Jewel Journal of Scientific Research (JJSR) 6(1&2): 98–105, 2021
©Federal University of Kashere-Nigeria (Print ISSN: 2384 – 6267, Online ISSN 2756-651X)
jjsr.fukashere.edu.ng

Phytochemical Screening and Antibacterial Effect of *Kigelia africana* L Stem Bark and Leaves Extracts on *Escherichia coli, Staphylococcus aureus* and *Proteus mirabilis.*

Babangida., M.I.[1], Bako., S.P.[2], Iortsuun, D.N.[2], Yahaya, H.S.[1] and Makeri, M. S.[1]
[1]Department of Biology, Faculty of Life Sciences,
[2]Ahmadu Bello University, Zaria-Nigeria
[*]**Corresponding author:** mbabangida72@gmail.com**,** 07069439381

Abstract
This study was carried out to determine the phytochemical composition and antibacterial effect of *Kigelia africana* on *Escherichia. coli, Staphylococcus. aureus* and *Proteus. mirabilis*. The plant materials of *Kigelia africana* stem bark and leaves were obtained from Yankari Game reserve, Bauchi state, Nigeria. One hundred 100g of the plant powder each, was extracted with methanol and chloroform by Soxhlet extraction method. All the extracts were subjected to standard phytochemical screening for the presence or absence of various secondary metabolites. The susceptibility test of the plant extracts on *Escherichia coli, Staphylococcus aureus* and *Proteus mirabilis* were done using agar well diffusion method. Ciprofloxacin 10 µg was used as positive control. The phytochemical screening of the extracts revealed the presence of alkaloids, flavonoids, tannins, saponins, cardiac glycoside, steroids and carbohydrates. The antibacterial activity showed that, the plant extract was effective on all the bacterial isolates, it also indicated that, the activities increased as the concentration of the extract increased. The highest activity of *K. africana* stem bark extracted with chloroform was observed on *P. mirabilis* with a mean zone of 17mm.

Keywords: Phytochemicals, Antibacterial effect, *Kigelia africana*,

Received: 26th May, 2021 *Accepted:* 19th Aug, 2021 *Published Online:* 12th Nov, 2021

Introduction

Plants are the most common source of antimicrobial agents. Their usage as traditional health remedies is the most popular for 80% of world population in Asia, Latin America and Africa and is reported to have minimal side effects (Mezouar *et al*., 2014). Drugs resistance in microorganism has become an unsolvable problem and reducing the efficiency of the existing drugs. This encourage researcher's on alternative drug discovery from different sources, among others plant-based drugs. Medicinal plants are valuable for getting novel drugs that form the ingredients in traditional systems of medicine, modern medicine, food supplements, bioactive principles and lead compounds in synthetic drugs (Nehad *et al*., 2012). There have been some extensive searches for potential natural antimicrobials with a broad spectrum of antimicrobial activities that can be used to extend the shelf life of perishable foods (Calo *et al*., 2015).
Kigelia is a genus of flowering plants within the family Bignoniaceae. The sausage tree is fast growing and can mature in 4 to 5 years.

It begins to flower at the age of 6 years. Mature fruits can be found on trees throughout the year (Jackson and Beckett 2012). Fruits grow up to 2 feet long and appear like sausage. Previous studies of the fruits of *K. africana* showed some antibacterial activity (Grace *et al*., 2002).

The understanding of the phytochemical constituents of medicinal plants like *Kigelia africana* is imperative not only because of the understanding of the scientific rationale for its usage but also for the discovery of novel compounds of pharmaceutical value (Fennell *et al*.,2004). Several phytochemical studies revealed that the extracts from many species of Bignoniaceae contained secondary metabolites such as saponins, tannins, flavonoids, quinones, alkaloids, anthralene derivatives, reducing sugars, glycosides, carbohydrates, querletin, kaempferol, α-sitosterol, terpenes, steroids, coumarins secondary metabolites and their derivatives (Gouda *et al*., 2006; Choudhury *et al*., 2011). A notable number of bioactive compounds have been recorded from the Bignoniaceae family of plants that reportedly demonstrates a number of important activities which are beneficial to human beings.

The various activities included anti-oxidant (Olaleye and Rocha, 2007), anti-plasmodial (Zofou *et al*., 2011), anti-diarrheal (Owolabi and Omogbai,2009), anti-inflammatory (Owolabi and Omogbai, 2007), antibacterial (Grace *et al*., 2002; anti-depressant/central nervous system (CNS), stimulant effects (Owolabi *et al*., 2008), anti-cancer Picerno *et al*., 2005; Bharti *et al*., 2006;), anti-diabetic, anti-snake venom and neurotrophic (Rahmatullah *et al*., 2010). This research aiming to screen for the phytochemicals and antibacterial effect of *Kigelia africana* leaves and stem bark extracts on *E. coli*, *S. aureus* and *P. mirabilis***.**

Materials and Methods

Study area.

Kigelia africana stem bark and leaves were collected from Yankari Game Reserve, Bauchi State, and the GPS coordinate of the collection area is 9.7529345N, 10.5114725E.

Authentication of plant materials

The stem bark and leaves of *Kigelia africana* were carefully washed with tap water and rinsed with distilled water. All the plants material was spread in a clean stainless-steel and air dried under shade at room temperature. Dried leaves and bark were crush and ground into powder using mortar and pestle. The powdered materials were kept in a nylon bag.

Preparation of the extracts

This was carried out according to the procedure describe by Sigaroodi *et al*. (2008) Ten gram (10g) each of the dried powdered plant materials were soaked in chloroform and methanol at room temperature for 36hours. The extraction was carried out with solvent under shaking conditions. The respective extracts were then filtered through Whatmann No.1 filter paper and methanol and chloroform extracts were obtained by Soxhlet extraction method. and solvents were removed completely under reduced pressure. Filtered extract was collected in conical flask. The chloroform and methanol extracts were evaporated by rotary evaporator at 45^{o}C and the crude extracts were obtained for the determination of antibacterial activity.

Phytochemical screening of *Kigelia africana.*

All the extracts were subjected to standard phytochemical qualitative screening for secondary metabolites as described by Sofowora (2006).

Test for carbohydrates

Molisch's test

Three drops of Molisch's Reagent was added to a 0.1g of each extract in a test tube and 1ml of concentrated Sulphuric acid was allowed to run down the side of the test tube to form a layer. Purple to violet at the interface indicates the presence of carbohydrates.

Fehling's test

Two (2ml) of each extract, 5ml of Fehling's solution A and B in the ratio of 1:1 was added and the mixture boiled for three minutes. A brick red precipitate indicates the presence of free reducing sugar.

Test for saponins (Frothing test)

Half (0.5g) of each extract was dissolved in 10ml of distilled water and then shaken

vigorously for 30 seconds and allow to stand for 30 minutes. A honey comb-like froth formed for more 30minutes indicates saponin.

Test for steroids

Two (2ml) acetic anhydride was added to 2ml of each extract in a test tube.1ml of concentrated sulphuric acid was added down to the side of the test tube. A colour change was observed immediately and later. Blue-green colour indicates steroids.

Test for Cardiac glycoside

Keller kiliani test

A portion of the extract was dissolved in 1ml of glacial acetic acid containing traces of ferric chloride solution. This was then transferred into a dry test tube and 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer at the bottom. Observe carefully at the interface for purple brown ring. This indicates the presence of desoxy sugars and a pale green color in the upper acetic acid layer indicates the presence of cardiac glycosides (Evans, 1996).

Test for Anthraquinones

Bontrager's test

To a portion of the extract in a dry test tube, 5ml of chloroform was added and was shaken for at least 5minutes. This was then filtered and the filtrate shaken with equal volume of 10% ammonia solution, bright pink color in the aqueous (upper) layer indicates the presence of free anthraquinones (Evans, 1996).

Test for flavonoids

Half (0.5g) of each extract was dissolved in 2ml of 50% methanol in the heat. Metallic magnesium and four drops of concentrated HCl were added. A red or orange colour indicates the presence of flavonoids.

Sodium hydroxide test

Five drops of aqueous NaOH was added to 5ml of each extract, a yellow colouration shows the presence of flavonoid.

Test for tannins

Lead sub-acetate test

Three drops of lead sub-acetate solution were added to 2ml of each extract. A colour precipitate indicates tannins.

Ferric chloride test

Half (0.5ml) of each extract was dissolved in 10ml of distilled water, and then filtered. Two drops of Ferric chloride solution were added to the filtrate. Formation of a blue-black precipitates indicates hydrolysable tannins and green precipitates indicates the presence of condensed tannin.

Test for alkaloids

Mayers Test

Two drops of the mayers Reagent were added to 2ml of the extract in a test tube, cream precipitates indicate alkaloids.

Dragendorffs Test

Two drops of the Dragendorffs Reagents were added to 2ml of each extract. A rose red precipitate indicates the presence of alkaloids.

Wagner's Test

Two drops of the Wagner's Reagents were added to2ml of each extract. A reddish-brown precipitate indicates the presence of alkaloids.

Preparation of different concentrations of the extract

Ten 10mg each of the plant (stem bark and leaf) extracts were dissolved in 10ml of distilled water to form two stock solutions of each extract. From the first stock solutions of each extracts, serial dilutions were carried out to prepare 4 different concentrations as,100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml respectively.

Standardization of inoculum

The bacterial isolates of *Escherichia coli*, *Staphylococcus aureus* and *Proteus mirabilis* were collected from microbiology laboratory, Department of microbiology, Ahmadu Bello University, Zaria. An overnight culture of the bacterial isolates was prepared in nutrient broth0.1 ml of the nutrient broth was emulsified into 20ml of physiological saline, until the turbidity of the suspension of test organism matches with 0.5 Mcfarland turbidity standards (Deeni and Hussain, 1994).

Determination of inhibitory activity (susceptibility test) of the extract using agar well-diffusion:

The standardized inucula of the bacterial isolate were streaked on sterilized Mueller hinton agar plates respectively with the aid of

sterile wire loop. Four wells were bored on each inoculated agar plate with a sterile cork borer. The well was properly labeled according to different concentrations of the extract prepared which were 100, 50, 25 and 12.5mg/ml respectively. Each well was filled up with approximately 0.2ml of the extract.

The inoculated plates with the extract were allowed to stay on the bench for about one hour; this is to enable the extract to diffuse on the agar. The plates were then incubated at 37ºC for 24 hours. At the end of incubation period, the plates were observed for any evidence of inhibition which will appear as a clear zone that was completely devoid of growth around the wells (zone of inhibition). The diameter of zones was measured using transparent ruler calibrated in millimeter and the results were recorded.

Statistical analysis

Data obtained were subjected to one-way analysis of variance (ANOVA) using SPSS version 20.0 to determine the mean zones of inhibition of the plant extract on *E. coli, S. aureus* and *P. mirabilis*.

Results

A total of three bacterial isolates have been used in the present study to assess the antibacterial effect of methanolic and chloroform extract of *Kigelia africana*. This result showed that, *Kigelia Africana.* Table 1 contained secondary metabolites such as flavonoids, carbohydrates, cardiac glycoside and steroids. This is in line with the report of (Choudhury *et al*., 2011).

The antibacterial activity of the extract was determined by agar well diffusion method. Table 2 shows the antibacterial activity of *Kigelia africana* extracts against *E. coli, S. aureus* and *P. mirabilis*. The extract was effective against all three bacterial isolates with the activities increasing as the concentrations of the extract increased. Highest activity was observed on *P. mirabilis* with mean zone of 17mm.

Table 1: Phytochemical screening of *Kigelia africana* stem bark and leaves extracts.

S/N	Phytochemicals	SME	SCE	LME	LCE
1	Alkaloids	+	+	+	+
2	Flavonoids	+	-	+	-
3	Tannins	+	-	+	-
4	Saponins	+	-	+	-
5	Cardiac glycoside	+	-	+	-
6	Streriods	+	+	+	-
7	Anthraquinon	-	-	-	-
8	Carbohydrates	+	+	+	+

Key: SME=Stem bark methanol extract, SCE=Stem bark chloroform extract, LME=Leaf methanol extract, LCE=Leaf chloroform extract, +=Present, - =Not present.

Table 2: Antibacterial activity of *Kigelia africana* stem bark and leaves extracts

Bacteria		Extracts			
S. aureus	Concentration	SME	SCE	LME	LCE
	DMSO (-)	0.00±0.00^{e}	0.00±0.00	0.00±0.00^{d}	0.00±0.00^{d}
	C (+)	21.00±1.16^{a}	21.00±1.16^{a}	21.00±1.16^{a}	21.00±1.16^{a}
	100	14.00±0.48^{b}	10.00±0.58^{b}	8.00±1.16^{b}	8.00±1.15^{b}
	50	12.00±0.58^{b}	8.00±1.15^{b}	4.00±1.15^{c}	6.00±1.73b^{c}
	25	7.00±1.15^{c}	6.00±0.58^{c}	0.00±0.00^{d}	4.00±0.58^{c}
	12.5	3.00±0.58^{d}	1.33±0.67^{d}	0.00±0.00^{d}	0.00±0.00^{d}
	P-value	0.000	0.000	0.000	0.000
E. coli					
	Concentration	SME	SCE	LME	LCE
	DMSO (-)	0.00±0.00^{d}	0.00±0.00^{e}	0.00±0.00^{d}	0.00±0.00^{d}
	C (+)	15.00±1.15^{a}	15.00±1.15^{a}	15.00±1.15^{a}	15.00±1.15^{a}
	100	12.00±1.16^{b}	11.00±1.15^{b}	10.00±0.58^{b}	7.00±0.58^{b}
	50	8.00±0.58^{c}	6.00±0.58^{c}	6.00±1.15^{c}	5.00±0.58^{c}
	25	6.00±0.57^{c}	3.00±1.16^{d}	4.00±0.58^{c}	0.00±0.00^{d}
	12.5	2.00±0.58^{d}	0.00±0.00^{e}	0.00±0.00^{d}	0.00±0.00^{d}
	P-value	0.000	0.000	0.000	0.000
P. mirabilis					
	Concentration	SME	SCE	LME	LCE
	DMSO (-)	0.00±0.00^{c}	0.00±0.00^{f}	0.00±0.00^{e}	0.00±0.00^{d}
	C (+)	18.00±1.16^{a}	18.00±1.16^{a}	18.00±1.16^{a}	18.00±1.16^{a}
	100	16.00±1.73^{a}	17.00±1.16^{b}	15.00±0.58^{b}	8.00±0.57^{b}
	50	7.00±0.58^{b}	13.00±0.58^{c}	8.00±0.58^{c}	6.00±0.58^{c}
	25	4.33±0.88^{b}	9.00±0.57^{d}	3.00±0.58^{d}	5.00±0.29^{c}
	12.5	0.00±0.00^{c}	6.00±0.58^{e}	0.00±0.00^{e}	0.00±0.00^{d}
	P-value	0.000	0.000	0.000	0.000

Mean values with different superscripts in the same column are significantly different.

SME=Stem bark methanol extract, SCE=Stem bark chloroform extract, LME=Leaf methanol extract, LCE=Leaf chloroform extract, DMSO=Negative control, C=Ciprofloxacin positive control

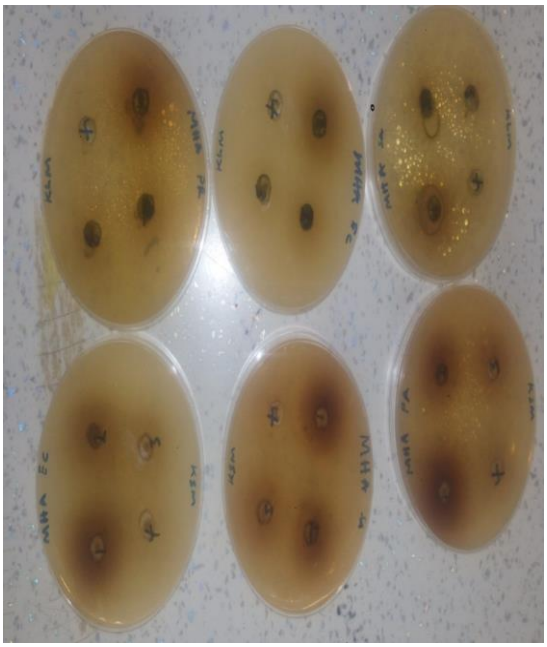

Plate1. Antibacterial activity of *Kigelia africana*.

Plate 2. *Kigelia africana* plant

Discussion

Kigelia africana contained most of the secondary metabolites such as, alkaloids, flavonoids, saponin, steroids and cardiac glycosides (Table1) And these agrees with the findings ofsofowora,1993 who reported that these classes of secondary metabolites are known to show medicinal activity as well as exhibiting physiological activity. It was also in line with the work of Njoku and obi, 2009 who reported that these classes of phytochemical compounds are known to show curative activity against several bacteria related ill-health. The result of phytoconstituents of *K. africana* (Table1) was also similar to what have been reported by (Subramanya *et al.*, 2012). Results of antibacterial activity of *K.africana* (Table2) showed that, *K. africana* extracts have antibacterial activities and the effect of the extract concentrations differ with antibacterial activity being reduced as the concentration of the extract decreased. This result was in line with Robert *et al*., (2003), who reported that the inactivation of a susceptible bacterial population is dependent on the relative concentration of the two reactants, the bacteria and the chemical. The antibacterial activity of *K. africana* (Table2) stem methanol extract has the highest activity on *S. aureus* at 100mg/ml concentration, with mean zone of 14mm, while the least activity occurred in SCE at 12.5mg/ml with mean zone of 1.33mm, but showed no activity on LME and LCE at 12.5mg/ml. Stem methanol extract (SME) also have the highest activity on *E. coli* at 100mg/ml with mean zone of 16mm, while on *P. mirabilis*, SCE has the highest activity at 100mg/ml with mean zone of 17mm. The result also showed that, *K. africana* stem bark methanol extract presents higher activity than chloroform extract. This result agrees with Silver *et al*. (2003), who reported that, methanol solvent was found as the best extract solvent to be used compared to chloroform, this was due to methanol as universal solvent, thus was able to bound various compound or secondary metabolites. The result of this (Table2) agrees with the findings of Rasool and Varalakshmi, (2006) who reported that, the effectiveness of an extract depends on the solvent used.

Conclusion

Kigelia africana contained significant phytochemicals (secondary metabolites). *Kigelia africana* extract revealed antibacterial activity against the bacterial isolates, *E. coli*, *S. aureus* and *P. mirabilis*. The activity of the extracts on the test organisms increased with increase in

concentration. However, based on the findings of this research, *Kigelia africana* showed significant anti-bacterial effect.

Acknowledgement

All praises be to Almighty Allah, the beneficent the most merciful for the gift of life and protection during this research work. My profound and utmost gratitude goes to my team of supervisors Prof. D.N. Iortsuun H.O.D Botany department and Prof S.P Bako for their guidance and diligent supervision during this research work.

References

Bharti, N., Singh, S., Fermida, N., Amir, A. (2006). Isolation and *in vitro* antiamoebic activity of iridoids isolated from *Kigelia pinnata*, Arkivoc. 69-76.

Calo, J. R., Crandall, P. G., O'Bryan, C. A. and Ricke, S. C. (2015). Essential Oils as antimicrobials in food systems. *A review. Food control*. 54: 111-119.

Choudhury, S., Datta, S., Talukdar, A.D. and Chaudury, M. D. (2011). Phytochemistry of the family Bignoniaceae- A review. *Assam University Journal of Sci. Tech.:Bio. Environ. Sci*, 7(1): 145-150.

Deeni, Y. Y. and Husain, H.S.N. (1994). Antibiotic sensitivity testing. *Journal of Ethnomedicine* 35: 91-96

Fennel, C.W., Lindsey, K.L., McGaw, L.J., Sparg, S.G., Stafford, G.I., Elgorashi, E.E., Grace, O.M., Van staden, J. (2004). Assessing African medicinal plants for efficacy and safety: pharmacological screening and toxicology. *J. Ethnopharmacol*. 94:205-217

Gouda, Y.G., Abdel-baky, AM., Mohammed, K.M., Darwish, F.M., Kasai, R., Yamasaki, K. (2006). Phenyl propanoid and phenyl ethanoid derivatives from *Kigelia pinnata* DC. Fruits, *Nat Prod Res*. 20(10): 935-939.

Grace, O.M., Light, M.E., Lindsey, K.I., Mulholland, D.A., Van Staden, J. and Jager, A.K. (2002). Antibacterial activity and isolation of active compounds from fruit of the traditional African medicinal tree *Kigelia africana*, *South African Journal of Botany*, 68(1): 220-222.

Jackson, S., and Beckett, K. (2012). Sausage Tree *Kigelia pinnta*: An Ethnobotanical and Scientific Review, Herbal Gram; *American Botanical Council*, 94:48-59.

Khan, S.S., and Malhotra, D. (2002). Plantagentic diversity exploration, evaluation, conservation; Angiospermic diversity in Bhopal-reassessment with particular reference to endangered species and their conservation Vol.232, East:west press pvt Ltd; p.234.

Mathad, P., and Shrishail, C (2008). Floristic diversity of a famous historical barid-shahi fort of boidarin Karnataka. *Indian Journal*, 31:577-80.

Mezouar, D., Lahfa, F.B., Abdelouahid, D.E., Adida, H., Rahmoun, N.M., Boucherit. and Otmani, Z. (2014). Antimicrobial activity of Berberis vulgaris. *Phytotherapie*, 12(6):380-5.

Nehad, M., Gumgumjee, Khedr and Hajara, A.S. (2012). Antimicrobial activities and chemical properties of *Tamarindus indica* L. leaves extract. *African Journal of Microbiology, Res*, 6(32): 6172-6181.

Njoku, O.V. and Obi, C. (2009). Phytochemical constituents of some selected medicinal plants. *African journal of Pure and Applied chemistry*. 3(11): 228-233.

Olaleye, M.T. and Rocha, J.B. (2007). Commonly used medicinal plants exhibit distinct *in-vitro* antioxidant activities against hepatotoxins in rat liver. *Expl Toxicol Path*. 58(6): 433-8.

Owolabi, O.J., Amaechina, F.C., Eledan, A.B. (2008). Central nervous system stimulant effect of the ethanolic extract of *Kigelia africana, Journal of Medicinal Plants Res*. 2(2): 20-23.

Picerno, P., Autore, G, Marzocco, S., Meloni, M., Sanogo, R., Aquino, R.P. (2005). Antiinflammatory activity of verminoside from *Kigelia africana* and evaluation of cutaneous irritation in

cell cultures and reconstituted human epidermis. *Journal of Nat Prod*. 68: 1610-16.

Rahmatullah, M., Samarrai, W., Jahan, R., Rahman, S., Sharmi, N., Emdad Ullah, Miajee ZUM., Chowdhury, M.H., Bari, S., Jamal, F., Anwarul Bashar, A.B.M., Azad, A.K., Ahsan, S. (2010). An Ethno-medicinal, pharmacological and phytochemical review of some Bignoniaceae plants in folk medicinal uses in Bangladesh. *Adv. Nat. Appl. Sci*. 4(3): 236-253.

Rasool, M., and Varalkshmi, P. (2006). Suppressive effect of *Withanian sonifera* on

experimental goutyarthritis: An *Invivo* and *invitro* study. *Chem. Biol. Interaction*, 164: 174-180.

Roberts, C.W., Mcleod, R., Rice, D.W., Ginger, M., Chance, M.L., Goad, L.J. (2003). Fatty acid and sterol metabolism: Potential antimicrobial targets in apicomplexan and trypanosomatid parasitic protozoa. *Molecular Biochemical Parasitology*. 126(2): 129-142.

Sigaroodi, F., A. Hadjiakhoondi, M. Ahvazi, M. Taghizadeh, Yazdani D. and Sigaroodi, S. (2008). Cytotoxity evaluation of two species from *Caesalpinia* genus. *Journal of Medicinal Plants*, 7: 60-70

Silver, H.T., Marcos, J.S., Evandro, W., Izabel, Y.I., Dionela, C.R.O. (2003). Antimicrobial activity of flavonoids and steroids isolated from two *Chromolaena* species. *Brazilian Journal of Pharmaceutical Sciences.* 39(4): 403-408.

Subramanya, J.K., Muttagi, S. (2012). Invitro colour change of three dental veneering resins in tea, coffee and tamarind extracts. *Journal of Dentistry Tehran University of Medical Sciences*. 8(3): 138-145.

Sofowora, A. (1993). Medicinal plants and traditional medicine in Africa. Spectrum books limited, Ibadan, Nigeria. 150pp

Sofowora, A. (2006). *Medicinal plants and Traditional medicine in Africa* (2nde.d). Spectrum books ltd, Ibadan. Nigeria, Pp. 150-153.

Zofou, D., Kengne, A.B., Tene, M., Ngemenya, M.N., Tane, P., Titanji, V.P. (2011). *Invitro* anti plasmodial activity and cytotoxicity of crude extracts and compounds from the stem bark of *Kigelia africana* (Lam.) Benth (*Bignoniaceae*). *Parasitol Res.* 108(6):1383-90.