºC for 24 hours. The development of blue colour denoting alkalinisation will observe (Igekele et al., 2012).

Hydrogen Sulphide Production

This was done as described by Oyeleke and Manga (2008). This test detects the ability of a bacterial species to produce hydrogen sulphide, e.g. by the reduction of sulphate or from the metabolism of Sulphur-containing amino acids (Singleton, 1997).

A speck of each isolates was inoculated by streaking and stabling into triple sugar iron agar and incubate at 37oC for 24 hours. Evolution on blackening on the medium indicates a positive test while no blackening indicates negative.

Indole Test

Indole test was done as described by Singleton (1997). A speak of each isolate was inoculated onto 5mls of sterile peptone water enriched with 1% tryptophan and incubate at 37°C for 48 hours; to the culture, 0.5 ml Kovac'sindole reagent were added and was gently shaken. In a positive test, indole (present in the culture) dissolves in the reagents which then become pink or red, and forms a layer at the surface of the medium. A yellow layer at the surface of the medium denotes a negative result.

Methyl Red Test

MR test was done as described by Oyeleke and Manga (2008). A speck of each isolate was inoculate onto the glucose phosphate peptone water medium and will be incubate at 37°C for 48 hours. Few drop of methyl red was added to the

culture. MR positive test indicate red color formation while no change denote negative.

The Voges-Prokeur Test

This test was done as described by Oyeleke and Manga, (2008). A speak of each isolate were inoculated into glucose phosphate peptone water medium and was incubate at 37OC for 24hours. Ethanolic solution of 5% α-nephthool (1.2ml) and 0.4ml of 40% potassium hydroxide solution will sequentially added to 2ml of culture, it will shaken vigorously and placed in a sloping position (for maximum exposure of the culture to air), and examine after 10 to 15 minutes. The evolution of red color indicates a positive test for voges-prokeur

Standardization of Bacterial Culture

About 0.1ml of 1% Barium chloride was added to 9.9ml of 1% surphuric acid which was later reconstituted into 10ml of sterile distilled water to make 0.5ml Mcfarland standard solution. The test bacteria culture were sub-culture on nutrient ager and incubated at 37C for 24 hours. After incubation, a sterile Wireloop was used to pick up the colonies of test bacterium and suspended in a test tube containing 10mL of sterile normal saline. The turbidity of the innocula suspension was adjusted and standardized to that of Mcfarland standard (Aibinu et al., 2007).

Determination of Antibacterial Activity of Cochlospermum tinctorium root Extract

The well plate diffusion method was used to determine the growth inhibition of bacteria by plant extracts as described by Mohammad and Dabai, (2008). The Muller Hinton plate were prepared and seeded with the test organisms. Four holes of 6.0 mm diameter each were made on the plate with a sterile cork borer and filled with the following concentration of the extract 2000µg/ml, 1000µg/ml, 500µg/ml and 250µg/ml. The inoculated plates were allowed to congeal for 30 min

To allow pre diffusion time and incubated at 37C for 24 hrs .The plates were examined for zone of inhibition (Cheesbrough, 2001). The diameter of such zone of inhibition was measured using a transparent meter ruler and the value was recorded and expressed to the nearest millimeter.

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

The estimation of MIC of the plant extract was carried out by using the method of Akinpelu and kolawole (2004).The minimum inhibitory concentration of the extracts was determined using the broth dilution method in nutrient broth. Five hundred microliters (500µL) of the bacterial suspension were aseptically inoculated in each of the four tubes containing the extract in order of increasing dilution (250, 500, 1000, 2000). 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) (Williams L, Wilkins S 2007). MBC was determined as described by Spencer (2004). 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 37ºC 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 plate (Akinjogunla et al., 2009).

RESULTS AND DISCUSSIONS

The results of the quantitative phytochemical screening of Chochlospermum tinctorium root powder using methanol extract revealed the presence of alkaloids, flavonoids, saponins, tannin and phenolics, while steroids are absent as showed in (Table 1).

The morphological and biochemical characterization of the bacterial isolate were presented in table (Table 2). Biochemical reaction of the test bacteria was confirmed by comparing the reaction with that in Bergy's manual of determinative bacteriological 2nd edition. The confirmed organisms are E. coli, S. aureus and Klebsiella.

The antibacterial activity of the methanol and aqueous extracts of the root powder of Cochlospermum tintorium against Staphylococcus aureus, Escherichia coli and Klebsiella was presented on (Table 3). The methanol extract revealed maximum zone of inhibition of 15.00 mm against Staphylococcus aureus, 13.00 mm against E. coli and 10.00 mm against Klebsiella at concentration of 2.0×10^3 µg/ml with the minimum zone of inhibition of 5.00 mm against Staphylococcus aureus, 6.00 mm against E. coli and 2.00 mm against Klebsiella at concentration of 0.25×10^3 µg/ml. The aqueous extract revealed maximum zone of inhibition of 9.00 mm against Staphylococcus aureus, 10.00 mm against E. coli and 8.00 mm against Klebsiella at concentration of 2.0×10^3 µg/ml with the minimum zone of inhibition of 4.00 mm against Staphylococcus aureus, 3.00 mm against E. coli and 1.00 mm against Klebsiella at concentration of 0.25×10^3 µg/ml.

The result of the minimum inhibitory concentration (MIC) of the methanol and aqueous extract of Cochlospermum tinctorium root powder against Staphyloccoccus aureus, Escherichia coli and Klebsiella pneumonia are presented on (Table 4). From the results obtained, isolate Staphyloccoccus aureus showed MIC at 0.25×10^3 µg/ml, 0.5×10^3 µg/ml for methanol extract and 0.5×103 µg/ml for aqueous extract, isolate Escherichia coli showed MIC at 0.5×10^3 µg/ml for methanol extract and 1.0×10^3 µg/ml for aqueous extract and isolate Klebsiella showed MIC at 1.0×10^3 µg/ml for both methanol and aqueous extract.

The result of the minimum Bactericidal Concentration (MBC) of the methanol and aqueous extract of Cochlospermum tinctorium root powder against Staphyloccoccus aureus, Escherichia coli and Klebsiella are presented on (Table 5). From the results obtained all isolates tested Staphyloccoccus aureu, Escherichia coli and Klebsiella showed MBC at 1.0×10^3 µg/ml and 2.0×10^3 µg/ml for methanol extract, isolate Staphyloccoccus aureus showed MBC at 1.0×10^3 µg/ml and 2.0×10^3 µg/ml for aqueous extract and isolate Escherichia coli and Klebsiella showed MBC at 2.0×10^3 µg/ml

Table 1: Results of quantitative phytochemical screening of methanol extract of *Cochlospermum tinctorium* root powder

Plants constituents'	methanol extract	aqueous extract
Alkaloids	Mayer's reagent	++
Flavonoids	Ferric chloride	+
Tannins	Ferric chloride	+
Phenols	Ferric chloride	+++
Saponins	Frothing test	+++
Steroids	Salkowski reaction	-

Key: += present - = absent

Table 2: Results of quantitative phytochemical screening of Aqueous extract of *Cochlospermum tinctorium* root powder.

Plants constituents'	methanol extract	aqueous extract	Method	Inference
Alkaloids	Mayer's reagent	+		
Flavonoids	Ferric chloride	+		
Tannins	Ferric chloride	+		
Phenols	Ferric chloride	+		
Saponins	Frothing test	+		
Steroids	Salkowski reaction	-		

Key: += present - = absent

Table 3: Morphological and biochemical characterization of the test bacteria.

Isolates	Gram	shape	Cat	Coa	Glu	Suc	Lac	Gas	H_2S	Cit	Urea	MR	VP	Mot	Ind	Organisms
a	-ve	rod	+	-	+	-	+	+	-	-	-	+	-	+	+	*E. coli*
b	+ve	cocci	+	+	+	+	+	-	-	+	+	+	-	-	-	*S. aureus*
c	-ve	rod	+	-	+	+	-	+	-	-	+	-	-	-	-	*Klebsiella*

Key: += positive, -= Negative, Cat= Catalase

Table 4: Zone of inhibition diameter (mm) produced by aqueous and methanol extract of *cochlospermum tinctorium* root powder against *Staphyloccoccus aureus, Escherichia coli* and *Klebsiella.*

Solvents/ test organisms	concentration of the extract ($\times 10^3$ µg/l)				
	0.25	**0.5**	**1.0**	**2.0**	**Amoxicillin**
Methanol					
S. aureus	5	8	12	15	18
E. coli	6	9	10	13	20
Klebsiella pneumonia	2	3	8	10	15
Aqueous extract					
S. aureus	4	5	7	9	17
E. coli	3	5	6	9	16
Klebsiella pneumonia	1	3	5	8	10

Table 5: Minimum Inhibitory Condition (MIC) of the *cochlospermum tinctorium* root powder of methanol and aqueous extract against *Staphyloccoccus aureus, Escherichia coli* and *Klebsiella*

Extract\organism's	concentration of the extract ($\times 10^3$ µg/ml)			
	0.25	**0.5**	**1.0**	**2.0**
Methanol extract				
S. aureus	©	©	-	-
E. coli	+	©	-	-
Klesiella	+	+	©	-
Aqueous extract				
S. aureus	+	©	-	-
E. coli	+	+	©	©
Klesiella	+	+	+	©

Key: ©= MIC, += turbidity, - = non turbidity

Table 6: Minimum Bactericidal Condition (MBC) of the *Cochlospermum tinctorium* root powder of methanol and aqueous extract against *Staphyloccoccus aureus, Escherichia coli* and *Klebsiella.*

Extract\organism's	concentration of the extract ($\times 10^3$ µg/ml)			
	0.25	0.5	1.0	2.0
Methanol extract				
S. aureus	+	+	¢	¢
E. coli	+	+	¢	¢
Klesiella	+	+	¢	¢
Aqueous extract				
S. aureus	+	+	¢	¢
E. coli	+	+	+	¢
Klesiella	+	+	+	¢

Key: ¢= MBC, += growth

The phytochemical screening revealed the presence of phenolic compounds, tannins, flavonoids, saponins and alkaloids, while steroids were absent in the roots of *C. tinctorium* (Table 1). The qualitative analysis of the extracts (Tables 1) confirms the richness of the roots of *C. tinctorium* in phenolic compounds, particularly tannins and flavonoids. This observation is correlated with the conclusions of many studies that have highlighted the variations in concentration of secondary metabolites from plant to plant species as well as in the different parts of a plant, leaves and roots being the preferential sites of accumulation of these compounds (Hyder *et al.,* 2002; Springer *et al.,* 2002; Isah *et al.,* 2013; Kantati *et al.,* 2016).In addition, the presence of so important pharmacological groups would justify the traditional use of the plant in the treatment of several diseases; burn, snake bites, malaria (Evans and Gaiere, 2017), palpitations, typhoid fever, urinary tract infections, hypertension, jaundice, wounds, viral hepatitis (Togola *et al.,* 2008), hepatitis, diarrhea dysentery, infertility, diabetes (Igoli *et al.,* 2005).

Saponins are promising anticancer agents that works by stopping cellular mutations that could inevitably lead to cancer (Mohammed *et al.*, 2009). Phytochemical test revealed the presence of saponins in the *C. tinctorium* root powder extract; this study was similar to that of Akpemi Audu Musa (2012) on his study of Cytotoxicity Activity and Phytochemical Screening of *Cochlospermum tinctorium* Perr Ex A. Richrhizome who reported that The presence of saponins and terpenes in both extracts justified their potency as antitumor and anticancer agents (Man *et al.*, 2010) based on the LC50 values determined from brine shrimp lethality bioassay extracts or compounds that are cytotoxic have good correlation to be effective antitumor agents (Meyer *et al.*, 1982; McLaughlin *et al.*, 1998).

Recently, plant phenolic have demonstrated to be very effective antitumor agents, some of them have been determined to be flavonoids, polyphenols, anthraquinones, coumarins, alkaloids etc. (Lee, 1992). Flavonoids are polyphenolic compounds that are ubiquitously found in plants that accounts for their ability to prevent cancer and tumor growth (Ren *et al.*, 2003). Tannins are also polyphenols that have anticancer properties, and it is believed that tannins achieve this property by blocking the production of enzyme required for cancer cell line growth (Mohammed *et al.*, 2006). The extract contain flavonoids as shown in table 4.1, similar study was reported by Akpemi Audu Musa 2012 who reported that 80% acetone extract of *Cochlospermum tinctorium* contains flavonoids and tannins. He concluded that; the presence of these secondary metabolites accounts for the potent cytotoxic activity of the extract. This implies that it may also possess potent anticancer and antitumor activity (Meyer *et al.*, 1982).

The aqueous extract of *C. tinctorium* rhizomes was reported to possess antibacterial activity (Diallo *et al.,* 1987). Additionally, the antibacterial activity of the methanol root extract of *C. tinctorium* (250, 500, 1000, and 2000 µg/ml) was evaluated using hole-in-plate bioassay technique with ciprofloxacin (10 µg/ml) as described by Tijjani *et al.,* (2009).

The results of the antibacterial activities of methanol extracts showed that the *C. tinctorium* was active against the various test bacteria at different concentrations tested. The bacteria tested are *Staphyloccoccus aureus, Escherichia coli* and *Klebsiella.* However, the plant showed good inhibition toward test bacterial isolates. It was observed that at higher concentration of 2.0×10^3 µg/ml the inhibition activity fall within 10.00 – 15.00 mm for methanol extract and 8.00 – 10.00 mm for aqueous extract which very closed to the control antibiotic with inhibition zone of 15 - 20mm for methanol extract and 10.00 – 17.00 mm for aqueous extract. The reason for high antibacterial activity on S. aureus could be attributed to fact that *Staphyloccoccus aureus* are gram-positive bacteria whose outer peptidoglyan layer is not an effective permeability barrier. However, the high activity of 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 (Antara and Amla, 2012). This was closely related to the finding of Tijjani et al., (2009) who reported the significant antibacterial activity of *Cochlospermum tinctorium* root powder at 2000 µg/ml against *Staphylococcus aureus* (19.00 mm), *Corynebacterium ulcerans* (17.20 mm), *Klebsiella pneumonia* (11.00 mm), *Escherichia coli* (14.30 mm), *Proteus mirabilis* (11.00 mm), and *Shigella dysentriae* (19.00 mm) (Tijjani *et al.,* 2009). The highest activity of the extract was observed against *S. aureus* and *S. dysentriae* (19.00 mm).

Cowan 1999 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 (Rosina *et al.,* 2009).

Elsewhere, this study also showed that the Methanol and aqueous extracts from the roots of *C.tinctorium* were active on all the germs tested. Based on the bacterial parameters MIC and MBC (tables 4 and 5) it was confirm that the antibacterial activity of root extracts of *C. tinctorium* is stronger on *Staphylococcus aureu* with MIC at (1.0, 2.0) $\times10^3$ µg/ml and MBC at (1.0, 2.0) $\times10^3$ µg/ml for both methanol and aqueous extracts of C. tinctorium root powder. *Klebsiella pneumoneae*

was the bacterial isolate with least sensitive to both methanol and aqueous extracts of C. tinctorium root powder root with at MIC and MBC at 2.0×10^3 µg/ml for both methanol and aqueous extracts. It can therefore be said that both methanol and aqueous extracts of C. tinctorium root powder have bactericidal activities on the test bacteria. This study was correlated with that of Okou *et al.*, (2018) who conclude that hydroethanolic extracts of roots and leaves of *Cochlospermum tinctorium*have bactericidal activities on the strains studied. These results are confirmed by those of Isah et al. (2013) who demonstrated that the hydroethanolic extract of young leaves of *C. tinctorium* at the concentration of 80 mg/mL was sensitive on *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli.* This antibacterial activity of the plant is also reported by the work of Alain et al. (2014) who found that the plant extracts are active on several germs, including *Pseudomonas aeruginosa* and *Staphylococcus aureus.* The antibacterial activity observed would be related to the metabolites found in the plant. Indeed, flavonoids and triterpenes have a known antimicrobial activity (Ayeni, 2018), anti-ulcer properties (Madivoli *et al*., 2018), and anti-inflammatory properties (Özçelik *et al*., 2011).

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 *Staphylococcus aureus, Escherichia coli and Klebsiella*, 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. High zone of inhibition of 10.00 – 15.00 mm and 8.00 to 10.00 mm was observed on both methanol and aqueous extract at high concentration of 2000 µg/ml against test bacteria. MIC was observed at 500 µg/ml and 1000 µg/ml on methanol extract and 1000 µg/ml and 2000 µg/ml for aqueous extract. MBC was observed at 1000 µg/ml and 2000 µg/ml for methanol extract and d 2000 µg/ml for aqueous extract against all organisms tested

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