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ANTIBACTERIAL, PHYTOCHEMICAL AND TOXICOLOGICAL STUDIES OF *Entada africana* EXTRACTS.

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ABSTRACT

The study was carried out to determine the antibacterial activity, phytochemical and toxicological studies of Entada africana extracts against Escherichia coli strains isolated from diarrhoeal stool of children (0-5) five years. The antibacterial activity was carried out using agar well diffusion method, while phytochemical screening was conducted using Sofowora, Harbone and Hassan's methods. The acute toxicity (LD_{50}) test was also carried out using Lorke and Hassan's methods. The phytochemical screening of Entada africanaleaf extract revealed the presence of alkaloids, steroids, tannins and glycosides, while stem-bark extracts indicated the presence of alkaloids,

Introduction:

Plants that possess therapeutic properties or exert beneficial pharmacological effect on the human body are generally designated as medicinal plant (Motaleb, 2011). Therefore a medicinal plant is that plant in which one or more of its organs contain active ingredients which is used for therapeutic purposes or contains active ingredients that can be used for synthesis of drugs (Tijjani *et al*., 2017). Medicinal plants are used by traditional medicinal healers' inform of decoction, concoction, infusion and tisane (Culei, 1982).

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saponins, steroids, saponins and tannins.The antibacterial activity of methanol leaf and stem-bark extracts of Entada africana indicated highest activity against Escherichia coli, while hexane leaf and stem-bark extracts exhibited lowest activity against the test isolates. Based on the result of acute toxicity $(LD)_{50}$ test greater than5000mg/kg body weight, showed that the plant extracts are safe for consumption. The result has justified their utilization by the traditional medicine practioners for the treatment of diarrhoea and other related ailments associated with Escherichia coli strains.

Keywords: *Diarrhoea, Extract, Phytochemical, Toxicity, Entada africana*

Medicinal plants naturally synthesize and accumulate some secondary metabolites like alkaloids, steroids, tannins, terpenes, flavonoids, saponins, glycosides, cyanogenics, resins, lactones and carotenoids (Melkamu *et al*., 2018). Plants have elaborate remarkable array of natural products many of which have antimicrobial activities (Kiyasar *et al.,* 2002).

Medicinal plants are the back bone of traditional medicine which more than 3.3 billion people in developing countries utilize on a regular basis (Davidson-Hunt, 2000). Moreso, World Health Organization (WHO), estimated that more than 80% of the world population still relies on traditional medicine for their primary health care needs (Sasidharan *et al*., 2011).The discovery of modern drugs such as quinine, vincristine, digoxin, digitoxin and artemisinin from medicinal plants signifies the huge potential that still exists for the production of more novel pharmaceuticals (Geyid *et al*., 2005).

Entada africana is commonly known as Dorot and belongs to *Mimosaceae* Family. The plant is called Tsawata (Hausa), Ogumba (Yoruba), Kawonuwanchi (Nupe) (Mann *et al*., 2003). It is a tropical perennial plant predominant in the savannah especially the central and eastern tropical Africa (Katende, 1995). Ethno-botanical uses include treatment of

dysentery, cough, fever, wound and also as an arbotifacient (Mann *et al.*, 2003).

Materials and Methods

Collection and Identification of Plant.

The fresh leaves and the stem- bark of *Entada africana* were collected within Ibrahim Badamasi Babangida University, Lapai Niger state. The plant was identified and authenticated by a botanist Dr. M.O Adebola from the Department of Biological Sciences, Ibrahim Badamasi Babangida University Lapai, where a voucher document has been deposited in the school herbarium.

Preparation of Plant Sample.

The leaves and stem-bark of *Entada africana* (Dorot) were rinsed with distilled water, cut into pieces and air-dried in the laboratory for three weeks at room temperature (22^0C) in a shaded area. The dried plant materials were ground into powder by the aid of a clean mortar and pestle. The ground particles or powder form were sieved with mesh sieve size (0.26mm) to obtain a fine powder.

Extraction of Pulverized Plant Sample

Extraction of plant samples was carried out using Soxhlet extractor. Acetone, water, hexane and methanol were used as extractants (solvents). Methanol being polar solvent has capacity of extracting hydrophilic compound, while hexane (non-polar) has capacity of extracting lipophilic compounds.

Hundred gram (100g) of the powdered leaves of each plant *Entada africana* was weighed and wrapped in a plain paper and placed in a Soxhlet extractor and extracted with water, acetone, hexane and methanol. The extraction was done until solvent in the Soxhlet turned colourless. The solution (filtrate) was transferred into Porcelain dish then allowed to dry. The extract obtained was labeled, weighed and kept for further analysis. The same procedure was followed for stem-bark extraction.

Preliminary Phytochemical Screening of Plant Extracts.

The method of Hassan *et al.* (2005) and El-Mahmood and Doughari (2009) were used to detect the presence of phytochemical constituents. The crude aqueous, acetone, hexane and methanolic extracts were subjected to phytochemical constituent's test for alkaloids, anthraquinones, flavonoids, glycosides, resin, saponins, steroid and tannins.

Test for Alkaloids.

To 3ml of extract, 1ml of 1% of HCl was added. The mixture was treated with two drops of Meyer's reagent. A creamy white precipitate indicated the presence of alkaloids. Absence of white precipitate indicated negative result (Ogukwe *et al.*, 2004)

Test for Anthraquinones

To 4ml of extract, 4ml of 100% ammonia solution was added. Pink violet or red colour in the ammonical layer (lower layer) indicated the presence of anthroquinones (Danlami, 2009)

Test for Flavonoids

To 1ml of extract, 3 drops of ammonia solution was added. Half (0.5) ml of conc. HCl was further added to the mixture. A pale brown coloration indicated the presence of flavonoids (Odebiyi and Sofowora, 1978).

Test for Glycosides

To 1ml of the extract, 2ml of acetic acid was and added and then cool in an ice bath at (4^0c). To the mixture, 1ml of conc. H_2SO_4 was added drop wise. Oil layer formed on top of the solution indicated the presence of glycosides (Odebiyi and Sofowora, 1978; Ogukwe *et al.*, 2004; El-Mahmood and Amey, 2007).

Test for Resins

To 5ml of extract, 5ml of copper acetate solution was added. The mixture was shaken vigorously and the allowed to separate. A reddish brown

precipitate indicated the presence of resin (El-Mahmood and Doughari, 2008).

Test for Saponins

To 2ml of the extract, 5 drops of olive oil was added. The mixture was vigorously shaken, a stable emulsion forms in the extract indicated the presence of saponin (Hassan *et al.*, 2005)

Test for Steroids

To 1ml of extract, 1ml of conc.H_2SO_4 was added. A red coloration indicated the presence of steroid (Hassan *et al.*, 2005).

Test for Tannins

Two drops of 5% $FeCl_3$ was added to 1ml to 1ml of extract and a dirty green precipitate indicates of tannins (Ogukwe *et al.*, 2004).

Experimental Animal (Albino Rats)

Young Albino rats (male) with an average body weight (70 – 130g) were used for this study. They were obtained from the Animal House Unit of the Department of Biochemistry, Ibrahim Badamasi Babangida University Lapai, Niger State. They were housed in a polypropylene cage and maintained at 30-37ºC for 12 hours' dark/light cycle. The animals were acclimatized to laboratory condition for 7 days prior to experiment. They were fed with standard mash and water ad libitum. For hygienic purpose, the litter to the cage was cleaned twice a week.

Acute Toxicity Studies (Determination of LD_{50}).

The method of Hassan *et al.* (2005) and OECD, (2001) were used for acute toxicity study of the plant extracts. Water (aqueous) extract of *Entada africana* leaf extract (1000, 2000, 3000, 4000 and 5000 mg/kg) was administered to six (6) groups, (six Wister albino rat per group) of rats (one after the other at a grace observation of 48 hours) in single oral dose by using a feeding needle. The control group received distilled water.

Observation of toxic symptoms were made and recorded systematically at one, two and six hours after administration. The number of survivors was noted after 48 hours for each animal (rat). The toxicological effect was assessed on the basis of mortality, which was expressed as LD_{50} and was calculated using the limit test or fixed dose.

Reconstitution of the Plant Extracts

The dried residue was weighed into McCartney bottles. Zero point two grams (0.2g) of the dried residue of the leaf and stem-bark extracs of *Entada africana* were dissolved or dispensed into 10ml of glycerol to make a stock solution of 2 mg/mL respectively.

Preparation and Standardization of Inoculums

All test organisms were separately prepared by sub culturing the pure isolates in to nutrient agar and incubated at 37°C for 24 hours. One gram (1g) of Barium chloride was weighed and dissolved in 99ml of sterile distilled water. This was followed by the measurement of 1ml of concentrated sulphuric acid in 99ml of sterile distilled water. To prepare 10ml of McFarland Nephelometer, 0.2ml of 1% barium chloride was added to 9.8ml of concentrated sulphuric acid. Turbidity corresponds to 6.10^8ml bacteria cells referred to McFarland standard (McFarland, 1970).

Sources of Test Organisms

Pure clinical isolates used in this study were *Escherichia coli* isolated from diarrhoeic stool of children 0-5 five years attending selected Hospitals in Niger State, Nigeria. viz Etsu Umaru Sanda General Hospital, Bida; General Hospital, Minna and General Hospital, Kontagora.

Antibacterial Activity of the Plant Extracts.

Before antibacterial susceptibility testing was carried out, the isolates were sub-cultured unto fresh nutrient agar slants and incubated at 37°C for 24 hours. Suspension was prepared for the sub-culture isolates into clean sterilized tubes according to 0.5 McFarland standards. Eighteen milliliter

of molten Mueller-Hilton agar was poured into the Petri plate and was allowed to solidify. A standard cork borer of (6mm diameter) was used to punch five well aseptically on the surface of agar and filled with 0.2mL of the extract. The standardized suspension was used to inoculate the surface of agar plates using sterilized swab. The inoculated agar plates were then incubated at 37°C for 24 hours. The antibacterial activities were evaluated by measuring zone of inhibition using transparent metric ruler.

Results and Discussion

The preliminary phytochemical screening of *Entada africana* leaf extracts revealed that the acetone leaf extract contained alkaloids, anthraquinones, flavonoids, glycosides, resins and steroids, while the aqueous leaf extract contained alkaloids, flavonoids and glycosides. The hexane leaf extract contained alkaloids, flavonoids and glycosides, while methanol leaf extract contained all the phytochemical constituents considered except steroids. As for the stem-bark, acetone stem-bark contained alkaloids, anthraquinones, flavonoids, steroids and tannins, the aqueous stem-bark extract contained alkaloids, anthraquinones, flavonoids, saponins and tannins. The hexane stem-bark extract contained all except resins, while the methanol stem-bark contained alkaloids, anthraquinones, resins, saponins and steroids (Table 1).

Table 1: Phytochemical Constituents of Leaf and Stem-Bark Crude Extracts of *Entada africana*.

	Phytochemical constituents							
Extracts	Alkaloids	Anthraquinones	Flavonoids	Glycosides	Resins	Saponins	Steroids	Tannins
EAALE	+	+	+	+	+	-	+	-
EAQLE	+	-	+	+	+	-	-	-
EAHLE	+	-	+	+	-	-	-	-
EAMLE	+	+	+	+	+	+	-	+
EAASBE	+	+	+	-	-	-	+	+
EAQSBE	+	+	+	-	-	+	-	+
EAHSBE	+	+	+	+	-	+	+	+

EAMSBE	+	+	-	-	-	-	+	+

Key: EAALE=*Entada africana* acetonne leaf extract; EAASBE= *Entada africana* acetone stem-bark extract; EAHLE= *Entada africana* hexane leaf extract; EAHSBE= *Entada africana* hexane stem-bark extract; EAMLE=methanol leaf extrract; EAMSBE= *Entada africana* methanol stem-bark extract; EAQLE=*Entada africana* aqueous leaf extract; EAQSBE= *Entada africana* aqueous stem-bark extract; + = Present; - = Absent.

The result of acute toxicity test on albino rat at 3000mg/kg body weight for *Entada africana* aqueous leaf and stem-bark extracts produced no mortality after 48 hours of observation. Oral administration of low doses of 1000-3000mg/kg produced no obvious toxicity. However, higher doses of 4000-5000mg/kg caused slow movement (weakness) of the animals (Table 2).

Table 2: The Acute Toxicity Test (LD_{50}) of Crude Aqueous Leaf and Stem-Bark Extracts of *Entada africana* on Albino Rat.

Plant extract	Group	Albino Rat	Weight of animals (g)	Dosage (mg/kg)	Vol. of extract administered (g/mL)	Mortality
Leaf	Grp A	A1	182	1000	0.2	No-mortality
		A2	185	2000	0.4	"
		A3	190	3000	0.6	"
		A4	210	4000	0.8	"
		A5	220	5000	1.1	"
		Control	220	0.00	0.1mL dw	"
Stem-bark	Grp B	B1	184	1000	0.2	No-mortality
		B2	192	2000	0.4	"
		B3	196	3000	0.6	"
		B4	200	4000	0.8	"
		B5	205	5000	1.0	"
		Control	208	0.00	0.1 mL dw	"

Key: LD_{50}=Median lethal dose; Dw = Distill water; g = gram; ml= mililire; kg = Kilogram;
mg = Miligram; Vol = Volume.

The results of preliminary antibacterial activity of crude leaf extract of *Entada africana* showed that six *Escherichia coli* isolates were resistant to acetone leaf extract, while four isolates were susceptible to it with zones of inhibition which ranged from 8.00mm-11.00mm at 0.5mg/mL concentration. At concentration of 1.0mg/mL, all *Escherichia coli* isolates were susceptible to acetone leaf extract with zones of inhibition which ranged from 9.0mm-15.00mm, while at 2.0mg/mL, all *Escherichia coli* isolates were susceptible with zones of inhibition which ranged from 11.00mm-25.00mm in which *Escherichia coli* coded 186 recorded 25.00mm of zone of inhibition.

Aqueous leaf extract of *Entada africana* inhibited all the *Escherichia coli* isolates except the ones coded 149 and 445 that showed resistance at 0.5mg/mL concentration, while at 2.0mg/mL concentration, all the isolates were susceptible to aqueous leaf extract with zones of inhibition which ranged from 12.00mm-24.00mm. However, *Escherichia coli* coded 003 had highest zone of inhibition (24.00mm) and *Escherichia coli* coded 445 had lowest zone of inhibition (12.00mm). *Escherichia coli* coded 003, 010 and 166 respectively were fairly susceptible to hexane leaf extract with zones of inhibition which ranged from 8.00mm-9.00mm and 10.00mm-11.00mm at concentration of 1.0mg/mL and 2.0mg/mL respectively. The remaining isolates were resistant to hexane leaf extract at 0.5-2.0mg/mL concentrations.

All *Escherichia coli* isolates were susceptible to methanol leaf extract at 0.5-2.0mg/mL concentrations but at 2.0mg/mL concentration, *Escherichia coli* coded 003 and 268 showed highest zones of inhibition of 36mm and 34mm respectively (Table 3).

Table 3: Preliminary Antibacterial Activity of *Entada africana* Crude Leaf Extracts against *Escherichia coli* Isolates.

Zone of inhibition (mm) and Concentration of extract (mg/ml)												
Code	EAALE			EAQLE			EAHLE			EAMLE		
	0.5	1.0	2.0	0.5	1.0	2.0	0.5	1.0	2.0	0.5	1.0	2.0
EUS003	10.00	15.00	22.00	9.00	14.00	24.00	0.00	9.00	10.00	14.00	22.00	36.00
EUS010	0.00	10.00	13.00	8.00	12.00	16.00	0.00	8.00	10.00	8.00	12.00	18.00
EUS149	0.00	9.00	11.00	0.00	8.00	12.00	0.00	0.00	0.00	9.00	12.00	16.00

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GHM166	11.00	15.00	25.00	10.00	13.00	20.00	0.00	9.00	11.00	10.00	14.00	25.00
GHM220	0.00	10.00	14.00	9.00	13.00	18.00	0.00	0.00	0.00	9.00	13.00	20.00
GHM268	10.00	14.00	20.00	10.00	14.00	20.00	0.00	0.00	0.00	14.00	21.00	34.00
KGH349	0.00	11.00	16.00	8.00	11.00	16.00	0.00	0.00	0.00	12.00	17.00	28.00
KGH360	0.00	11.00	15.00	8.00	14.00	18.00	0.00	0.00	0.00	10.00	14.00	20.00
KGH399	0.00	9.00	12.00	8.00	12.00	16.00	0.00	0.00	0.00	8.00	12.00	16.00
KGH445	8.00	11.00	15.00	0.00	8.00	12.00	0.00	0.00	0.00	8.00	10.00	14.00

Key: EUS=Etsu Umaru Sanda General Hospital, Bida; GHM=General Hospital, Minna; KGH=General Hospital, Kontagora; EAALE=*Entada africana* acetone leaf extract; *E. coli=Escherichia coli*; EAQLE= *Entada africana* aqueous leaf extract; EAHLE=*Entada africana* hexane leaf extract; EAMLE= *Entada africana* methanol leaf extract

The results of antibacterial activity of stem-bark extract on some *Escherichia coli* isolates indicated that hexane stem-bark extract had lowest activity at 1.0mg/mL concentration with zones of inhibition ranging from 8.00mm-10.00mm, while at 2.0mg/mL concentration, eight isolates were susceptible with zones of inhibition which ranged between 10.00mm-12.00mm except *Eschericheria coli* isolates coded 360 and 445 that were resistant to the extract. At 2.0mg/mL methanol stem-bark extract inhibited all *Escherichia coli* isolates with zones of inhibition which ranged between 22.00mm-36.00mm, followed by aqueous stem-bark extract that inhibited all the tested *Escherichia coli* isolates with zones of inhibition which ranged between 14.00mm-22.00mm (Table 4).

Table 4: Preliminary Antibacterial Activity of *Entada africana* Crude Stem-Bark Extracts against *Escherichia coli* Isolates.

Zone of inhibition (mm) and Concentration of extract (mg/ml)												
Code		EAASBE			EAAQSBE			EAHSBE			EAMSBE	
	0.5	1.0	2.0	0.5	1.0	2.0	0.5	1.0	2.0	0.5	1.0	2.0
EUS003	9.00	14.00	22.00	11.00	15.00	22.00	0.00	9.00	12.00	12.00	17.00	26.00
EUS010	8.00	11.00	15.00	8.00	11.00	16.00	0.00	0.00	0.00	9.00	14.00	20.00
EUS149	9.00	15.00	17.00	9.00	13.00	20.00	0.00	8.00	10.00	10.00	14.00	22.00
GHM166	8.00	10.00	15.00	8.00	14.00	18.00	0.00	0.00	0.00	10.00	15.00	23.00
GHM220	9.00	15.00	17.00	9.00	12.00	19.00	0.00	0.00	0.00	9.00	13.00	20.00
GHM268	8.00	11.00	16.00	11.00	15.00	16.00	0.00	8.00	11.00	10.00	14.00	22.00

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KGH349	0.00	8.00	12.00	8.00	10.00	14.00	0.00	0.00	0.00	11.00	15.00	22.00
KGH360	8.00	10.00	16.00	8.00	12.00	16.00	8.00	10.00	12.00	10.00	15.00	24.00
KGH399	9.00	12.00	16.00	9.00	12.00	18.00	0.00	8.00	10.00	11.00	16.00	26.00
KGH445	8.00	11.00	16.00	11.00	14.00	20.00	0.00	0.00	0.00	13.00	19.00	36.00

Key: EUS=Etsu Umaru Sanda General Hospital, Bida; GHM=General Hospital, Minna; KGH=General Hospital, Kontagora; EAASBE=*Entada africana* acetone stem-bark extract; *E. coli=Escherichia coli*; EAQSBE=*Entada africana* aqueous stem-bark extract; EAHSBE=*Entada africana* hexane stem-bark extract; EAMSBE=*Entada africana* methanol stem-bark extract.

From this work, the phytochemical analysis of *Entada africana* leaf extracts indicated the presence of glycosides, alkaloids and flavonoids as the phytochemical constituent's common to all the extracts. This could be attributed to the facts that these phytochemical constituents are the ones mostly found in the photosynthetic part of the plant such as leaves, and also in the vegetables, fruits, grains and seeds. This work is similar to the findings of Agbaku *et al*. (2015) who report the presence of flavonoids, glycosides and alkaloids in their studies of phytochemical analysis of ethanolic leaf extract of *Entada africana.* The work is also in conformity with the findings of Abu *et al*. (2017) who reported the presence of cardiac glycosides, flavonoids and saponins in their studies of phytochemical screening and toxicological studies of aqueous, acetone and ethanolic leaf extracts of *Entada africana.*

This result is however contrary to findings of Adisa *et al*. (2015) who reported the presence of flavonoids, glycosides, alkaloids and anthraquinones in their studies on the antibacterial activity and phytochemical analysis of *Entada africana* leaf extracts as well as to that of Abdullahi *et al.* (2016) who reported the presence of alkaloids, anthraquinones and glycosides in their studies on the evaluation of phytochemical constituents and antimicrobial activity of *Entada africana* leaf and stem-bark extracts. This could be attributed to geographical area and season of plant collection because the more the environmental stressor, the more phytochemical constituents are produced. Shuaibu and

Abdullahi (2016) investigated the phytochemical constituents, antiplasmodial and toxicological studies of *Entada africana* leaf extracts and reported the presence of protein, balsam, flavonoids, alkaloids, glycosides, saponnins and tannins which is contrary to findings in this study. The reason is also attributed to geographical location and time of collection which was March and in Tangaza town, Sokoto State.

The phytochemical screening of *Entada africana* stem-bark extracts revealed the presence of anthraquinones, saponins, steroids, alkaloids and tannins. The reason may be as a result of anthraquinones, resins, alkaloids and tannins reported to be found in the stem-bark, rhizome and bulb of the plant. Presence of flavonoids could be attributed to shift of phytochemical constituents in response to the seasonal change. The study agrees with the findings of Patrick *et al*. (2016) who reported the presence of alkaloids, flavonoids, tannins, saponins, steroids and phenol in their studies on in-vitro antioxidant activity and phytochemical evaluation of aqueous ad methanol stem-bark extract. The work is however contrary to the findings of Abdullahi *et al*. (2012) who reported the presence of flavonoids, alkaloids, glycosides, saponnins, steroids, terpenes, carbohydrates, balsam and tannins in their studies on the evaluation of some medicinal plants from Nupe land for their *in-vitro* antitrypanosomal activity of *Entada africana* extracts.

Comparatively, stem-bark of *Entada africana* contained more phytochemical constituents than leaf extracts. This could be attributed to the season of the plant collection which was the dry season (heat period), in which plant shed their leaves and therefore could be minimizing metabolic cost by retieving the metabolites to the stem-barks and the roots or rhizomes for senescing leaf tissues and transporting constituents to the stem and underground parts.

The result of acute toxicity test (LD_{50}) of *Entada africana* extracts on the albino rats in the present study indicated that all albino rats tested survived at 5000mg/kg body weight. No mortality was recorded and one of the toxicological indices accepted for the determination of the safety of drug/substances/extract is lethal dose of 50% (LD_{50}), which is the amount

of acute dose required to kill half of the test population (Dawang, 2015). However, since there was no death recorded during acute toxicity experiment by oral administration, then it is evidence that the median lethal dose (LD_{50}) is greater than 5000mg/kg weight (Assao *et al.*, 2011), this implies that *Entada africana* extract is safe (non-toxic). The result in this study is in conformity with Shuaibu and Abdullahi, (2016) and Patrick *et al*. (2016) who reported that *Entada africana* leaf extract had LD_{50} of greater than 5000mg/kg bodyweight.

The results of preliminary antibacterial activity of *Entada africana* crude leaf extracts indicated that the ten *Escherichia coli* isolates tested were susceptible to acetone leaf extract at 1.0 and 2.0mg/mL concentration with zones of inhibition ranging from 9.00mm-15.00mm and 11.00mm-22.00mm respectively. This finding is contrary to the report of Yusuf *et al*. (2019), where all *Escherichia coli* isolates were susceptible, with zones of inhibition ranging from 5.00mm-9.00mm and 6.00mm-19.00mm at 1.0 and 2.0mg/mL concentration, and similar to what was reported by Kwuje *et al*. (2017) with zones of inhibition ranged from 5.00mm-18.00mm at 2.0mg/mL concentration. The reason may be attributed to different phytochemical constituents detected in acetone leaf extract.

In a related development, a study carried out on phytochemical analysis, antibacterial and antioxidant activities of *Entada africana* stem-bark extract on *Echerichia coli* isolates showed that these *Echerichia coli* isolates were susceptible to methanol stem-bark extract with zones of inhibition ranging from 8.40mm-11.00mm (Kwuje *et al.*, 2017) which is similar to the finding in the present study. The study is also similar to the findings of Olarenwaju and Ahmed (2018) who reported that methanol stem-bark extract inhibited the growth of *Echerichoa coli* isolates with zones of inhibition which ranged from 9.00mm-12.00mm. This could be attributed to the fact that methanol being polar solvent was able to extract all phytochemical constituent tested except steroids. Tannin as one of the phytochemical constituent detected was reported to have potent inhibitor of many hydrolytic enzyme used by pathogens. However, there are contrary reports to this, for instance Marthe *et al*. (2014) reported zones

of inhibition of 8.00mm and 14.00mm at 20 and 40mg/mL concentration. This could be attributed to flavonoids and glycosides that were detected. Flavonoid was reported to have abilty to achieve complex formation with extracellular soluble proteins and the cell wall of bacteria. Some lipid soluble flavonoids can penetrate bacteria cell and cause the disruption of cell membrane (Cushnie and Lamb, 2008).

The methanol stem-bark extracts of *Entada africana* significantly inhibited the growth of the tested *Escherichia coli* isolates with zones of inhibition which ranged from 9.00mm-13.00mm, 13.00mm-19.00mm and 20.00mm-36.00mm at 0.5, 1.0 and 2.0mg/mL concentrations respectively. The reason may not only be attributed to genetic factor but also to agroclimatic conditions since the plant was harvested during the dry and heat season, while phytochemical constituents might have migrated to stem-bark and root-bark organs. This study is contrary to the findings of Yusuf *et al*. (2019) who reported zones of inhibition which ranged from 14.00mm-17.00mm exhibited by *Escherichia coli* isolates at 50mg/mL concentration.

Conclusion

In conclusion, the phytochemical constituents commonly present in *Entada africana* leaf extracts includes alkaloids, flavonoids and glycosides, while alkaloids, anthraquinones, saponin and tannins were commonly detected in stem-bark extracts. The lethal dose (LD_{50}) of *Entada africana* extracts on albino rats was greater than 5000mg/kg, meaning that the plant is safe for consumption. Presence of phytochemical constituents contributed immensely to their antibacterial activity.

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