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Lawal Danjuma *

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Molecular Assessment and Antibacterial Activity of Synthesized Silver Nanoparticles using Stem Bark of *Sclerocarya birrea* and Leaf of *Guiera senegalensis* against Some Bacterial Isolates Causing Diarrheal Infection

Lawal Danjuma [1]*, Hafeez Muhammad Imam [2], Nura Muhammad Sani [1], Muslim Ismail [1]

[1]Department of Microbiology and Biotechnology, Federal University Dutse, Duse, Nigeria

[2]Department of Biological Sciences, Sule Lamido University Kafin Hausa, Kafin Hausa, Nigeria

***Corresponding Author:** Lawal Danjuma, Department of Microbiology and Biotechnology, Federal University Dutse, Duse, Nigeria.

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Abstract

Nigeria is among well-known countries that are rich in ethnos botanical medicinal plants which are traditionally used in the treatment of illness and therefore become a good source for discovery of new, safe and biodegradable drugs. Herbalist processed Sclerocarya birrea stem bark and leaf of Guiera senegalensis in the treatment of diarrhoreal diseases. This research was aimed at determining the in vitro antibacterial activity of AgNPs synthesized from extracts of Sclerocarya birrea stem bark and leaf of Guiera senegalensis against some bacterial isolates capable of causing diarrhoreal diseases. The elemental analysis on the used plants materials revealed the presence of mineral elements (Ca, Zn, Mg, Fe, Cu, Na and k) in various concentrations. Calcium was found to be highest among the elements tested in both plants while zinc and copper were the least cocentration. Quantitative phytochemical analyses showed high content of alkaloids in all plant's materials fallowed by Flavanoids and Saponin, while low concentration of tannins and phenolic compound. Standard phenotypic and genotypic techniques were used for the identification of the isolate. Analysis for bioactive compounds of the both plants showed the presence of tannins, alkaloids, flavonoids, cardiac glycosides, phenols, saponins and terpenoids. The AgNPs were synthesized using various extracts of the used plants. Antibacterial profile of crude extract of Sclerocarya birrea stem bark and leaf of Guiera senegalensis at 1:1 of aqueous are not active on the E. coli, Salmonella enterica and Klebsiealla pneumonia, while methanolic and ethyl acetate on E. coli showed zones of growth inhibition ranged from 6.5 6.0 to 16.0 0.0 mm and 13.0 0.0 to 17.0 0.0 mm for ethyl acetate and petroleum ether extracts. Where methanolic extracts zones of growth inhibition on Salmonella enterica ranged from 6.5 6.0 to 13.0 0.0 mm and 10.0 0.0 to 14.0 0.0 mm for ethyl acetate with no significant difference (P> 0.05). The antibacterial activity of the combined biologically synthesized AgNPs at 1:1 from each extract showed zones of growth inhibition on all tested isolate ranged from 12.0 0.0 to 17.0 0.0 mm with no significant difference (P> 0.05). The additive antibacterial activity of mixed crude extract of S. birrea stem and G. senegalensis leaf at the ratio of 1:1 was observed on the E. coli, Salmonella enterica using methanol and ethyl acetate extract as compared with separate extract. Where antagonism was observed on Salmonella enterica using petroleum ether extracts. Similarly additive activity was observed on mixed biosynthesized AgNPs from S. birrea stem and G. senegalensis leaf at the ratio of 1:1 on all tested isolate using aqueous, methanol and ethyl acetate extract as compared with separate extracts, with exception of petroleum ether on Klebsiealla pneumonea were antagonism was observed as compared with separate extracts.

Keywords: sbirrea stem; g. senegalensis; silver nanoparticles; antbacterial; diarrhoe; bacteria

Introduction

Diarrhea is one of the most common ailments and a leading cause of mortality especially among the children in the world, and remains high in the international public health agenda. The United Nations Children's Fund and World Health Organization defined diarrhea as unusual increase in having

loose or watery stools at least three times per day or more frequently than normal for an individual [1 – 3]. The causes of diarrhea are broad and varied; mostly related to poor sanitary conditions and low socio-economic status. Viruses, bacteria and protozoa are regarded as the causative agents of infections worldwide [4]. The bacterial pathogens that are usually causative agent of diarrhea diseases include *E. coli, Shigella* spp, *Salmonella* spp, *K. pneumonia*, *Campylobacter, Yersinia* and *Aeromona*. *E. coli* remain one of the major causative agents of infectious diarrhea that lead morbidity and mortality among infants and young children in Nigeria. However, *Shigella* spp were reported by some researchers in Saudi Arabia to have the highest incidence among other bacterial pathogens causing diarrheal disease in that country. *K. pneumoniae* were detected in the stool specimens from outpatients with diarrhea syndromes in Beijing [5].

In Nigeria and some other African countries, the stem bark, roots and leaves of *S. birrea* are being process and use in managing human ailments, including: malarial fever, diarrhea and dysentery, stomach ailments, headache, toothache and body pains [6] G. *senegalensis* is widely recognize in traditional medicine for the remedy of many diseases as ethnobotanical studies carried out by many authors on its medicinal properties confirmed that *G. senegalensis* has a good reputation as medicinal plant [7]. Some part of the Northern Nigeria combined powdered leaves with food are being used as a general tonic and blood restorative. In addition, processed *G. senegalensis* leaves are widely used for pulmonary and respiratory diseases, for coughs, febrifuge, diarrhea, syphilis, beriberi, leprosy, impotence, rheumatism, diuresis and expurgation [8]. Similarly, herbalist combined powdered of the stem bark of *S. birrea* and the leaf of *G. senegalensis* soak in water or mixed with beverage to treat diarrhea and abdominal pain. Recently, researches showed that the Nanoparticles of silver serve as enhancer of antimicrobial agent. Silver Nanoparticles (AgNPs) can be synthesize biologically using plants extract as a reducing and capping agent. The use of medicinal plants in the synthesis of AgNPs is not only used for size and shape control, but also to enhance plant antimicrobial properties [9].

This research was aimed at finding out scientific bases of combining powdered of the stem bark of *S. birrea* and the leaf of *G. senegalensis* in the treatment of diarrheal diseases using *in vitro* antimicrobial activity of crude extracts of *S. birrea* stem bark and leaf of *G. senegalensis* and its synthesized AgNPs against some bacterial isolates causing diarrheal diseases.

2. Materials and Methods:

2.1. Materials

Stem Bark of *Sclerocarya birrea* and Leaf of *Guiera senegalensis*. Solvents (methanol and ethyl acetate, petroleum ether and distilled water), Dimethyl sulfoxide (DMSO). Media (Mueller Hinton agar, Mueller Hinton broth and Nutrient Agar), $AgNO_3$ and other reagents (Sigma-Aldrich Laboratories Pvt. Ltd., USA). Phytochemical screenings reagents. The pure clinical isolates (*E. coli*, *Salmonella* sp. and *Klebsiella pneumonia*). Gram staining reagents, Biochemical test reagents, Genomic DNA extraction kits, primers, Water bath, Atomic Absorption Spectrophotometer, UV/Visible spectrophotometer, thermocycler, hot air oven, incubator.

2.2. Methods

2.2.1. Collection, Authentication and Preparation of Plants Materials

The plants (stem bark of *Sclerocarya birrea* and leaf of *Guiera senegalensis*) were selected through ethno medicinal survey among the traditional healers in four local governments area of Jigawa state, Nigeria. The local governments include: Hadejia, Malam Madori, Auyo and Kafin Hausa. The plant materials were identified and authenticated at the Herbarium of the Department of Plant Biology, Bayero University Kano where a voucher specimen numbers of BUKHAN 435 and BUKHAN 32 was assigned to *S. birrea* and *G. senegalensis* respectively. The fresh plants materials were washed four times with de-ionized water to remove dust particles and air dried at room temperature. Then they were grinded in to powder form and then sieved to obtained fine powder using 20 µm mesh size sieve.

2.2.2. Elemental Analyses

This was carried out according to method described by Mohammed [10] as follows: About 0.5 g of dried powdered were digested using 10 cm^3 of a mixture of conc HNO_3 and conc HCl (3:1 v/v). Analytical grade reagents were used for the preparation of the standard solutions of these elements (Ca, Zn, Mg, Fe, Cu, Na and k). The diluted digests were analyzed using atomic absorption spectrophotometer (PerkinElmer PinAAcle 900H) for Ca, Cu, Fe, Mg Zn, Na and K.

2.2.3. Qualitative and Quantitative Phytochemical Screening

Standard procedures as described by Mamman and Isah, Somboro *et al.* and Jaradat *et al.* [8, 11, 12] used to qualitatively determine the presence of bioactive constituents such as alkaloids, flavonoids, tannins, saponins, phenols and glycosides, while standard procedures described by Theng & Korpenwar, Louis *et al.*, Orimadegun *et al*. and Danjuma *et al.* [13-16] were used for the quantitative determination of phytochemical such as alkaloids, flavonoids, tannins, saponins, phenols, and terpenoids.

2.2.4. Biosynthesis of AgNPs from *S. birrea* Stem Bark and Leaf of *G. senegalensis* Extracts

The AgNPs was biologically synthesized following standard procedures as described by Khan *et al*., Sriram and Pandidurai, and Bobboi *et al.* [9, 17, 18] as follows: For aqueous extracts, about 10 g of the powdered plant was added to 100 ml of de-ionized water and stirred for 20 min at 60 ºC. After boiling, the extract was allowed to cool at room temperature and filtered. 0.1M of aqueous solution of silver nitrate ($AgNO_3$) was prepared and used for the synthesis for AgNPs. About 10 ml of plants extract was added to 90 ml of aqueous solution of 0.1M $AgNO_3$ drop-by-drop until an initial color changed observed. The mixture was held at 60 °C for 60 minutes to control color rapid changes. It was then incubated at room temperature for 24 hours in a dark chamber to minimize photo-activation of $AgNO_3$ at room temperature until the color changes to brown which confirmed the reduction of silver ions to AgNPs. The AgNPs solution was then centrifuged at 10,0000 rpm for 18 min. The supernatant was discarded and the pellet was dried in hot air oven at 25℃ and stored at 4℃ before used.

For methanol, petroleum ether and ethyl acetate extracts, about 10 g of powdered plant were dispensed into 100 ml of each solvent and allowed to stand for 24 hours with continues shaking in the first 6 hours. After filtration, 10 ml of plants extract was added to 90 ml of aqueous solution of 0.1M $AgNO_3$ and kept at room temperature in a dark chamber to minimize photo-activation of $AgNO_3$ at room temperature until the color changes to brown which confirm the reduction of silver ions to AgNPs. The AgNPs solution was then centrifuged at 10,0000 rpm for 18 min. The supernatant was discarded and the pellet was dried in hot air oven at 25℃ and stored at 4℃ before used. The concentrations of 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml for the AgNPs were prepared using 5% Dimethyl sulfoxide (5% DMSO).

2.2.5. Collection of Test Bacteria

The pure clinical isolates of *E. coli, Salmonella* sp. and *Klebsiella pneumoniae* were obtained from Microbiology unit of Hadejia General Hospital laboratory and identified using biochemical tests, and further confirmed using molecular characterization.

2.2.6. Molecular Characterization of Test Bacteria

The molecular characterization of bacterial isolates was conducted using DNA extraction, Polymerase Chain Reaction (PCR) amplification and 1.5% agarose gel electrophoresis of 16S rRNA genes Using the forward primer - GGACTACAGGGTATCTAAT 16S (RIBOSE-1) and reverse primer - AGAGTTTGATCCTGG 16S (RIBOSE-2). The DNA extraction was done usimg bioneer bacterial extraction kits following the protocols described by Bobai *et al.* [18]. The PCR of the extracted genomic DNA was carried out following the protocol described by Bobai *et al.* (18). The electrophoresis of the PCR product was carried out using 1.5% agarose gel at 125 volts for 35 min and gel DNA bands were visualized using UV Biorad gel imaging system.

2.2.7. Evaluation of Antibacterial Activity of the Crude Extracts and its Synthesized AgNPs against Test bacteria

Agar well diffusion method as described by Garba *et al*., [19] was used to carried out Bioassay as follows: All the test bacteria to be used were sub cultured in Mueller Hilton broth at 37 ºC and incubated for 24 h. About 15 ml of sterile molten Mueller Hilton agar was dispensed in a Petri dishes and allowed to solidified. About 0.1 ml of test bacterial (0.5 McFarland standard) suspension were swabbed uniformly on the surface of solidified media. The wells were made on the surface of agar with 6 mm diameter sterile corn borer. The 200, 100, 50, 25, 12.5 and 6.25 mg/ml concentrations of the crudes extracts and its synthesized AgNPs were dispensed into the wells. The plates were incubated at 37°C for 24 h and the inhibition zones formed were measured with transparent ruler in millimeter (mm) and average zone of inhibition was calculated.

2.2.8. Statistical Analyses

The data obtained were analyzed using One-Way Analysis of Variance (One-Way ANOVA), Duncan's multiple range Post Hoc using SPSS. The results were presented as the mean ± standard deviation. Significance level for the differences was set at $p< 0.05$ while $p> 0.05$ show no significant.

3. Results:

3.1. Elemental Analysis

The results for elemental analysis of the stem bark of *S. birrea* and the leaf of *G. senegalensis* (Table 1) revealed that all the elements tested were present in various concentrations. Calcium was found to be highest among the elements tested in both stem bark of *S. birrea* and the leaf of *G. senegalensis*, even though the concentration varies which is higher in the stem bark of *S. birrea* than in the leaf of *G. senegalensis*. Sodium, Potassium and Magnesium are among the detected elements especially magnesium which is in higher concentration in the leaf of *Guiera senegalensis*. Zinc and copper were the least among the detected elements tested in both stem bark of *S. birrea* and the leaf of *G. senegalensis.*

Plants	Part Used	Element Concentrations (PPM)						
		Ca	Cu	Fe	K	Mg	Na	Zn
S. birrea	**Stem bark**	400.5	0.560	1.169	53.86	23.18	187.2	0.160
G. senegalensis	**Leaves**	363.3	0.410	1.818	37.87	191.2	121.1	0.194

Key: Ca = Calcium: Cu = Copper: Fe = Iron: K = Potassium: Mg =Magnesium: Na=Sodium: Zn = Zinc: PPM = Parts per million.

Table 1. Result of Elemental Analyses (per 0.5 g of plant sample)

3.2. Qualitative Phytochemical Screening Tests of the Various Extracts

Phytochemical screening test for the bioactive components present in the extract of stem bark of *S. birrea* and the leaf of *G. senegalensis* (Table 2) revealed that the extracts were rich in secondary metabolites, including alkaloids, saponin, tannins**,** flavones and glycoside. The ethyl acetate extract of the leaf of *G. senegalensis* has the highest number of phytochemicals in the plants extract followed by the methanolic extract of the both plants, the least among is the aqueous extract.

		Alkaloid	Tannin	Phenol	Saponnin	Glycocide	Flavones
		Wagner's reagent	Sodium chloride	Ferric chloride Foam test		Sulphuric acid	Sodium hydroxide
Methanol	***S. birrea***	+	+	-	+	+	+
	G. senegalensis	-	+	+	-	+	-
Ethyl acetate	***S. birrea***	-	-	-	+	+	-
	G. senegalensis	+	+	+	+	+	-
Aqueous	***S. birrea***	+	+	+	-	-	-
	G. senegalensis	-	-	+	-	+	-
Pet. ether	***S. birrea***	+	+	-	+	-	-
	G. senegalensis	+	+	+	-	+	-

Key: += Present = Absent

Table 2. Qualitative Phytochemical Test

3.3. Quantitative Phytochemical Analyses

The results of Quantitative phytochemical analyses of stem bark of *S. birrea* and the leaf of *G. senegalensis* (Tables 3) showed high content of alkaloids in all plants materials fallowed by Flavanoids and Saponin, while tannins and phenolic compounds had the lowest concentrations. The results also revealed that phytochemical compounds present in the stem bark of *S. birrea* are higher than that of leaf of the *G. senegalensis*

Phytochemical	Plants Concentrations (%)		Amount of sample used (g)
	S. birrea **(stem bark)**	***G. senegalensis*** **(Leaves)**	
Alkaloid	19.37	17.45	5
Phenol	0.09	0.28	1
Saponin	15.13	12.49	10
Tannin	0.60	0.53	1
Flavanoids	14.72	13.72	10
Terpenoids	7.59	5.33	10

Key: g = gram, %= percentage

Table 3. Quantitative Phytochemical Analyses

3.4. Molecular Identification

The results for gel electrophoresis of amplified PCR product of 16S rRNA genes of the test bacteria (Figure 1) showed bands at 16S rRNA genes of *Escherichia coli*, *Salmonella enterica* and *Klebsiella pneumoniae* at 972 bp of the 100 bp plus DNA marker.

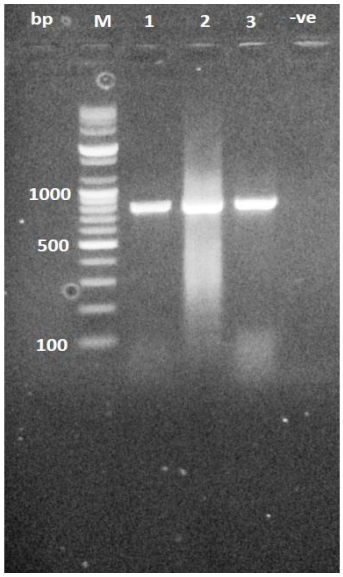

Figure 1. Agarose gel electrophoresis image of amplification of 16S rRNA genes of test bacteria

Key: M**=** hyperladder IV DNA ladder [Bioline 100–1000 bp (40–200 ng/band)], bp= base pair**,** --ve= Negative control, 1**=** *Escherichia coli*, 2= *Salmonella enterica*, 3= *Klebsiella pneumoniae*

3.5. Antibacterial Activity of Crude Extracts

The results of antibacterial activity of crude extracts of stem bark of *S. birrea* and the leaf of *G. senegalensis* (Tables 4 to 5) showed that the methanol and ethyl acetate extract of *S. birrea* stem bark was found to be active against *E. coli* and *Salmonella enterica*, where petroleum ether extract were only active on *Salmonella enterica*. Methanol and ethyl acetate extract produce 13.0± 0.0 mm diameter zone of inhibition on *E. coli* at concentration of 100 mg/ml, methanol, ethyl acetate and petroleum ether extract produce inhibition zone of 11.0± 0.0 mm, 11.0± 0.0 mm and 10.0± 0.0 mm diameters respectively on *Salmonella enterica* at concentration of 50 mg/ml, while aqueous extract found to be not active against all tested isolates. *Klebsiealla pneumoniae* resist all tested extracts (Table 4). Similarly, the activity of the *G. senegalensis* leave extracts on the tested isolates varies; methanol, ethyl acetate and petroleum ether extract were found to be active on *E. coli* and *salmonella enterica* with inhibition zone of 12.0± 0.0 mm 10.0± 0.0 mm and 10.0± 0.0 mm diameter respectively at 50 mg/ml concentrations. *Klebsiella pneumoniae* resist both plant extracts. Aqueous extract found to be not active against all tested isolates (Table 5).

Extract Type	**Concentration (mg/ml)**	**Mean zone of inhibition (mm) ±SD**		
		E. coli	***S. enteric***	***K. pneumoniae***
Methanol	200	14.0± 0.0	12.0± 0.0	NZI
	100	13.0± 0.0	11.0± 0.0	NZI
	50	NZI	11.0± 0.0	NZI
	25	NZI	10.0± 0.0	NZI
	12.5	NZI	6.8± 0.0	NZI
	6.25	NZI	NZI	NZI
	P-value α= 0.05	0.00 (p< 0.05)	0.24 (P> 0.05)	
Ethyl acetate	200	14.0± 0.0	13.0± 0.0	NZI
	100	13.0± 0.0	12.0± 0.0	NZI
	50	12.0± 0.0	11.0± 0.0	NZI
	25	NZI	10.0± 0.0	NZI
	12.5	NZI	10.0± 0.0	NZI
	6.25	NZI	6.8± 0.0	NZI
	P-value α= 0.05	0.00 (p < 0.05)	0.18 (P> 0.05)	
Petroleum ether	200	NZI	12.0± 0.0	NZI
	100	NZI	11.0± 0.0	NZI
	50	NZI	10.0± 0.0	NZI
	25	NZI	NZI	NZI
	12.5	NZI	NZI	NZI
	6.25	NZI	NZI	NZI
	P-value α= 0.05		0.15 (P> 0.05)	

Aqueous	200	NZI	NZI	NZI
	100	NZI	NZI	NZI
	50	NZI	NZI	NZI
	25	NZI	NZI	NZI
	12.5	NZI	NZI	NZI
	6.25	NZI	NZI	NZI
	P-value α= 0.05			
Control	200	NZI	NZI	30.0± 0.0
	100	NZI	NZI	20.0± 0.0
	50	NZI	NZI	18.0± 0.0
	25	NZI	NZI	15.0± 0.0
	12.5	NZI	NZI	10.0± 0.0
	6.25	NZI	NZI	6.9± 0.0

Key: SD = Standard Deviation; NZI= No zone of Inhibition

Table 4. Antibacterial susceptibility of test isolates to S. birrea stem bark crude extracts

Extract Type	**Concentration (mg/ml)**	**Mean zone of inhibition (mm) ±SD**		
		E. coli	***S. enteric***	***K. pneumoniae***
Methanol	200	15.0± 0.0	15.0± 0.0	NZI
	100	13.0± 0.0	13.0± 0.0	NZI
	50	12.0± 0.0	12.0± 0.0	NZI
	25	12.0± 0.0	12.0± 0.0	NZI
	12.5	NZI	6.8± 0.0	NZI
	6.25	NZI	NZI	NZI
	P-value α= 0.05	0.15 (p< 0.05)	0.15 (p< 0.05)	
Ethyl acetate	200	12.0± 0.0	12.0± 0.0	NZI
	100	11.0± 0.0	11.0± 0.0	NZI
	50	10.0± 0.0	10.0± 0.0	NZI
	25	NZI	NZI	NZI
	12.5	NZI	NZI	NZI
	6.25	NZI	NZI	NZI
	P-value α = 0.05	0.15 (p< 0.05)	0.15 (P> 0.05)	
Petroleum ether	200	12.0± 0.0	12.0± 0.0	NZI
	100	11.0± 0.0	11.0± 0.0	NZI
	50	10.0± 0.0	10.0± 0.0	NZI
	25	NZI	NZI	NZI
	12.5	NZI	NZI	NZI
	6.25	NZI	NZI	NZI
	P-value α = 0.05	0.15 (p < 0.05)	0.15 (P< 0.05)	
Aqueous	200	NZI	NZI	NZI
	100	NZI	NZI	NZI
	50	NZI	NZI	NZI
	25	NZI	NZI	NZI
	12.5	NZI	NZI	NZI
	6.25	NZI	NZI	NZI
	P-value α= 0.05			
Control	200	NZI	NZI	30.0± 0.0
	100	NZI	NZI	20.0± 0.0
	50	NZI	NZI	18.0± 0.0
	25	NZI	NZI	15.0± 0.0
	12.5	NZI	NZI	10.0± 0.0
	6.25	NZI	NZI	6.9± 0.0

Table 5. Antibacterial susceptibility of test isolates to G. senegalensis crude extract

3.6. Additive Antibacterial Activity Test of Crude Extracts of *S. birrea* stem and *G. senegalensis* leaf at the Ratio of 1:1

The result of mixed crude extract of *S. birrea* stem and *G. senegalensis* leaf at the ratio of 1:1 varied base on the extraction solvents, methanol, ethyl acetate and petroleum ether extract were found to be active on *E. coli* at concentration of 25 mg/ml each, with 13.0± 0.0 mm diameter of inhibition zone. The activity was also recorded using methanol and ethyl acetate extract on *Salmonella enterica* at concentration of 25 mg/ml with 11.0± 0.0 mm and 13.0± 0.0 mm diameter of inhibition zone respectively. Aqueous extract were found to be not active on all tested isolates (Table 6).

Extract Type	Concentration (mg/ml)	Mean zone of inhibition (mm) ±SD		
		E. coli	*S. enterica*	*K. pneumoniae*
Methanol	200	16.0± 0.0	13.0± 0.0	NZI
	100	16.0± 0.0	12.0± 0.0	NZI
	50	14.0± 0.0	11.0± 0.0	NZI
	25	13.0± 0.0	11.0± 0.0	NZI
	12.5	6.5± 6.0	6.5± 6.0	NZI
	6.25	NZI	NZI	NZI
	P-value α= 0.05	0.00 (p< 0.05)	0.17 (P> 0.05)	
Ethyl acetate	200	17.0± 0.0	14.0± 0.0	NZI
	100	17.0± 0.0	14.0± 0.0	NZI
	50	13.0± 0.0	14.0± 0.0	NZI
	25	13.0± 0.0	13.0± 0.0	NZI
	12.5	NZI	10.0± 6.0	NZI
	6.25	NZI	NZI	NZI
	P-value α= 0.05	0.00 (p< 0.05)	0.17 (P> 0.05)	
Petroleum ether	200	17.0± 0.0	NZI	NZI
	100	17.0± 0.0	NZI	NZI
	50	13.0± 0.0	NZI	NZI
	25	13.0± 0.0	NZI	NZI
	12.5	NZI	NZI	NZI
	6.25	NZI	NZI	NZI
	P-value α= 0.05	0.00 (p< 0.05)		
Aqueous	200	NZI	NZI	NZI
	100	NZI	NZI	NZI
	50	NZI	NZI	NZI
	25	NZI	NZI	NZI
	12.5	NZI	NZI	NZI
	6.25	NZI	NZI	NZI
	P-value α= 0.05			
Control	200	NZI	NZI	30.0± 0.0
	100	NZI	NZI	20.0± 0.0
	50	NZI	NZI	18.0± 0.0
	25	NZI	NZI	15.0± 0.0
	12.5	NZI	NZI	10.0± 0.0
	6.25	NZI	NZI	6.9± 0.0

Table 6. Result of Additive Antibacterial Activity Test of Crude Extracts of S.birrea stem bark and G.senegalensis leaf at the Ratio of 1:1

3.7. Antibacterial Activities of Biosynthesized Silver Nanoparticles

The antibacterial activity of the biosynthesized silver nanoparticles of S. birrea stem were investigated and the result was presented in (Table 7). The activity of biosynthesized AgNPs obtained with aqueous and petroleum ether extract of S. birrea were recorded at 25 mg/ml concentration on E. coli with 14.0± 0.0 mm zone of inhibition. While that obtained with methanol extract gave a zone of 11.0± 0.0 mm at the same concentration. However, no activity recorded with ethyl acetate extracts. Where Methanol and aqueous extracts produce 11.0± 0.0 mm diameter of inhibition zone each on Salmonella enterica at the concentration of 25 mg/ml and 50 mg/ml respectively. The activity of all biosynthesized AgNPs using S. birrea stem were observed on Klebsiella pneumoniae.

The antibacterial activities of the biosynthesized AgNPs of *G. senegalensis* leaf were investigated and the result was presented in (Table 8). The activity of ethyl acetate extracts on *E. coli* were obtained at low concentration of 6.25 mg/ml with inhibition zone of 13.0± 0.0 mm, while 13.0± 0.0 mm diameter of inhibition zone was recorded at 50 mg/ml using methanol extract, and 14.0± 0.0 mm at concentration of 200 mg/ml using petroleum ether. The activity of biosynthesized silver nanoparticles using methanol was observed on *Salmonella enterica* with 11.0± 0.0 mm diameter zone of inhibition at 25 mg/ml. However, the activities were observed on *K. Pneumoniae* using both extracts biosynthesized AgNPs.

Extract Type	Concentration (mg/ml)	Mean zone of inhibition (mm) ±SD		
		E. coli	*S. enterica*	*K. pneumoniae*
Methanol	200	15.0± 0.0	14.0± 0.0	18.0± 0.0
	100	12.0± 0.0	13.0± 0.0	17.0± 0.0
	50	11.0± 0.0	13.0± 0.0	13.0± 0.0
	25	11.0± 0.0	11.0± 0.0	12.0± 0.0
	12.5	NZI	NZI	11± 0.0
	6.25	NZI	NZI	11.0± 0.0

	P-value α= 0.05	0.23 (p> 0.05)	0.18 (P> 0.05)	0.14 (P> 0.05)
Ethyl acetate	200	NZI	NZI	11.0± 0.0
	100	NZI	NZI	12.0± 0.0
	50	NZI	NZI	10.0± 0.0
	25	NZI	NZI	NZI
	12.5	NZI	NZI	NZI
	6.25	NZI	NZI	NZI
	P-value α= 0.05			
Petroleum ether	200	16.0± 0.0	NZI	16.0± 0.0
	100	16.0± 0.0	NZI	12.0± 0.0
	50	15.0± 0.0	NZI	12.0± 0.0
	25	14.0± 0.0	NZI	11.0± 0.0
	12.5	NZI	NZI	11.0± 0.0
	6.25	NZI	NZI	0.0± 0.0
	P-value α= 0.05	0.23 (P> 0.05)		0.14 (P> 0.05)
Aqueous	200	16.0± 0.0	14.0± 0.0	13.5± 0.7
	100	16.0± 0.0	12.0± 0.0	13.0± 0.0
	50	15.0± 0.0	11.0± 0.0	13.0± 0.0
	25	14.0± 0.0	NZI	13.0± 0.0
	12.5	NZI	NZI	6.4± 0.0
	6.25	NZI	NZI	6.4± 0.0
	P-value α= 0.05	0.23 (P> 0.05)	0.18 (P> 0.05)	0.03 (P< 0.05)
Control	200	NZI	NZI	30.0± 0.0
	100	NZI	NZI	20.0± 0.0
	50	NZI	NZI	18.0± 0.0
	25	NZI	NZI	15.0± 0.0
	12.5	NZI	NZI	10.0± 0.0
	6.25	NZI	NZI	6.9± 0.0

Table 7. Antibacterial susceptibility of test isolates to Biosynthesized silver nanoparticles using S. birrea stem bark

Extract Type	**Concentration (mg/ml)**	**Mean zone of inhibition (mm) ±SD**		
		E. coli	***S. enteric***	***K. pneumoniae***
Methanol	200	16.0± 0.0	14.0± 0.0	18.0± 0.0
	100	16.0± 0.0	13.0± 0.0	18.0± 0.0
	50	13.0± 0.0	13.0± 0.0	16.0± 0.0
	25	NZI	11.0± 0.0	16.0± 0.0
	12.5	NZI	NZI	12± 0.0
	6.25	NZI	NZI	12.0± 0.0
	P-value α= 0.05	0.23 (p> 0.05)	0.18 (P> 0.05)	0.00 (P< 0.05)
Ethyl acetate	200	15.0± 0.0	NZI	12.0± 0.0
	100	14.0± 0.0	NZI	11.0± 0.0
	50	16.0± 0.0	NZI	NZI
	25	16.0± 0.0	NZI	NZI
	12.5	13.0± 0.0	NZI	NZI
	6.25	13.0± 0.0	NZI	NZI
	P-value α= 0.05	0.20 (p> 0.05)		0.00 (P< 0.05)
Petroleum ether	200	14.0± 0.0	NZI	17.0± 0.0
	100	NZI	NZI	15.0± 0.0
	50	NZI	NZI	12.0± 0.0
	25	NZI	NZI	11.0± 0.0
	12.5	NZI	NZI	0.0± 0.0
	6.25	NZI	NZI	0.0± 0.0
	P-value α= 0.05			0.00 (P< 0.05)
Aqueous	200	NZI	NZI	14.0± 0.7
	100	NZI	NZI	14.0± 0.0
	50	NZI	NZI	14.0± 0.0
	25	NZI	NZI	11.0± 0.0
	12.5	NZI	NZI	NZI
	6.25	NZI	NZI	NZI
	P-value α= 0.05			0.00 (P< 0.05)
Control	200	NZI	NZI	30.0± 0.0

	100	NZI	NZI	20.0± 0.0
	50	NZI	NZI	18.0± 0.0
	25	NZI	NZI	15.0± 0.0
	12.5	NZI	NZI	10.0± 0.0
	6.25	NZI	NZI	6.9± 0.0

Table 8. Antibacterial susceptibility of test isolates to Biosynthesized silver nanoparticles using G. senegalensis leaf

3.8. Additive Antibacterial Activity of Biosynthesized Silver Nanoparticles of *S. birrea* stem and *G. senegalensis* leaf at the ratio of 1:1

The result of mixed extract of biosynthesized AgNPs of *S. birrea* stem and *G. senegalensis* leaf at the ratio of 1:1 varied base on the extraction solvents and concentration of the extract indicated in (Table 9) revealed that, all the extract are found to be active on *E. coli* at low concentration of 6.25 mg/ml. The result of mixed extract of biosynthesized AgNPs of *S. birrea* stem and *G. senegalensis* leaf at the ratio of 1:1 are found to be active on *Salmonella enterica* at low concentration stating from 6.25 mg/ml using the aqueous extract, while the methanol extract activity observed from 12.5 mg/ml while ethyl acetate activity observed from 25 mg/ml.

The result of mixed extract of biosynthesized silver nanoparticles of *S. birrea* stem and *G. senegalensis* leaf at the ratio of 1:1 are found to be highly active on *Klebsiella pneumoniae* at low concentration ranged from 6.25 mg/ml using all the extract.

Extract Type	**Concentration (mg/ml)**	**Mean zone of inhibition (mm) ±SD**		
		E. coli	***S. enteric***	***K. pneumoniae***
Methanol	200	15.0± 0.0	11.0± 0.0	12.0± 0.0
	100	14.0± 0.0	11.0± 0.0	17.0± 0.0
	50	14.0± 0.0	11.0± 0.0	12.0± 0.0
	25	14.0± 0.0	11.0± 0.0	12.0± 0.0
	12.5	13.5± 0.0	6.4± 0 0	11± 0.0
	6.25	13.5± 0.0	6.4± 0.0	11.0± 0.0
	P-value α = 0.05	0.03 (p< 0.05)	0.00 (P< 0.05)	0.14 (P> 0.05)
Ethyl acetate	200	15.0± 0.0	13.0± 0.0	17.0± 0.0
	100	15.0± 0.0	12.0± 0.0	15.0± 0.0
	50	15.0± 0.0	12.0± 0.0	13.0± 0.0
	25	15.0± 0.0	11.0± 0.0	12.0± 0.0
	12.5	15.0± 0.0	6.4± 0.0	11.0± 0.0
	6.25	15.0± 0.0	6.4± 0.0	11.0± 0.0
	P-value α= 0.05			0.00(P< 0.05)
Petroleum ether	200	16.0± 0.0	13.0± 0.0	14.0± 0.0
	100	16.0± 0.0	12.0± 0.0	13.0± 0.0
	50	15.0± 0.0	12.0± 0.0	13.0± 0.0
	25	12.0± 0.0	11.0± 0.0	11.0± 0.0
	12.5	6.4± 0.0	6.4± 0.0	11.0± 0.0
	6.25	6.4± 0.0	6.4± 0.0	11.0± 0.0
	P-value α= 0.05	0.07 (P> 0.05)	0.15 (P> 0.05)	0.14 (P> 0.05)
Aqueous	200	14.0± 0.0	15.0± 0.0	16.5± 0.7
	100	13.0± 0.0	15.0± 0.0	16.0± 0.0
	50	13.0± 0.0	15.0± 0.0	16.0± 0.0
	25	12.0± 0.0	13.0± 0.0	16.0± 0.0
	12.5	11.5± 0.0	12.0± 0.0	14.0± 0.0
	6.25	11.5± 0.0	12.0± 0.0	14.0± 0.0
	P-value α= 0.05	0.00 (P< 0.05)	0.03 (P< 0.05)	0.00 (P< 0.05)
Control	200	NZI	NZI	30.0± 0.0
	100	NZI	NZI	20.0± 0.0
	50	NZI	NZI	18.0± 0.0
	25	NZI	NZI	15.0± 0.0
	12.5	NZI	NZI	10.0± 0.0
	6.25	NZI	NZI	6.9± 0.0

Table 9. Antibacterial susceptibility of test isolates to Biosynthesized silver nanoparticles using S. birrea stem bark and G. senegalenis leaf 1:1

4. Discussions:

The result of this studies show that stem bark of *S. birrea* and the leaf of *G. senegalensis* are rich in mineral elements (Ca, Zn, Mg, Fe, Cu, Na and k) in various concentrations which is in agreement with finding of Darinka *et al.* [26]. These variations of concentrations of plants depend on the factors including composition of the soil, water and fertilizers used as well as permissibility, selectivity and absorbability of plants for the uptake of these elements. Hence, the observed variations in concentration of the elements are attributed to the nature of the plant as well as its environment [27]. The results of calcium analysis obtained in *S. birrea* stem bark 400.5 ppm and *G. senegalensis* leaf 363.3 ppm in this research were lower than those reported by Mohammed [10] but higher than that reported by Mohammed and Sulaiman [28]. This might be attributed to the nature of the plant as well as its environment Muhammad *et al.* [27]. Calcium helps in the transport of long chain fatty acids which aid in prevention of diseases, high blood pressure and other cardiovascular diseases [10]. Magnesium works with calcium in transmitting nerve impulse in the brain. Both elements give relief in patients having depression. In this result the magnesium content in *G.*

senegalensis leaf (191.2 ppm) is higher than that of S. *birrea* stem bark (23.18 ppm) and also higher than the finding of Mohammed and Sulaiman [28], while lower than what reported by Mohammed [10], this might be attributed to the nature of the plant as well as its environment. Zinc (Zn) is important in wound healing and also functions as an antioxidant. Iron (Fe) is a necessary trace element found in nearly all living organisms, it plays an important role in biology, forming complexes with molecular oxygen in hemoglobin and myoglobin which are oxygen transport proteins in vertebrates. Many enzymes vital to life also contain iron, such as catalase and lipoxygenase. The color of blood is also due to iron containing hemoglobin [10], the distribution pattern of iron recorded in this study, are closer to the content recorded by Mohammed and Sulaiman [28] but much lower than what was recorded by Mohammed [10], these mineral elements play an important role in metabolic process of the plants in the production of bioactive compound that make them medicinally important.

Preliminary phytochemical screening of *S. birrea* stem bark using methanol extract shows the presence of tannins, flavonoids, alkaloid terpenoid and glycoside. These results support the finding of Nicoline and Roland [29] and Louis *et al.* [14]. The aqueous extract revealed the presence of alkaloid, tannin and phenol while the petroleum ether and Ethyl acetate revealed the presence of saponin which reported to have ability to disturbed bacterial cells permeability by binding to the outer membrane [9]. The preliminary phytochemical screening of *G. senegalensis* leaves using methanol as a solvent revealed the presence of phenol, tannins, terpenoid and glycosides, where by alkaloid, saponin and flavonoid were not found, which is not in accurate with the finding of Nabaa *et al*. [30] where they are detected. Similarly, the preliminary Phytochemical screening of *G. senegalensis* leaves using aqueous extract revealed only phenol and glycoside unlike the finding of Abubakar *et al.* [31] and Wegdan [32], where they detected the presence of alkaloid, saponin and flavonoid.

The quantitative phytochemical results in this research showed high content of alkaloids in all tested plants materials, stem bark of *S. birrea* possess 19.37% of the total sample used (i.e. 5 g) and the leaf of *G. senegalensis* 17.45%, this is lower than alkaloids contents of stem bark of *S. birrea* reported by Louis *et al.* [14], but the content present in the leaf of *G. senegalensis* is in line with what was recorded by Mohammed [10]. Alkaloids as an organic base usually form salts with mineral acids such as hydrochloric acid, sulfuric acid and organic acid. Its salts are usually more water-soluble than their free base form [33]. It was revealed that alkaloid have ability to causes leakage of cytoplasmic contents and also inhibit nucleic acid synthesis after observing inhibition of type I topoisomerases in cell-free assays [34]. Flavanoids and Saponin, are also found to be 15.13 and 14.72% in the stem bark of *S. birrea* and 12.49 and 13.72% in the leaf of *G. senegalensis* respectively, these are in line with previous finding of Louis *et al* [14]. The flavonoids have multiple cellular targets in antibacterial activity, rather than one specific site of action their mode of antimicrobial action is also related to their ability to inactivate microbial adhesins, enzymes, cell envelope transport proteins, and may also disrupt microbial membranes [35]. Saponins disturbed bacterial cells permeability by binding to the outer membrane [9]. In this study the phenolic compounds and tannins are the least among the phytochemical present in all tested sample, the results is contradiction with findings of Louis *et al* [14] who reported the high contents of the compounds in *S. birrea* stem, while the tannin contents is in line what was recorded by Mohammed [10]. Tannins have ability to inhibit the enzymes activity through protein binding action as the property of tannin [36].

The result of these studies showed the methanol extract of *S. birrea* stem bark found to be active against *E. coli* and *Salmonella enterica*, in which the activity against *E. coli* at 100 mg/ml produce 13 mm diameter zone of inhibition which is in line with the finding of Lois *et al.*, Nicoline and Roland and Manzo *et al.* [14, 33, 37]. The results also revealed that the methanolic extract of *S. birrea* were found to be inactive against *Klebsiella pneumonia*, this supported the data recorded by Abdulhamid *et al.* [6]. The ethyl acetate extract show activity on *E. coli* and *Salmonella enterica* but not active on *Klebsiella pnemoniae*, this may be due to presence of saponin which have the ability to disturbed bacterial cells permeability [9]. The petroleum ether extract activity on *Salmonella spp* is in support of the result recorded by Moyo *et al.* [38]. The aqueous extract of *S. birrea* stem bark found to be not active against all tested isolate, this may be due to inadequate phytochemical compound in the extract. The activity of methanolic extract of *G. senegalensis* leaf against *E. coli* and *Klebsiella pneumonea* is supported by the finding of Mamman and Isah [8] where the activity observed on *E. coli* while no activity on *Klebsiella pneumoniae*. However, the petroleum ether extract of *G. senegalensis* activities on *E. coli* and *Klebsiella pneumoniae* is in agreement with the finding of Simon and Aminu [39]. The activity of aqueous extract against all tested isolates were not observed, the result is in contrary with finding of Ogbeba *et al* [40] where he recorded its activity against *E. coli* and *Salmonella enterica*.

The activity of mixed crude extract of *S. birrea* stem and *G. senegalensis* leaf at the ratio of 1:1 were increase against the *E. coli* using methanol, ethyl acetate and petroleum ether extracts, compared with the activity of the separate extract, which clearly shows their additive activity. The additive activities might be attributed to the presence of all the extracted phytochemicals with exception of flavanoids which was only present in methanolic extract of *S. birrea*. The additive activity was also observed in ethyl acetate against *Salmonella enterica*. However, the activities of combined crude extracts of *S. birrea* stem and *G. senegalensis* leaf at the ratio of 1:1 from methanol and petroleum ether were weak against the *Salmonella enterica* which showed an antagonistic activity. This study shows that the additive activities of *S. birrea* stem and *G. senegalensis* leaf. Ethyl acetate is the best when used on *E. coli* followed by Methanol and petroleum ether, while for *Salmonella enterica* ethyl acetate has the highest activities.

Biosynthesis of nanoparticles from plant's extracts was reported as the latest and most favorite method of production of nanoparticles, this is because plants are widely distributed and easily available as well as safe to handle. AgNPs synthesis using plants as the production assembly has drawn attention of many researchers, because of its less hazardous, less expensive and the rate of synthesis is faster [20]. AgNPs have been acknowledged as a novel and effective elicitor in plant biotechnology for the production of bioactive compounds they serve as an enhancer of the bioactivity of phenolic compounds; since the rate of bioactivity in plants is increased by the effect of NPs, it is expected that biological activities will also increase Samantha *et al.* [21]. It was reported that, when silver ions are transformed into a metal AgNPs by biological process of synthesis, their toxicity are seen to decrease while their antimicrobial activities get increase markedly Jain and Pradeep [22]. These characteristics of AgNPs make it wonderful weapons for the clinical management of microbial diseases, most specially as their selectivity towards bacterial cells have been proven and no case of antimicrobial resistance has been so far reported [21]. These result indicates an improved antibacterial action by AgNPs formation when compared to the results of the extract alone, as the bioactivity of some crude extract of *S. birrea* stem and *G. senegalensis* leaf especially aqueous extract increased when used to produce biosynthesized AgNPs, these results supported the finding of Samantha *et al.* [21] who reported that, the biosynthesized AgNPs using aqueous extract of *S. birrea* stem bark found to be active against all tested isolate unlike normal aqueous crude extract of *S. birrea* stem bark which doesn't show any activity on all tested isolates, this is in line with the finding of Stephen *et al.* [23]. The activity of methanolic and petroleum ether of crude extract of *S. birrea* stem increased on *E. coli* as it was used to produce AgNPs. It was also recorded in this studies that biosynthesized AgNPs of *S. birrea* stem and *G. senegalensis* leaf using methanol and ethyl acetate extract are found to be highly active on *Klebsiella pneumoniae* at lower concentration. However, no activity observed using crude extracts of *S. birrea* stem and *G. senegalensis* against *Klebsiella pneumoniae* at all used concentrations which is in line with the finding of Bello *et al.* [24]. In this study, the highly antibacterial activity seen may be due to the release of silver cation from AgNPs. The Ag^+ penetrated into bacteria through the cell wall as a consequence of which the cell wall ruptures leading to denaturation of protein and death. The antibacterial activity of AgNPs toward gram negative bacteria depends on its concentration. The nanoparticles form pits in the cell wall of microbes, get accumulated, and permeate into the bacterial cell leading to their death [41].

The activity of mixed biosynthesized AgNPs from *S. birrea* stem and *G. senegalensis* leaf at the ratio of 1:1 was increase on all tested isolate using aqueous extract at low concentration used. Similarly, there were and increased activity on the *E. coli* using methanolic and ethyl acetate mixed extract, there increased activity was also recorded on *Klebsiella pneumoniae* using ethyl acetate extract, which clearly shown their additive activity. The petroleum ether extracts show very impressive synergic activity on *Salmonella enterica* which resist the activity of separate extract, but when combined the activity were observed at low concentration of 6.25 mg/ml. Howevere, the antagonist activity was observed on *Klebsiella pneumoniae* using petroleum ether extracts in which activity decreased as compare with the activity of separate extract at concentration of 200 mg/ml while at concentration of 12.5 mg/ml the activity remained the same.

The results of this research show the activity of various extract *S. birrea* stem and *G. senegalensis* leaf on the *E. coli*, *Salmonella enterica* and *Klebsiella pneumoniae* and isolates were recorded to be among the bacterial pathogens capable of causing diarrhoeal diseases [4]. The ethno medicinal survey conducted among the traditional healers in four local government's area (Hadejia, Malam maduri, Auyo and Kafin Hausa) of Jigawa State Nigeria, revealed that the *S. birrea* and *G. senegalensis* were among medicinal plants used in curing diarrhoeal diseases is in line with finding of Dukku *et al.* [25] might have been supported by the finding of this research in which mixture of some extracts of this plants part enhance their activity although some extract lost their strength when compare with its activity separately.

5. Conclusion:

This research was aimed at determining the in vitro antibacterial activity of AgNPs synthesized from extracts *S. birrea* stem bark and leaf of *G. senegalensis* against some bacterial isolates capable of causing diarrhoreal diseases. Standard phenotypic and genotypic techniques were used for the identification of the isolates. Some mineral elements and bioactive compounds were detected and quantified in which both plants showed the presence of tannins, alkaloids, flavonoids, cardiac glycosides, phenols, saponins and terpenoids. Antibacterial profile of crude extract of *S. birrea* stem bark and leaf of *G. senegalensis* were tested individually and in combination at 1:1, aqueous extracts were inactive on the *E. coli*, *Salmonella enterica* and *Klebsiealla pneumonia*, while methanolic and ethyl acetate extracts on *E. coli* showed zones of growth inhibition, methanolic found to be active on *Salmonella enterica*. The antibacterial activity of the combined biologically synthesized AgNPs at 1:1 from each extract showed zones of growth inhibition on all tested isolates. The additive activity of mixed crude extracts was observed on the *E. coli*, *Salmonella enterica* using methanol and ethyl acetate extract as compared with separate extract while antagonist observed on *Salmonella enterica* using petroleum ether, similarly additive activity was observed using combined biosynthesized AgNPs on all tested isolate using aqueous, methanol and ethyl acetate extract as compared with separate extract in exception of petroleum ether on *Klebsiealla pneumonea* were antagonist observed as compared with separate extracts.

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Conflicts of Interest

The authors declare no conflicts of interest

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