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Phytochemical screening and antibacterial susceptibility of *Escherichia coli* O157:H7 isolates on *Acacia ataxacantha* leaves

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Acacia ataxacanta **leaves were screened for possible constituents, and the antimicrobial properties of the leaves were tested against 18 isolates of *Escherichia coli* O157:H7. The leaves were aseptically collected and crude extracts were prepared by cold maceration in 98% methanol. Phytochemical analysis for bioactive constituents revealed that it contained carbohydrates, cardiac glycosides, steroids, saponins, triterpenes, tannins, flavonoids and alkaloids. Partitioning of the crude extract yielded n-hexane, ethyl acetate and aqueous fractions were evaluated with their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The various concentrations of the crude extracts (600, 500, 400, 300, 200, 100, 50, 25, 12.5 and 3.15 mg/ml) were tested on the isolates. The crude extract demonstrated antibacterial activity against *E. coli* O157:H7 isolates with zones of inhibition ranging from 15.67±0.3 to 18.67± 0.3 mm at concentrations of 300 to 600 mg/ml. Ethyl acetate fraction was identified as the most potent of the fractions with bacteriostatic activity displayed as wide zone of bacterial growth inhibition of 18 and 13 mm at 200 mg/ml against the isolates. The aqueous fraction produced comparatively lower zones of bacterial growth inhibition of 12 and 15 mm at higher concentrations of 300 to 600 mg/ml. The MIC for the extracts was 100, 200 and 300 mg/ml for the crude extract, ethyl acetate and aqueous fractions respectively. It is therefore concluded that *A. ataxacantha* leaves tested contains bioactive constituents which are of high medicinal value. It is necessary that the extract should be further purified and exploited for toxicological use to confirm its safety in disease therapy, using higher molecular techniques.**

Key words: Photochemical screening, bioactive, *Escherichia coli* O157:H7 isolates on *Acacia ataxacantha* leave.

INTRODUCTION

Escherichia coli O157 and *E. coli* O157:H7 has caused devastating disease in animals and man, causing variety of illness ranging from gastroenteritis, abdominal cramps, vomiting haemolytic uremic syndrome (HUS) and

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thrombocytopenic purpura (CDC, 2017a). The very low dose of the organism is required to illicit infection. The fact that ingestion of very few as dose as low as 10 organisms, coupled with the short incubation period of 3 to 4 days in most clinical disease requires prominent attention to be paid in field outbreaks all over the world (Mailafia et al., 2017).

The estimated case fatality rate due to *E. coli* O157:H7 infection is very high in all ages in humans, ranging from children to adults. Young children and immune-compromised adults excrete the organisms in the feaces for more than three weeks (Nathan et al., 2013). The attempted administration of conventional antibiotics during episodes of infection is known to exacerbate shiga-toxin mediated cytotoxicity. Presently, there is no known specific cure for field outbreaks of these infectious diseases and efficient control regime (CDC, 2018). Also, the high cost of antibiotics coupled with the fact that infection with O157:H7 involves that stimulation of verocytoxic production have stimulated more interests in alternative plants sources which has become the last option in the management of this infection (Emmanuel et al., 2013).

The interest in plants sources with efficacious ethno veterinary properties have been revived due to current problems associated with the use of antibiotics coupled with the increased prevalence of multiple-drug resistant strains of a number of pathogenic bacteria including: methicillin resistant *Staphylococcus aureus* and multi-drug resistant *Klebsiella pneumonia*, *Helicobacter pylori* and many other emerging and re-emerging bacterial diseases (CDC, 2017b).

Plants offer prospects as sources of medicaments and novel drugs. *Acacia ataxacantha,* a *Fabaceae* and shrubby climber commonly called flame thorn tree is recognized in different parts of Nigeria due to their high potency in the management of several diseases. It is popularly referred to as *Ihun* in Igbo, *Sarkakiya* in Hausa and *Ewonadele* in Yoruba languages (Emmanuel et al., 2013) within Nigeria. The plant is widespread in tropical Africa from Senegal in the west to Sudan in the east, Namibia, Botswana, Zimbabwe, and in Kwazulu-Natal, Southern Africa. The stems grow up to 10 m long forming thicket of 4 to 5 m deep; leaves are alternate, pinnate with spine that carries 5 to 12 pairs of pinnae (Amoussa et al., 2014).

The plant has white flowers with long transition axillaries of 4 to 5 cm long and arranged on stem of 10 to 15 mm, sometimes isolated in pairs. The fruit pods are flattened, brownish red in the dry state (wikipedia.org). *A. ataxacantha* leaves, roots, pods and seeds were employed traditionally within Nigeria and other parts of Africa to relieve joint pain, back ache, stomach pain, dysentery, pneumonia and chronic cough (Joachim and Edwards, 2015). Preparations from the plant were found useful in the management of chickenpox and yellow fever (APG, 2014). The methanolic extract of *A. ataxacantha* leaves demonstrated ulcero-protective potency at 100 and 200 mg/kg in indomethacin and stress induced gastric ulcer models in rats. The aqueous extract exhibited a remarkable laxative activity at 400mg/kg (Akapa et al., 2014).

The use of herbal remedies in managing *E. coli* O157:H7 infection should be sought to reduce the high cost of conventional drugs, increased antibiotic-resistant strains in the case of infectious diseases and encourages researchers to ascertain a wide range of efficacious medicinal plant sources as alternative treatments for diseases caused by *E. coli* O157:H7, and there was no prior study on effects of extracts from this plant on isolates of *E. coli* O157: H7. The aim of this present research is to screen *A. ataxacantha* leaves for efficacious phyto constituents and ascertain its novel role as bioactive remedies for the management of infection caused by *E. coli* O157 and *E. coli* O157:H7.

MATERIALS AND METHODS

Source of *E. coli* O157 and *E. coli* O157:H7 isolates

The *E. coli* O157 isolates used for this study was a gift from Dr. Stella Madubuike of the bacteriology laboratory of Ahmadu Bello University Zaria. They were isolated from cattle faeces using standard bacteriological methods. They were therefore confirmed and preserved for the present study involving *A. ataxacanta* leaves against test strains of *E. coli* from cattle feaces.

Plants collection and identification

A fresh leaf of *A. ataxacantha* was collected in January 2016 from farm locations within Zaria. The plant was identified and authenticated at the herbarium laboratory, Department of Biological Sciences of Ahmadu Bello University, Zaria, were a voucher specimen was deposited and a specimen no. 1707 was given for future reference or correspondence.

Plants extraction

The leaves were cleaned air dried under shade and grounded into powder. The powder (150 g) was macerated in 98% methanol (ratio 1:3, w/v) at room temperature for 48 h. The entire process is repeated twice for a period of 24 h during the second and third extraction processes. The methanol extracts were pooled together and filtered using a filter paper (Whatmann size no.1). The filtrate was evaporated to dryness in a water bath at 40°C. The dried extract obtained was weighted and kept in airtight bottle in a refrigerator at 4°C until required for use (Sithara et al., 2016).

Phytochemical screening of crude methanol extract of *A. ataxacantha*

Phytochemical screening of *A. ataxacantha* leaves was carried out using standard analytical techniques (Mohammad et al., 2013). The tests conducted were: Molisch test, Fehling test, glycoside test, ferric test, sodium hydroxide test, Wagner's test, Mayer's test, Dragendoff's test, frothing test, Liebermann-Bucchard test, and Keller-Killiani test.

Figure 1. Minimal bactericidal concentration (MBC) test showing microbial growth.

Phytochemical screening of the fractions of *A. atxacantha*

The three fractions of the leaf extract which included: ethyl-acetate, N-hexane and aqueous methanol fractions were also subjected to phytochemical screening tests to detect the presence of its chemical constituents. The N-hexane fraction yield was very minimal and insignificant, thus it was discarded and not used (Akaba et al., 2014).

Susceptibility of *E. coli* isolates on the crude extract of *A. ataxacantha* leaves

The antimicrobial effect of the crude extract of the leaves of *A. ataxachantha* was tested against the bacteria isolates grown on Sorbitol-MacConkeyCefiximeTallurite agar (CT-SMAC, Oxoid, UK) as previously described and in accordance with standard recommendations (CLSI, 2014). The method allowed for determining of *in-vitro* efficacy of an extract by measuring the diameter of the zones of inhibition that results from diffusion of the agent into the medium surrounding the well. Graded concentrations of 600, 500, 400, 300, 200, 100, 50, 25, 12.5, .25 and 3.125 mg/ml of the crude extract were prepared by double serial dilutions. Using sterile saline, the broth cultures were adjusted to obtain optical turbidity comparable to 0.5 McFarland standards. Holes (wells) were created on the surface of MHA plate using a hole borer. This was to accommodate the graded concentrations of the crude extract. The sterile Mueller Hinton agar plates were then inoculated with 0.1 ml of the MTSB (Oxoid, UK) culture and spread over the entire sterile agar surface using sterile swab sticks. Thereafter, antibiotic controls (Ciprofloxacin (5µg) and gentamicin (10µg), were placed individually on the surface of the inoculated agar plates using a dispenser (Oxoid, UK). The inoculated plates were then incubated at 37°C for 24 h. Test assays were performed in triplicates. The clear zones of growth inhibition were then measured to the nearest millimeter with a transparent meter rule. The zone diameter value for each well was interpreted as sensitive, intermediate and resistant in accordance with standard breakpoints (CLSI, 2014) (Figures 1 to 3).

Minimum inhibitory concentration (MIC) of the crude extract of *A. ataxacantha*

The minimum inhibitory concentrations (MIC) of the crude extract of *A. ataxacantha* leaves that showed antimicrobial activity were determined for the bacterial isolates following a procedure adopted by Yusha'u et al. (2008). Serial dilutions of the extract (presenting different concentrations of the plant extract) in distilled water were prepared in test tubes. Each of the tubes was inoculated with 0.1ml of aliquot of the test organism (*E. coli* O157:H7) grown in MTSB and incubated at 37°C for 18 to 24 h. Broth tubes that appeared turbid were indicative of bacterial growth while tubes that remained clear indicated no growth. The least concentration of the extract that prevented visible growth of the organism was then accepted as the MIC.

Minimum bactericidal concentration (MBC) of the crude extract of *A. ataxacantha*

The minimum bactericidal concentration (MBC) was determined by inoculating the culture from MIC tubes, and 1 of 2 more before placing it on a plate of fresh selective media (CT-SMAC) that supports the growth of the organism using a sterile swab. After 24 h of incubation, the plate that showed no visible growth of the organism was then accepted for an MBC (Aamer et al., 2014).

Data analysis

Results were presented as descriptive statistics using percentages, tables and charts. Data were expressed as mean ±S.E.M and further analyzed using One Way analysis of variance. All the assays

Figure 2. Acacia ataxacantha leaves used for this study.

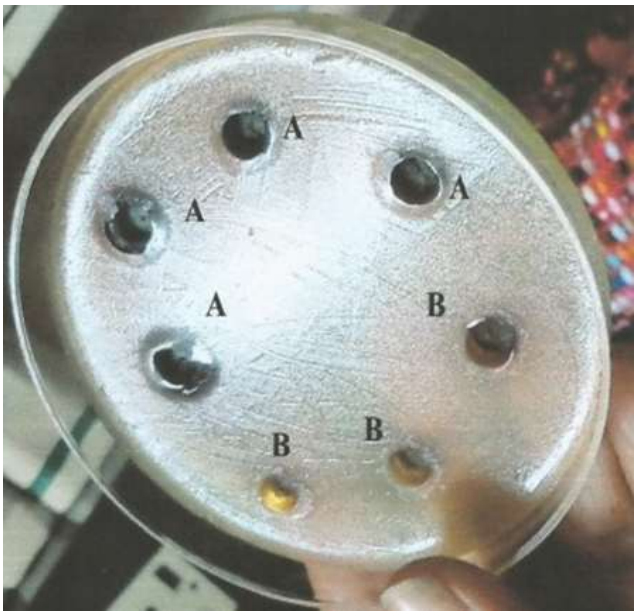

Figure 3. Susceptibility of the crude extract; A: shows inhibition while B: shows no inhibition.

of the mean zones of *E. coli* O157 and *E. coli* O157:H7 with the concentrations of the extracts were analyzed using graphpad Prism version 5.0 for windows. Values of P< 0.05 were considered significant (Scott, 2013).

RESULTS

The yield and percentage yield of the crude extracts of *A. ataxacantha* leaves are shown in Figure 2. It showed that powdered leaves (150g) yielded 26 g after crude extraction using 98% methanol which gave a percentage yield of 17.3%. The percentage yield of various solvents fractions from the crude extract leaves of *A. ataxacantha* leaves showed partitioning of 17g of the crude extract using n-hexane, ethyl acetate and distilled water yielded N-hexane fractions of 1.5 g (8.8%); ethyl acetate fraction

Table 1. Yield of solvent fractions from crude extract of *A. ataxacentha* leaves.

Fraction	No. of fractions	% yield
Aqueous distilled water	2.9	17.1
Ethyl-acetate	1.8	10.6
N-Hexane	1.5	8.8
Total	6.2	36.5

Table 2. Phytoconstituents of crude extracts and fractions of *A. ataxacantha* leaves.

Class of phyto component	Fraction			
	Aqueous	Crude	Hexane	Ethyl acetate
Alkaloids	+	-	+	+
Carbohydrates	+	-	+	+
Cardiac glycosides	+	-	+	+
Saponins	+	-	+	+
Steriods	+	-	+	+
Tannins	+	-	+	+
Triterpins	+	-	+	+

+ = Present; - =Absent.

Table 3. Mean zones of *E. coli* O157 and *E. coli* O157: H7 with concentrations of crude extract of the *A. ataxacantha* leaves.

Extract	Conc/ml	Mean DM zone of inhibition (mm)± S.E.M	
		***E. coli* 0157**	***E. coli* 0157: H7**
CME	600 mg	18.67±0.3	$17.33±0.9^{c}$
CME	500 mg	$18.33±0.3^{c}$	$16.33±1.3^{c}$
CME	400 mg	$17.00±1.0^{c}$	$15.67±0.3^{c}$
CME	300 mg	$17.33±1.2^{c}$	$16.00±1.5^{c}$
CME	200 mg	14.33±1.0	14.67±0.4
CME	100 mg	12.00±0.2	13.67±0.1
CME	50 mg	10.67±0.3	11.67±0.6
CME	25 mg	9.67±0.40	11.67±0.7
CME	12.5 mg	10.33±0.5	10.00±0.7
CME	6.25 mg	NILL	7.0±00.2
CME	3.125 mg	NILL	6.00±0.3

CME = Crude methanol extracts, no inhibition; means with different superscripts letters are significantly different ($p<0.05$).

of 1.8 g (10.6%) and aqueous fraction of 2.9 g (17.1%).

Table 1 shows percentage yield of solvent fractions of *A. ataxacantha* leaves. Aqueous distilled water has the highest yield of 17.1% while N-hexane had the lowest yield of 8.8%. Phytochemical analysis of the crude extract and fractions of the plant showed that the plant contains carbohydrates, cardiac glycosides, steroids, saponins, triterpenes, tannins and alkaloids (Table 2). The mean zones of inhibition of *E. coli* 0157 and *E. coli* 0157: H7 were seen in Figure 3 and it demonstrated significant ($p<0.05$) antibacterial effect at high test concentrations of 300 to 600mg/ml against the isolates with mean zone diameter between 15.67±0.3 and 18.67±0.3mm. The concentrations of crude extracts below 200 mg/ml exerted less than 15.0 mm average zones of inhibition (Table 3).

The mean zones of bacterial inhibition shown in Figure 1 produced by fractions of *A. ataxacantha* against *E. coli*

Table 4. Zones of inhibition of *E. coli* 0157 and *E. coli*O157: H7 produced by fractions of *A. ataxacantha* crude extracts.

Fraction	Isolate	Zones of growth inhibition (mm) at various concentrations (mg/ml)				
		200	300	400	500	600
N-hexane	*E. coli*O157	-	-	-	-	-
	E. coli O157:H7	-	-	-	-	-
Ethyle acetate	*E. coli* O157	13	-	-	-	-
	E. coli O157:H7	18	-	-	-	-
Acaeous	*E. coli* O157	-	12	12	11	12
	E. coli O157:H7	-	15	15	15	15

- = no zone of inhibition.

Table 5. Minimum inhibitory concentrations of the fractions and extracts of *A. ataxacantha* leaves on *E. coli* O157 and *E. coli* O157:H7.

Variable	Antibacterial activity in different concentrations of the extract							
	Isolate	50	100	200	300	400	500	600
Extracts	*E. coli* 0157	-	+	+	+	+	+	+
	E. coli 2157: H7	-	+	+	+	+	+	+
Fractions								
Ethylacetate	*E. coli* 0157	-	-	+	+	+	+	+
	E. coli 0157:H7	-	-	+	+	+	+	+
Aqueous	*E. coli* 0157	-	-	-	+	+	+	+
	E. coli 0157: H7	-	-	-	+	+	+	+

+ = Inhibition (Transparency); - = growth (Turbidity).

O157 and *E. coli* O157:H7 was assessed. The ethyl acetate fraction produced 18 and 13mm mean zones of bacterial growth inhibition on the isolates at 200mg/ ml, but exhibited no inhibitory activity at higher concentrations above 200mg/ ml. The aqueous fractions displayed zones of inhibition against the isolates at concentrations above 200mg/ml. The n-hexane fraction showed no activity against the isolates at various concentrations tested (Table 4).

The MIC crude fractions and MBC of the fractions of *A. ataxacantha* leaf extract on *E. coli* O157 and *E. coli* O157:H7 was determined. It showed that MIC for crude methanol extract to be 100 mg/ml and that of ethyl acetate fraction was 300 mg/ml. The extract did not show bactericidal activity (Table 5). Figure 1 shows the minimum bactericidal concentration of the test organisms showing bacterial growth. Figure 2 shows *A. ataxacantha* tree displaying the leaves that were used for this study, while Figure 3 dissipates the susceptibility of the crude extract in which parts labeled A showed zones of inhibition while B showed no zone of inhibition.

DISCUSSION

Multiple antibiotic resistances of bacterial pathogens are of great concerns to both veterinary and human medicine worldwide. Antimicrobial resistances pose serious problems in the treatment of animal and patients with infectious diseases associated with *E. coli* infections. These organisms have acquired resistances through the development of mobile genetic elements, thereby spreading resistance genes via over expression of endogenous multidrug transporters. Also, huge consumption of the antibiotics in the human and animal medical practice, coupled with the high cost of therapeutic remedies against infectious diseases has forced our limit of study to cheaper plant remedies (Martins, 2013).

The study findings indicated high yield of the crude leave extract of *A. ataxacantha* of 17.1% for the aqueous fraction, 10.6% for ethyl acetate fraction and 8.8% for N-hexane fraction. This yield was however lower than that obtained by El Mahmood et al. (2008) for *Sennaobtusfolia* who obtained yield of 52% for aqueous extract and 50% for hexane extract.

Ogbulie et al. (2007) also reported a different yield of 9.1% in aqueous extract of *Euphorbia hirta.* Several factors include age of the plant, polarity of the solvent used, and season of the year when the leaves were harvested. The topography of the soil upon which the plant is grown and the extraction methods used may

have been amongst the several factors that affected the yield. In this study, water seems to have produced the highest yield in terms of the extraction processes but that does not translate into proportionate medicinal value.

The phytochemical analysis of the crude extract revealed the presence of numerous phytochemicals including: flavonoids, carbohydrates, steroids, triterpenes, cardiac glycosides, tannins, saponins and alkaloids. Steroids are valuable as components of several drugs such as cardiac depressants, anti-hypertensives, sedatives and antibiotics (Joachim and Edward, 2015). Tannins are reported to have various physiological effects as anti-irritants, anti-secretolytic, antichloristic, antimicrobial and antiparasitic (Sieniawska and Baj, 2017), and are used in *Ayurveda* for the treatment of diseases like leucorrhoea, rhinnorhoea and diarrhoea. Alkaloids are employed as anti-malaria, anti-amoebic agents and as astringents (Kukula-Koch and Widelski, 2017), while saponins have been found to use in a wide range of pharmacological formulations including expectorants, anti-inflammatory, vasoprotective, hypocholesterolemic, immunomodulatory, hypoglycaemic, molluscicidal, antifungal, antiparasitic, hyperglycaemia, antioxidant, anti-cancer, weight loss and as natural antibiotics (Yun-Tao et al., 2017).

Flavonoids and tannins have been reported to possess antimicrobial activity. The antimicrobial activity of flavonoids is due to their ability to complex with extracellular and soluble proteins as well as lipids in the bacterial cell wall (Farina et al., 2014). Some of these bioactive compounds which are synthesized as secondary metabolites during growth of the plant also serve to protect plants against microbial attacks and predation by animals (Jitendra and Naveen, 2018). The increasing reliance on the use of medicinal products of plant origin by a large proportion of the populace in developing countries is due to their relative abundance, safety, high efficacy and cost effectiveness which invariably make such drugs cheaper.

Our observations also have shown that the crude extract produced inhibition remarkably wide zones of inhibition ranging from 15.67±0.3 to 18.67±0.3 mm when induced with maximum dose of 600mg/ml. Our crude extract appeared to be more potent with higher doses of 300 to 600mg/ml. The antibacterial effect of the crude extract particularly at high doses was more significant when compared with those of the lower doses. N hexane fractions had no activity against *E. coli* 0157:H7 and *E. coli* O157 at varying concentrations. Our findings are in concordance with Akapa et al. (2014) who reported zero activity in *A. ataxacantha* bark in *E. coli* isolates tested. The ethyl acetate fraction dissipated the most potent antibacterial susceptibility even at low concentrations as 200 mg/ml, compared with either aqueous or N- hexane fractions (Anil and Niraj, 2017).

The high antibacterial activity observed in ethyl acetate fractions may be due to some intrinsic constituents with high antibacterial effect. The variation in effectiveness of the extract against the isolates under study may be attributed to its phytochemical composition couple with better membrane permeability gradient of the bacterial organisms for chemicals and their possible metabolism. The high potency observed in our study is therefore a rapid response call for further analysis of this plant using higher molecular techniques to ascertain its safety in the management of human and animal diseases. The MIC and the MBC in our study indicated that the MBC at 200 and 300mg/ml may be accepted as the minimum dose required for usage especially when ethyl acetate and aqueous fractions were extracted.

Conclusion

The study has revealed the bacteriostatic potentials of the extracts of *A. ataxacantha* leaves against the isolates of *E. coli* O157 and *E. coli* O157:H7 particularly at high doses of 300 to 600 mg/ml for the crude extract. Ethyl acetate and aqueous fractions showed inhibitory effect with a maximal efficacy at 200 and 300 mg/ml respectively. The activity of *A. ataxacantha* leaf extract was due to the present of the secondary metabolites. However, we observed that ethyl acetate fraction produced maximal activity when compared to other fractions tested. Hence, *A. ataxacantha* leaf extract were effective against the tested bacteria. It therefore calls for rapid response by scientists globally to characterize, purify and determine the toxicological safety of the bioactive components of the extract. This is useful in understanding the mechanism of action and use of this plant for therapy of clinical infections caused by *E. coli* O157:H7 and other microorganisms.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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