Original Research Article

In Vitro Antibacterial and Antitubercular Activities of Leaf Extracts of *Senna occidentalis*

ABSTRACT

Aim: This study aimed to evaluate the antibacterial and antitubercular activities of ethylacetate and ethanol leaf extracts of *Senna occidentalis*.

Study Design: Fresh leaves of *Senna occidentalis* collected from Suleja, Niger state were used for this study against some medically important micro-organisms viz; *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, *Klebsiella pneumoniae, Mycobacterium bovis and Mycobacterium smegmatis*.

Place and Duration of Study: The study was conducted in Abuja, Nigeria at the Department of Microbiology and Biotechnology, National Institute for Pharmaceutical Research and Development [NIPRD], from February 2019 to April 2019.

Methodology: *Senna occidentalis* leaves were extracted successively with ethyl-acetate and ethanol. The obtained extracts were tested *in vitro* for antibacterial activity by agar well diffusion method, while anti-tubercular screening was carried out by broth micro-dilution method. A fixed-dose concentration of chloramphenicol was used as a control drug against the bacterial isolates while isoniazid was used as control drug against the mycobacterium isolates.

Result: The *in vitro* antibacterial screening showed that the crude extracts exhibited varying activity against the different microbes with highest zone of inhibition at 12 mm, and anti-tubercular activity with MICs ranging from 97.6-390.6 µg/mL. Among these extracts, ethyl-acetate extract showed significant antibacterial activity against most of the test micro-organisms. The most susceptible micro-organism was *P. aeruginosa* (12mm zone in ethyl-acetate at 80 mg/mL) followed by *B. subtilis* (10 mm zone in ethyl-acetate extract at 80 mg/mL) and *E. coli* (9 mm zone in ethyl-acetate extract at 80 mg/mL). The ethanol extract was the most effective in inhibiting the growth of *M. smegmatis and M. bovis* with MICs of 97.6 µg/mL and 195.3 µg/mL.

Conclusion: The activities observed could be attributed to the presence of some active metabolites contained in the extracts which could be useful in drug development for therapeutic purposes.

Keywords: *Senna occidentalis*; anti-bacterial activity; anti-tubercular activity; micro-organisms.

1. Introduction

Tuberculosis (TB) still remains one of the leading causes of death in the world. It is an infectious deadly disease caused by the organism, *Mycobacterium tuberculosis*, which emerged from East Africa for more than three million years ago [1]. About one-third of the world's population is at risk to develop active TB and contribute to the continued spread of *M. tuberculosis* [2, 3, 4]. Nigeria is ranked seventh in the world and second in Africa among the 30 countries with the highest burden of TB [5]. Globally, there are an estimated 9.3 million new cases and 13.7 million chronic active cases responsible for 1.7 million deaths worldwide yearly. Moreover, up to 50 million people are said to be infected with drug-resistant forms of TB from which about 500,000 cases of multidrug resistant (MDR) TB worldwide per a year [6]. Management of TB/MDR-TB patient requires intense multi-chemotherapy for at least six months to two years. It is very hurtful to a patient's health due to high levels of drug toxicity and its adverse effects [7, 8, 9]. The

emergence of MDR TB and extensively-drug resistant (XDR) TB to the medicines now in use makes urgent search for new anti-TB agents worldwide [10, 11].

Medicinal plants are important source of drugs; especially in traditional medicine [12]. It is a common practice in Nigeria and other parts of the world to use medicinal plant in the form of crude extracts, decoction, infusion or tincture to treat common infection and chronic conditions. Medicinal plants contain a lot of bioactive constituents or phytochemical compounds which are secondary metabolite because they are not required for growth, respiration, transpiration or any primary function in plants [13]. The major secondary metabolites including alkaloids, carbohydrates, flavonoids, tannins, terpenoids, and steroids [13]. Plant initially produces these phytochemical compounds to protect themselves from pathogens and predator [14].

Senna occidentals (Linn) (formerly *Cassia occidentalis* or *Ditremexa occidentalis*), also called Coffee Senna in English is a shrub that grows between 5 to 8cm in height, belongs to the plant family *Fabaceae* and the subfamily *Caesalpiniaceae* which are commonly found in the tropical and sub-tropical regions of the world [15]. It is a straight, somewhat branched, smooth, semi-woody, fetid herb, hard, stout, with a few lateral roots on mid-section. The stem of the plant is reddish purple and the leaves are alternate, each leaflet 4-6 cm long and 1.5-2.5 cm wide [16]. It can be found in open pastures and in fields cultivated with cereals such as soybean, corn, sorghum and others; thus, during the harvest it is almost impossible to prevent this plant from mixing with the cultivated crops [17, 18]. It is an ayurvedic plant with huge medicinal importance and also used for various therapeutic purposes in traditional medicine [19, 20, 21]. In Nigeria, this plant is locally called Sanga-sanga or Rai dore in Hausa language [22, 23]; Akidi agbara in Igbo language and Abo rere in Yoruba language [24].

The plant has been used in different parts of the world by the traditional healers in treating different forms of diseases. *Cassia occidentalis* plant extract (4–5 drops) is used in curing eye inflammations and also used in Jamaican folk medicines for curing diarrhoea, dysentery, constipation, fever, cancer, eczema and venereal diseases [25]. It has been documented in literatures that extract of *Senna occidentalis* has antimicrobial activity [26, 27], larvicidal and pupicidal activity [28], antioxidant and hepatoprotective activity [29], anti-inflammatory actions [20], antimalarial activity [30], antianxiety and antidepressant activity [31], analgesic activity [21] and antidiabetic activity [32, 33, 34]. Moreover, studies on this plant showed that the nature and amount of the phytochemicals varies according to the season and geographical location [35]. This present research was carried out to evaluate the antibacterial and antituberculosis activities of ethylacetate and ethanol leaf extracts of *Senna occidentalis*.

2. Materials and Methods

2.1 Plant Collection

Fresh leaves of *Senna occidentalis* were collected in the month of February, 2019 from Suleja, Niger State, identified and authenticated by the Taxonomist at the Herbarium Unit, National Institute for Pharmaceutical Research and Development (NIPRD). A voucher specimen was deposited at the herbarium of the institute.

2.2 Preparation of Plant Extract

The fresh leaves of *S. occidentalis* were dried at room temperature for 14 days, after which it was pulverized using a mechanical grinder. Ethyl-acetate and ethanol solvents of volume 500 mL each was used for the maceration for 24 hours. The solution obtained was filtered using a filter paper. The filtrate was allowed to dry on the water bath at 50˚C to obtain the various extracts and kept in the refrigerator until needed for use.

2.3 Test Organisms

Pure clinical isolates of *Bacillus subtilis, Klebsiella pneumoniae*, collected and biochemically confirmed from Diagnostic Laboratory of NIPRD clinic and American Typed cultures of *Escherichia coli* [ATCC 25952], *Staphylococcus aureus* [ATCC 25923], *Pseudomonas aeruginosa* [ATCC 27853], *Salmonella paratyphi* [ATCC 9150], *Mycobacterium bovis* [27290], *Mycobacterium smegmatis* [607] were used in this study.

2.4 Inoculum Preparation

A loopful of the test organism (*S. aureus*, *E. coli*, *P. aeruginosa*, *B. subtilis*, *K. pneumoniae* and *S. paratyphi*) was taken from their respective agar slants, sub-cultured into 5 mL of nutrient broth and incubated at 37°C. Following incubation at 37°C for 24 hrs, organisms were diluted with normal saline to a turbidity that was equivalent to 0.5 Mc Farland standard (10^6 CFU)/mL) [36] .
Fifty micro-litre (50 µL) of each freshly thawed stock test organism (*M. Bovis* and *M. smegmatis*) was inoculated into 50 mL of sterile Middle brook7H9/ADC media and incubated at 37˚C with shaking for 5-7 days. The activity grown *M. bovis* and *M. smegmatis* culture had its optical density adjusted to between 0.2 - 0.3 at a wavelength of 650 nm using Jenway 6405 UV-Visible spectrophotometer.

2.5 Antibacterial Activity

Prepared concentrations (80 mg/mL, 40 mg/mL, 20 mg/mL and 10 mg/mL) of each extract were tested against the test organisms using Agar well diffusion method as described by [37]. One hundred microliter (100 µL) of the suspension of standardized microorganisms was inoculated into sterile molten Mueller Hinton agar, swirled and poured into sterile Petri dishes and allowed to solidify. Holes for each concentration of the extracts and positive control were bored aseptically using a sterile cork borer of 5 mm. The bottom of the bored holes was sealed using a drop of Mueller Hinton agar. One hundred microliters of different concentrations of the extracts and a fixed dose (30 µg/mL) of the positive control, chloramphenicol being a drug of choice as a broad spectrum antibiotic was dispensed into appropriately labelled wells respectively. The plates were allowed to dry inside the biosafety cabinet as well as allowing the extracts to diffuse for about 2 hrs and then incubated at 37°C for 24 – 48 hours. Antibacterial activity was assessed by measuring the size of the zone of inhibition surrounding wells and taking the average of the readings of each duplicate plate post incubation.

2.6 Anti-tubercular Activity

The anti-tubercular test of the plant extracts was conducted using the broth micro-dilution method in 96 well micro-titre plates [38]. Each of the extract was first dissolved in tween 20 and then diluted in Middle brook 7H9 broth, to give a starting concentration of 100,000 µg/mL which was diluted across the 96-well micro-litre plate in a two-fold serial dilution to give final testing concentrations of 50,000 µg/mL to 97.7 µg/mL. Twenty five (25) mg of Isoniazid (Sigma Aldrich Inc) was dissolved in 1 mL dimethylsulfoxide (DMSO) and 25 µg/mL solution was made by diluting 25 µL in 25 mL 7H9 broth, sterile filtered was used as positive control drug and extracts/drug free medium with culture suspensions were used as negative control. Each extract concentration was assayed in duplicate. The plates were then incubated for 5-7 days at 37°C. After the 7th day, 25 µL of tetrazolium salt dye was added to all the wells, re-incubated over-night and observed for absence or presence of microbial growth by colour change in the wells. The MIC was defined as the lowest drug/extract concentration that prevented the color change of the tetrazolium dye to pink. Colourless well was interpreted as there is no mycobacterial growth and pink color was interpreted as growth occurrence.

3. Results

For the antibacterial activity, ethyl-acetate extract was found to be active against all the test organisms (*S. aureus*, *E. coli*, *B. subtilis*, *K. pneumoniae*, *P. aeruginosa*, *S. paratyphi*) while only *E. coli*, *P. aeruginosa* and *K. pneumoniae* had activity at 40 mg/mL concentration (table 1). The ethanol extract was active only against *S. paratyphi* at 80 mg/mL and 40 mg/mL respectively (table 1). All the organisms were resistant against both extracts at 20 mg/mL and 10 mg/mL concentrations.
For the anti-tubercular activity, the extracts were screened against *M. smegmatis* and *M. bovis*. Ethanol extract showed MIC of 97.6 µg/mL while ethyl-acetate showed MIC of 195.3 µg/mL against *M. smegmatis* whereas ethanol extracts showed MIC of 195.3 µg/mL while ethyl-acetate showed MIC of 390.6 µg/mL against *M. bovis* (table 2).

4. Discussion

Tuberculosis remains a global infectious disease and with emergence of multi-drug resistance strains, there is need for research and development of new compounds that will serve as leads in drug development. *Senna occidentalis* is a well-known herb used as ayurvedic traditional medicine for their effectiveness against wide range of diseases due to the presence of diverse secondary metabolites responsible for their antibacterial activity [39]. Egharevba *et al*., [24], reported the phytochemical screening of the plant showed the presence of carbohydrates, saponins, sterols, flavonoids, resins, alkaloids, terpenes, anthraquinones, glycoside and balsam. He also reported the isolation of anthraquinones; emodin and chrysophanone from the plant. Musa *et al*., [40], reported the presence of anthraquinones, tannins, flavonoids while saponins was not detected in the ethanolic extract of *S. occidentalis*. Odeja *et al*., [41], detected the presence of alkaloids, anthraquinones and resins in the ethyl acetate extract of S. occidentalis.
For instance, herbs that have tannins as their component are astringent in nature and are used for the treatment of gastrointestinal disorders such as diarrhoea and dysentery [42], as well as for soothing relief, skin regeneration, as anti-inflammatory and diuretics [43]. Saponins lower the cholesterol level; have anti-diabetic and anti-carcinogenic properties [44], and are also expectorants, cough suppressants and for haemolytic activities [45, 46]. Alkaloids are known to possess anti-malaria property [47], and also have antispasmodiac and analgesic properties [43]. Also, flavonoids and resins present in the plant might be responsible for its anti-inflammatory properties. Flavonoids are considered a key ingredient in Chinese folkloric medicine having anti-inflammatory effect on both acute and chronic inflammation [48, 49]. Emodin has been reported to have antibacterial effect against *Bacillus subtilis* and *Staphylococcus aureus* while chrysophanol has been reported to have some wound healing properties [50, 51].
The existence of these metabolites strongly suggests great potential of the plant as a source of phytomedicines. Various studies of phytochemicals of the plant shows that the amount and nature of phytochemicals varies according to climate condition for the growth of the plant and different geographical locations.
The antibacterial activities of ethanol and ethylacetate leaf extracts of *S. occidentalis* were investigated against two Gram-positive bacteria viz; *Staphylococcus aureus, Bacillus subtilis,* four Gram-negative bacteria such as *Escherichia coli, Pseudomonas aeruginosa, Salmonella paratyphi, Klebsiella pneumoniae* and two mycobacterium species namely; *Mycobacterium smegmatis* and *Mycobacterium bovis*.
From table 1 above, the highest activity (zone of inhibition of 12 mm) was shown by the ethylacetate extract against *Pseudomonas aeruginosa* and ethanol extract (zone of inhibition of 12 mm) against *Salmonella paratyphi* at 80 mg/mL while the lowest activity was at 6 mm. The result obtained for *P. aeruginosa* in this study is contrary to that of Egharevba *et al*., [24], which showed no activity against *P. aeruginosa* across all concentrations, which may be due to the level of concentration or strain of *P. aeruginosa* used in this study. This result suggests that the antibacterial activity of ethylacetate and ethanol extracts of *S. occidentalis* increases when used in higher concentrations, which is in agreement with a work done by Sadiq *et al*., [23], on the antibacterial activity of ethanol and water extracts of *Cassia ossidentalis*. Result of this study shows the ethylacetate extract inhibited the growth of various species of gram negative bacteria while ethanol extract only inhibited the growth of *Salmonella paratyphi*. However, *S. aureus* was resistant to both extracts at all concentrations except for ethylacetate extract which was

susceptible at 80 mg/mL concentration. This is in accordance with a report by Sadiq *et al*., [23], in which *S. aureus* was resistant to the ethanol and water extract used. This probably could be attributed to the cell membrane permeability or genetic factors. The result obtained from this study shows that ethyl acetate extract of *S. occidentalis* has higher antibacterial activity against the test organisms compared to the ethanol extract and this may be due to the different polarity of the solvents as well as the solubility of the active compounds that were able to dissolve.

Also in this study, the crude extracts of *S. occidentalis* showed varying degree of anti-tuberculosis activity. The ethanol extract was the most effective in inhibiting the growth of *M. smegmatis and M. bovis* with MICs of 97.6 µg/mL and 195.3 µg/mL respectively while the ethylacetate extracts had MICs of 195.3 µg/mL and 390.6 µg/mL respectively, but not as active as the control drug, isoniazid with an MIC of 0.02 µg/mL. In a previous study, Yeragamreddy *et al*., [52], reported an MIC of 25 µg/mL for the ethylacetate fraction of *Cassia ossidentalis* against *Mycobacterium tuberculosis*. Non-pathogenic tuberculosis strains are acceptable as surrogate in drug development research [53].

Table 1: Effect of ethylacetate and ethanol extracts of *S. occidentalis* at various concentrations (mg/mL) against bacterial isolates

	Zone of Inhibition (mm)							
	Ethylacetate				Ethanol			
Bacterial isolates	80	40	20	10	80	40	20	10
Salmonella paratyphi	7	-	-	-	12	7	-	-
Staphylococcus aureus	7	-	-	-	-	-	-	-
Escherichia coli	9	7	-	-	-	-	-	-
Bacillus subtilis	10	-	-	-	-	-	-	-
Klebsiella pneumoniae	8	6	-	-	-	-	-	-
Pseudomonas aeruginosa	12	8	-	-	-	-	-	-

- = No zone of inhibition

Table 2: Effect of ethylacetate and ethanol extract of *S. occidentalis* against *M. smegmatis* and *M. bovis*

	Minimum Inhibitory Concentration Values (µg/mL)		
Organisms	Ethanol	Ethylacetate	Isoniazid
M. smegmatis	97.6	195.3	0.02
M. bovis	195.3	390.6	0.02

CONCLUSION

From the present study conducted, it can be concluded that the antibacterial and antituberculosis activity of *Senna occidentalis* may be due to the phytochemicals present in the plant. The antibacterial activity of these plants shows the importance of the extracts in traditional preparations which may be helpful in treating diseases such as urinary tract infections and diarrhea. Thus, it may be considered as a natural source of antimicrobials and anti-tuberculosis for therapeutic purposes. Further research should be carried out to identify the active compounds responsible for the plant biological activity and also screen the plant against multi drug resistance tuberculosis (MDR-TB).

REFERENCES

1. Gutierrez MC, Brisse S, Brosch R, Fabre M, Omaïs B. Ancient Origin and Gene Mosaicism of the Progenitor of *Mycobacterium tuberculosis*. *PLoS Pathogens*. 2005;**1**, e5. http://dx.doi.org/10.1371/journal.ppat.0010005
2. Jasmer RM, Nahid P, Hopewell PC. Clinical practice: latent tuberculosis infection, N. Engl. J. Med. 2002;347:1860–1866.
3. World Health Organization (WHO). Multidrug and extensively drug resistant TB (M/XDR-TB): Global report on surveillance and response. 2010;1211 Geneva 27, Switzerland.
4. Maiga M, Abaza A, Bishai WR. Current tuberculosis diagnostic tools & role of urease breath test. Indian J. Med. Res. 2012;135:731–736.
5. World Health Organization (WHO). Global tuberculosis report 2017: http://www.who.int/tb/publications/global_report/en/
6. World Health Organization (WHO). Global Tuberculosis Control - Surveillance, Planning and Financing. Geneva, Switzerland. 2007;376. WHO/HTM/TB/2007.
7. Rivoire N, Ravololonandriana P, Rasolonavalona T, Martin A, Portaels F, Ramarokoto H. Evaluation of the Resazurin Assay for the Detection of Multidrug-resistant *Mycobacterium tuberculosis* in Madagascar. Int J Tuberc Lung Dis. 2007;11:683–688.
8. Aleme GA, Gebeyehu A. Clinical Improvement and Drug-adverse Effects among Patients Taking Anti-tuberculosis Drugs. Ethiopian J Health Biomed Sci. 2010;2:103–110.
9. Hannan A, Ullah MI, Usman M, Hussain S, Absar M, Javed K. Antimycobacterial activity of Garlic (*Allium sativum*) against Multi-drug resistant and non-Multi-drug resistant *Mycobacterium tuberculosis*. Pak J Pharm Sci. 2011;24:81–85.
10. Higuchi CT, Sannomiya M, Pavan FR, Leite SRA, Sato DN, Franzblau SG. Byrsonima fagifolia Niedenzu Apolar Compounds with Antitubercular Activity. Evid-Based Complement and Altern Med. 2008; 20(11):1–5.
11. Gupta R, Thakur B, Singh P, Singh HB, Sharma VD, Katoch VM. Anti-tuberculosis activity of Selected Medicinal Plants against Multi-drug resistant *Mycobacterium tuberculosis* isolates. Indian J Med Res. 2010;131:809–813.
12. Bako SP, Bakfur MJ, John I, Bala EI. Ethnomedicinal and phytochemical profile of some savanna plant species in Nigeria. Int J Bot. 2005;1(2):147–50.
13. Edoga HO, Okwu DE, Mbaebie BO. Phytochemicals constituents of some Nigerian medicinal plants. African Journal Biotechnology. 2005;4(7):685–688.
14. Poongothai A, Sreena KP, Sreejith K, Uthiral Ingam M, Annapoorani S. Preliminary phytochemical screening of *Ficus racemesa* Linn. Bark. *Int. J. Pharma. Bio. Sci*. 2011;2(2):1388-93.
15. Musa DD, Aliero AA, Bashir KA. Preliminary allelopathic activity of aqueous leaf extract of *Senna occidentalis* on the Germination and early seedling growth of Cowpea *int. Journal of Current Sciences and research.* 2017; 1(1):23-25.
16. Vijay VS, Jainendra J, Arun KM. Pharmacological and Phytochemical Profile of *Cassia occidentalis* L: A Review. Journal of Drug Delivery & Therapeutics. 2016;6(5):91-96.

17. Lar J, Gupta PC. Anthraquinone glycosides from the seeds of *Cassia occidentalis* Linn. Experientia. 1973;29:142–3.
18. Barbosa-Ferreira M, Dagli ML, Maiorka PC, Górniak SL. Sub acute intoxication by *Senna occidentalis* seeds in rats. Food Chem Toxicol. 2005;43:497–503.
19. Arya V, Yadav S, Kumar S, Yadav JP. Antimicrobial activities of *Cassia occidentalis* l. (leaf) against various human pathogenic microbes. Life Sciences and Medicine Research. 2010; 9:1-11.
20. Yadav JP, Vedpriya A, Sanjay Y, Manju P, Sandeep K, Seema D. *Cassia occidentalis* L.: A review on its ethnobotany, phytochemical and pharmacological profile. *Fitoterapia*. 2010; 81(4):223-230.
21. Silva MG, Aragao TP, Vasconcelos CF, Ferreira PA, Andrade BA, Costa IM, Costa-silva JH, Wanderley AG, Lafayette SS. Acute and sub-acute toxicity of *Cassia occidentalis* L. stem and leaf in Wistar rats. Journal of Ethnopharmacology. 2011;136(2): 341-346.
22. Nuhu AA, Aliyu R. Effects of *Cassia occidentalis* aqueous leaf extract on Biochemical markers of Tissue damage in Rats. Trophical Journal of Pharmaceutical Research. 2008; 7(4):1137-1142.
23. Sadiq IS, Shuaibu M, Bello AB, Tureta SG, Isah A, Izuagie T, Nasiru S, Kamaru MB. Phytochemistry and antimicrobial activities of *Cassia occidentalis* used for herbal remedies. Journal of Chemical Engineering. 2012;1: 38-41.
24. Egharevba HO, Odigwe AC, Abdullahi MS, Okwute SK, Okogun JI. Phytochemical Analysis and Broad Spectrum Antimicrobial Activity of *Cassia occidentalis* L. (whole plant*). New York Science Journal*. 2010;3(10):74-81.
25. Payne-Jackson A, Alleyne MC. Jamaican Folk Medicines: A Source of Healing. University of West Indies Press. 2004; p. 1–228.
26. Mariano-Souza DP, Paulino CA, Maiorka PC, Gorniak SL. Administration *Senna occidentalis* seeds to adult and juvenile rats: Effects on Thymus, Spleen and in Haematological parameters. Journal of Pharmacology and Toxicology. 2010;5:46-54.
27. Mohammed M, Aboki MA, Saidu HM, Victor O, Tawakalitu A, Maikano SA. Phytochemical and Some Antimicrobial Activity of *Cassia Occidentalis* L. (Caesalpiniaceae). International Journal of Science and Technology. 2012;2:4.
28. Ibrahim MA, Aliyu AB, Sallau AB, Bashir M, Yunusa I, Umar TS. *Senna occidentalis* leaf extract possesses antitrypanosomal activity and ameliorates the trypanosome-induced anaemia and organ damage. Pharmacognosy Research. 2010; 2(3):175-180.
29. Gowrisri M, Sarita K, Vrushabendra SBM, Archana SP, Vishwanath KM. Anti-oxidant and Nephroprotective Activities of *Cassia occidentalis* Leaf Extract against Gentamicin Induced Nephrotoxicity in Rats. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2012; 3(3):684-694.
30. Gwarzo US, Gimba CE, Adeyemo DJ, Paul ED. Neuron Activation Analysis (NAA) of *Senna occidentalis* Linn. Journal of Natural Sciences Research. 2014;(11):22-28.
31. Shafeen S, Srinath RT, Arafath S, Nagarjuna S, Padmanabha RY. Evaluation of antianxiety and antidepressant activity of *Cassia occidentalis* leaves. Asian Journal of Pharmaceutical and Clinical Research. 2012;5(3):47-50.
32. Emmanuel S, Rani MS, Sreekanth MR. Antidiabetic activity of *Cassia occidentalis* Linn in streptozocin-induced diabetic rats: A dose dependent study. International Journal of Pharmacology and Bioscience. 2010; 1(4):14-25.
33. Laxmi V, Singour PK, Chaurasiya PK, Rajak H, Pawar RS, Patil UK. Effect of ethanolic extract of *Cassia occidentalis* Linn. For the management of alloxan-induced diabetic rats. Pharmacognosy Research. 2010;2(3):132-137.
34. Onakpa MM, Ajagbonna OP. Antidiabetic Potentials of *Cassia occidentalis* leaf extract on Alloxan-induced Diabetic albino mice. International Journal of Pharm Tech Research. 2012;4(4): 1766-1769.
35. Yadav JP, Arya V, Yadav S, Panghal M, Kumar S, Dhankhar S. *Cassia occidentalis*: a review on its ethnobotany, phytochemical and pharmacological process. Fitoterapia. 2009;10: 1016.
36. Woods G, Washington JA. Antimicrobial susceptibility test; dilution and disk diffusion methods. Manual of Clinical Microbiology; 6th Ed. 1995;1327-1332.

37. Jayaraman S, Manoharan MS, Illanchezian S. *In-vitro* antimicrobial and antitumor activities of *Stevia rebaudiana* (Asteraceae) leaf extracts. Tropical J. Pharma. Res. 2008;7(4):1143-1149.
38. Clinical Laboratory Standards Institute (CLSI). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically: Approved Standard, 9th ed. 2012;32(2): 18-19.
39. Janaky R, Sivasankari K, Sekar T. Screening of Antimicrobial activities of an indigenous herb *Cassia occidentalis.* Applied Botany. 2011; 39:4584-4588.
40. Musa DD, Bashir KA, Hassan KY. Phytochemical Screening and Antibacterial Activity of Leaves Extract of *Senna Occidentalis* (L.). FUDMA Journal of Science. 2018;2(1):59-65.
41. Odeja O, Obi G, Ogwuche CE, Elemike EE, Oderinlo Y. Phytochemical Screening, Antioxidant and Antimicrobial activities of *Senna occidentalis* (L.) leaves Extract. Clinical Phytoscience. 2015;1-6.
42. Dharnananda SG. The uses of tannins in Chinese medicine. *In Proceedings of Institute for Traditional Medicine* Portland, Oregon. 2003.
43. Okwu DE, Okwu ME. Chemical composition of *Spondias mombin* Linn plant parts. Journal of Sustainable Agriculture and Environment. 2004;6(2):140-147.
44. Trease GE, Evans WC. Pharmacognosy. Thirteenth Edition. Balliere Tindall, London. 1989; pp. 882.
45. Sofowora AE. Medicinal plants and traditional medicines in Africa. *2nd Edition*. Spectrum Books Limited, Ibadan, Nigeria. 1993; pp.289.
46. Okwu DE. Phytochemicals, vitamins and mineral contents of two Nigerian medicinal plants. International Journal of Molecular Medicine and Advance Sciences. 2005;1(4): 375-381.
47. Ronan B, Ademir JSJ, Alaide BO. Plant-derived Antimalarial Agents: New Leads and Efficient Phytomedicine. Part II. Non- Alkaloid Natural Products – A Review. Molecules. 2009;14:3037-3072.
48. Sadique J, Chandra T, Thenmozhi V, Elango V. Biochemical modes of action of *Cassia occidentalis* and *Cardiospermum halicacabum* in inflammation. J Ethnopharmacol. 1987; 19(2): 201-212.
49. Kunle OF, Egharevba HO. Preliminary studies on *Vernonia ambigua*: Phytochemistry and Antimicrobial Screening of the Whole Plant. Ethnobot Leaf. 2009;13:1216-21.
50. Chukwujekwu JC, Coombes PH, Mulholland DA, van Staden J. Emodin, an antibacterial anthraquinone from the roots of *Cassia occidentalis*. South African Journal of Botany. 2006;72(2):295-297.
51. Sheeba M, Emmanuel S, Revathi K, Ignacimuthu S. Wound healing activity of *Cassia occidentalis* L. in Albino Wistar rats. IJIB. 2009;8(1):1-6.
52. Yeragamreddy PR, Peraman R, Chilamakuru NB, Routhu H. *In vitro* Antitubercular and Antibacterial activities of isolated constituents and column fractions from leaves of *Cassia occidentalis, Camellia sinensis* and *Ananas comosus*. Afr. J. Pharmacol. Ther. 2013; 2(4):116-123.
53. Chatterrjee TK. Medicinal Plants with Hepatoprotective Properties. Herbal Options. Books and Applied Allied (P) Ltd., Calcutta. 2000; 143.