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In vitro Antibacterial Activity and Phytochemical Screening of Crude Extracts from *Jatropha curcas* Linn.

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Research Article

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ABSTRACT

Aims: This study aims at investigating the antibacterial activity of crude methanolic and aqueous extracts of leaves and root barks of *Jatropha curcas* against Gram-positive and Gram-negative bacteria isolated from urinary tract infections (UTIs) and to confirm the effective use of this plant against the uropathogenic strains in traditional medicine in Democratic Republic of Congo (D.R.C).
Study Design: Laboratory experimental tests; Extraction of *J. curcas* leaf and root bark, susceptibility tests (zones of inhibition) and minimum inhibitory concentration (MIC) determination and phytochemical screening and high performance liquid chromatography (HPLC) analysis.
Place and Duration of the Study: Department of Phytobiology, Department of Biotechnology and Department of Microbiology, General Atomic Energy Commission. Regional Center of Nuclear Studies of Kinshasa P.O BOX 868 Kin. XI DRC during October and November 2011.
Methodology: Fresh leaves and root barks of *J. curcas* were collected, oven- dried at 45ºC, powdered and extracted with water and methanol. The aqueous extracts were lyophilized. Agar disc diffusion method was used to test antibacterial activity of the crude extracts of *J. curcas* against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and

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Citrobacter diversus isolated from UTIs. The extracts were subjected to phytochemical tests. HPLC method was used to screen phenolic compounds.
Results: The crude extracts exhibited a significant antibacterial activity against four of seven tested bacterial isolates. MIC values ranged from 1.0 to 7.5 mg/L. The extracts phytochemical screening revealed the presence of saponins, tannins, alkaloids, steroids and flavonoids. The presence of phenolic compounds was screened by HPLC analysis.
Conclusion: The inhibitory effects of the crude extracts from leaves and root barks against uropathogenic strains have justified the usefulness of *J. curcas* for the treatment of UTIs and sexually transmitted Infections (STIs) in traditional medicine of D.R.C.

Keywords: Jatropha curcas; antibacterial activity; urinary tract infections; phytochemical screening; HPLC; traditional medicine; Democratic Republic of Congo (D.R.C.).

1. INTRODUCTION

The interest in herbal medicine is steadily increasing in Democratic Republic of Congo (D.R.C.) as other developing countries. People in the rural regions of D.R.C. prefer herbal and inexpensive therapy. Therefore, primary health care depends on medicinal plants. Some Congolese traditional "healers" use *J. curcas* to cure urinary tract infections (UTIs) and sexual transmitted infections (STIs). It is claimed to be able to treat several diseases. However, there is no sufficient scientific validation of these claims.

J. curcas L. (Euphorbiaceae) or physic nut is a large drought-resistant shrub with used for several purposes. This tropical plant has received extensive attention of many scientists in view of its great economic importance, medicinal significance and for its seed oil as commercial source of fuel (Grimm, 1996; Heller, 1996; Henning, 2000). *J. curcas* is widely used in traditional medicine in Africa, Asia and Latino America to cure various ailments such as skin infections, diarrhea, gonorrhea, fever and several other diseases caused by microorganisms (Burkill, 1994; Kambu, 1999).

Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in developing countries (WHO, 2002). Previous studies have reported that *J. curcas* exhibits antimicrobial activity (Aiyela-agbe et al., 2007; Akinpelu et al., 2009; Igbnosa et al., 2009; Namuli et al., 2011; Ekundayo et al., 2011). The search for new antibacterial drugs of natural origin is urgently needed in the light of growing cases of microbial resistance to the available synthetic antibiotics (Iwu et al., 1999; Wurochekker et al., 2008; Krishnaiah et al., 2009).

The aim of the present paper is to screen the *in vitro* antibacterial properties of crude extracts of *J. curcas* and to establish the effective use of this plant by Congolese traditional "healers" against diseases caused by the uropathogenic strains.

2. MATERIALS AND METHODS

2.1 Plant Materials and Preparation of Extract

Fresh leaves and root barks were collected from the experimental garden of Regional Center for Nuclear Studies of Kinshasa, D.R.C. The plant parts were dried to a constant weight in hot-air oven at 45ºC powdered and stored in a container for further use.

Ten grams of each prepared sample were submitted to cold extraction in methanol. Another 10 gr each of plant material was extracted by decoction. Extracts were recovered by filtration using Whatman filter paper. Excess of methanol was evaporated in hot-air oven at 45ºC and the residues stored for other uses. The aqueous extracts were lyophilized using FREEZE DRYING 7670530 LABCONCO to obtain dry powder extracts. The extraction yields collected were respectively 10.8% and 20.5% for leaves and root barks.

2.2 Bacterial Strains and *In Vitro* Culture

The bacterial strains used in this investigation were obtained from Microbiology Department, University Hospital of Mont-Amba, Kinshasa,D.R.C., i.e. the Gram-positive *Staphylococcus aureus*, *Staphylococcus epidermidis, Bacillus subtilis* and the Gram-negative *Escherichia coli*, *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Citrobacter diversus* isolated from UTIs.

Cultures were routinely maintained in nutrient agar slants at 4ºC. For the experiment the bacteria were sub-cultured in nutrient broth and incubated at 37ºC for at least 24 hours.

2.3 Sensitivity of Bacterial Species to *J. Curcas* Extracts

Filter paper discs (Whatman, 6mm diameter) were soaked with 20 µl of each extract at a final concentration of 25 mg/ml and dried at room temperature (27ºC). The discs of 500 µg of crude extracts were then placed on soft nutrient agar Petri plates which were previously seeded with 100 µl suspension of each of seven bacterial species. After incubation at 37ºC for 24 hours, zones of inhibition were measured. Antibacterial activity was expressed in terms of diameter of the zone of inhibition. The effects were compared with those of kanamycin (K30) and ampicilin (AM10) standard antibiotics. All the experiment was carried out in three replicates.

2.4 Determination of Minimal Inhibition Concentration (MIC) of Extract

The MIC of the crude extracts was estimated for four sensitive bacterial species. Dilutions of the crude extract was prepared and 1 ml aliquot of different concentrations of the solution was added to 9 ml of pre-sterilized molten nutrient agar at 40ºC to give final concentration regimes of 0.1 and 10 mg/ml. The medium was then poured into sterile Petri dishes.

The surface of the medium was allowed to dry before streaking with 24 hours old bacterial. The plates were later incubated at 37ºC for 24 hours and they were examined for the presence or absence of growth. The lowest concentration of the sample required to inhibit the growth of the test organism was recorded for each organism as the MIC.

2.5 Phytochemical Analysis of the Plant Extract

The extracts were subjected to phytochemical tests for plant secondary metabolites alkaloids, saponins, steroids, tannins and flavonoids using methods of Harborne (1998) and Evans (2000).

2.6 High Performance Liquid Chromatography Analysis (HPLC)

Hydrolysis of samples was carried out in Soda test tubes (75x12x0, 8-1.00 mm) (Germany). 0.5 ml of 2 N HCl was added in 0.5 ml of each sample. All Soda test tubes were closed by a Pasteur pipette, tubes and Pasteur pipettes were attached with parafilm at the neck. The extract was heated at 80ºC for 20 minutes and 0.5 ml of distilled water was added to each tube after removing the Pasteur pipette, followed by addition of 0.5ml, resulting in the immediate appearance of two phases: upper organic and lower aqueous layer. The separation of two phases with a minipipette came after vortexing for several seconds (Hoist et al., 2001).

The hydrolyzed and pure extracts were used for HPLC. To do this, 18 µl of each samples (pure or hydrolyzed extracts) was placed in Waters 2996 HPLC tubes which was then placed in the rack by placing HPLC 18 µl of the control. The racks containing the samples were placed in the HPLC, which was then switched on after programming. The machine used for HPLC Waters 2695 Alliance was meeting the conditions set out in Table 1.

Table 1. Gradient elution HPLC of phenolic compounds of *J. curcas*

Time (minutes)	Acetonitrile%	(H_2O+1%HCL)
0	22	78
7	24	76
17	40	60
25	100	0
30	100	0
35	22	78
40	22	78

The flow rate was 1 ml/min in the Alltech Altima C18 column (5 µm) 250 x 4.6 mm. The detection was made by a diode array detector (Waters 2996) and the chromatogram was observed at 275 nm.

3. RESULTS AND DISCUSSION

3.1 Antibacterial Activity

The effects of the crude extracts of *J. curcas* on bacterial isolates were compared favourably with those of two standard antibiotics (kanamycin and ampicillin). Extracts from leaves and root barks of *J. curcas* were active against the strain of *E. coli* but this bacterial isolate was resistant to synthetic antibiotic Kanamycin. On the other hand, *S. aureus* and *B. subtilis* were sensitive to the crude extracts of *J. curcas* but ampicillin couldn't inhibit their growth. Unfortunately, this is due to the fact that microorganisms gain resistance to some synthetic antibiotics with long term use while the lower resistance was found in medicinal plants (Acar

et al., 2010). The crude extracts of the plant tested showed varying degree of antibacterial activities against the test bacterial. The crude methanol extracts of leaves and root barks were found to be more effective against both Gram-positive and Gram-negative bacteria than the aqueous extracts which showed low antibacterial activity against all organisms tested (Table 2). The inhibitory activity of plant extract is largely dependent on the concentration, parts of the plant used and the microbes tested (Kalimuthu et al., 2010).

The methanol extract had the highest activity against bacterial isolates; this may be attributed to the presence of soluble phenolic and polyphenolic compounds. Phenols have been found to be useful in the preparation of some antimicrobial compounds such as dettol and cresol (Kowalski and Kedzia, 2007). Methanol has a high polarity index (Cowan, 1999) than the water and thus is able to extract more phenolic and flavonoid compounds**.** Namuli et al. (2011) have founded that the root bark methanolic extract of *J. curcas* containing high phenolics (11.51 mg gallic acid equivalents/g Dry weight) and flavonoids (0.94 mg rutin equivalents/g Dry weight). These phytocompounds have been reported to be effective antimicrobial agents (Cowan, 1999; Vaquero et al., 2007).

In this study, the low antibacterial activity of the aqueous extract is in agreement with previous works which show that aqueous extracts of plant generally showed little or no antibacterial activities (Koduru et al., 2006; Ashafa et al., 2008). This explains why the amount of aqueous extracts of drug is high in the treatment of different forms of infections and diseases by the Congolese traditional healers.

The MIC of the methanol extract of leaves for different organisms ranged between 5.0-7.5 mg/ml and while that of methanol extract of stem bark ranged between 1.0-5.0 mg/ml (Table 3). The MIC was not determined for water extract since the antibacterial activity was low.

The results obtained had corroborated those reports for *J. curcas* leaves, stem bark, root bark, root wood and kernel meal extracts. Indeed, these extracts elicited good antimicrobial activity against several test organisms (Akinpelu et al., 2009; Igbinosa et al., 2009; Namuli et al., 2011; Ekundayo et al., 2011). Ayelaagbe et al., (2007) reported that the presence of some secondary metabolites in the root extract of *J. curcas* inhibited some microorganisms isolated with STIs which are in agreement with this present study.

3.2 Phytochemical Screening

The qualitative estimation of the phytocompounds conducted on *J. curcas* extracts revealed the presence of alkaloids, saponins, flavonoids, tannins and steroids (Table 4). Using qualitative analysis, Igbinosa et al. (2009) and Akinpelu et al. (2009) observed the presence of the same compounds in *J. curcas* stem bark and leaves extracts respectively. These phytocompounds are known to support bioactive activities in medicinal plant and therefore aid the antimicrobial activity of *J. curcas* (Motar et al., 1985). These compounds have been associated with medicinal uses for centuries and were reported as the most efficient, therapeutically significant plant substance (Nobori et al., 1994; Njoku and Akumefula, 2007). The active principles of many drugs found in plant are secondary metabolites. Fortunately, these compounds exert antibacterial activity through different mechanisms (Rabe, 2000; Shimada, 2006).

Table 2. Antibacterial activities profiles of crude extracts from *J. curcas*

Bacterial isolates	Zones of inhibition (mm) *(Mean± SD)					
	Methanol		Water		Kanamycin (30µg /disc)	Ampicillin (10µ /disc)
	Leaves (500µg/disc)	Root barks (500µg/disc)	Leaves (500µg/disc)	Root barks (500µg/disc)		
Staphylococcus aureus	15 ±0.5	20±0.1	9±1.5	10±1.0	10±0.4	0±0
Staphylococcus epidermidis	0±0	0±0	0±0	0±0	Nd	Nd
Bacillus subtilis	13±1.1	17±1.5	0±0	8±1.5	18±1.2	0±0
Escherichia coli	14±0.2	16±0.8	9±0.8	10±0.3	0±0	13±1.2
Citrobacter diversus	7±0.5	8±1.4	0±0	0±0	Nd	Nd
Klebsiella pneumoniae	13±0.1	14±0.5	8±2.0	9±0.5	17±1.1	12±0.5
Pseudomonas aeruginosa	8±1.5	10±0.4	0±0	0±0	Nd	Nd

**: Values are means of 3 replicates; Nd: not determined.*

Table 3. MIC of crude extracts from *J. curcas*

Test bacteria	M.I.C. (mg/ml)	
	Methanol	
	Leaves	Root barks
Staphylococcus aureus	5. 0	1. 0
Bacillus subtilis	7.5	2.5
Escherichia coli	5. 0	5. 0
Klebsiella pneumoniae	5. 0	5. 0

Table 4. Phytochemical compounds present in the crude extracts of *J. curcas*

Phytocompounds	*J. curcas*	
	Leaves	Root barks
Saponins	+	+
Tannins	+	+
Alkaloids	+	+
Steroids	+	+
Flavonoids	+	+

+ indicates Present

3.3 High Performance Liquid Chromatography (HPLC)

The examination of HPLC chromatograms revealed the presence of several compounds. The results in Fig. 1 indicated the qualitative composition of phenols in the unhydrolyzed extracts of *J. curcas* leaves. Two major products of *J. curcas* with relative areas of 9.1 and 8.7 were eluted at 3.01 minutes and 4.95 minutes respectively. The spectrum showed two maxima at 222.6 nm and 335.5 nm for the first product and a maximum at 272.3 nm for the second product.

The results of the hydrolyzed extracts of *J. curcas* (Fig. 2) showed two major products. The first one with a relative area of peak of 1.6 for which the retention time is 7.88 minutes with a spectrum having three maxima at 237.9 nm, 271.1 nm and 329.5 nm. The second product has a retention time of 5.89 minutes for which the relative area was 1.3. The spectrum of this product showed two maxima at 279.4 nm and 312.8 nm.

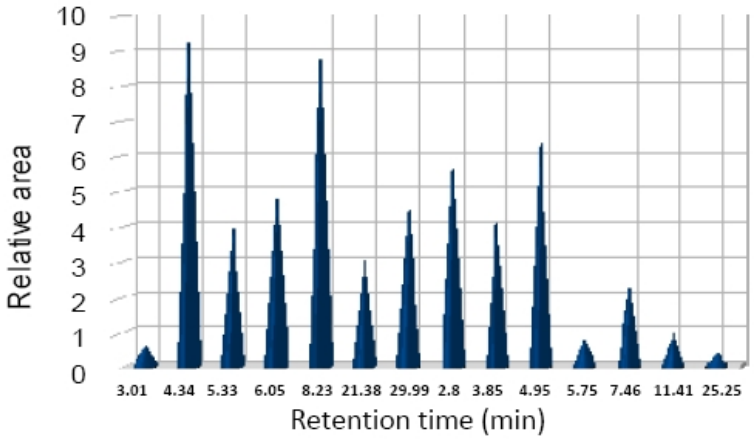

Fig. 1. Relative peak areas of unhydrolyzed extracts of *J. curcus* leaves by HPLC

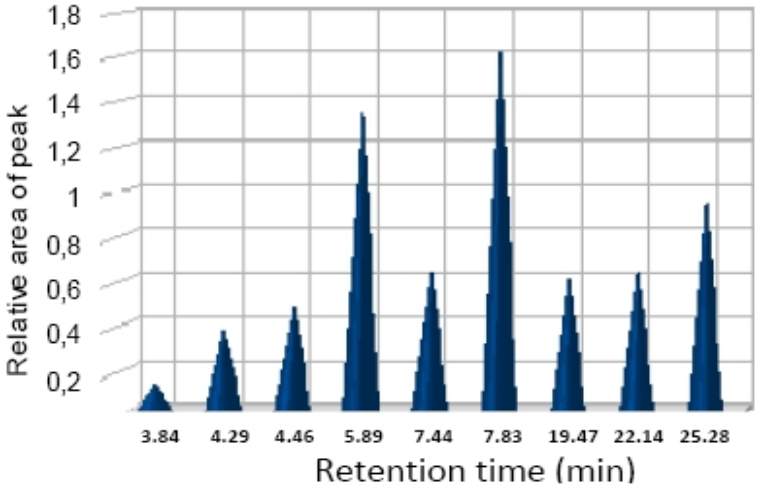

Fig. 2. Relative peak areas of hydrolyzed extracts of *J. curcus* leaves by HPLC

Using RP-HPLC analysis, Namuli et al. (2011) reported the presence of pyrogallol, gallic acid, vanillic acid, rutin and nargingin in different extracts of various plant parts of *J. curcas.* Phenolic compounds were detected at 280 nm while isoflavonoids and flavonoids were detected at 350 nm. The extracts of *J. curcas* leaves would be contain phenolic compounds which are eluted at **5.89** minutes with a relative area of **1.3** (max_1: 279.4 nm; max_2:312.8 nm).

The differences in the change of absorption spectra of many compounds would depend on the chemical change: hydroxylation, methylation, glycosylation and condensation with other phenolic molecules or not (Macheix et al., 2005).

4. CONCLUSION

The investigations carried out *in vitro* on the crude extracts of *J. curcas* leaves and root barks showed a broad spectrum action towards Gram-positive and Gram-negative microorganisms with the MIC values ranging from 1.0 to 7.5 mg/L.

The ability of the crude extracts to inhibit the growth of tested bacterial isolates has confirmed the usefulness of *J. curcas* for the treatment of UTIs and STIs in traditional medicine of D.R.C.

The phytochemical screening of leaves and root barks extracts of *J. curcas* revealed the presence of saponins, alkaloids, steroids; tannins and flavonoids. HPLC method has been found to be an excellent tool for screening of phenolic compounds in *J. curcas*. The amount of the main phytocompounds observed may vary according to the extraction method and the solvent used. Their presence corroborates the previous observed antibacterial activities. To be complete, the quantitative analysis of detected phytocompounds should be carried out in view to characterize efficiently this medicinal plant.

Thus, the inhibitory effect of *J. curcas* against uropathogenic strains can introduce this plant as a potential candidate in bioprospecting for antibiotic drugs.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Acar, G., Dogan, N.M., Duru, M.E., Kivrak, I. (2010). Phenolic profiles, antimicrobial and antioxidant activity of the various extracts of *Crocus* species in Anatolia. Afr. J. Microbiology Res., 4, 1154-1161.

Aiyelaagbe, O.O., Adeniyi, B.A., Fatunsin, O.F., Arimah, B.D. (2007). *In vitro* antimicrobial activity and phytochemical analysis of *J. curcas* roots Intern. J. Pharmacol., 3, 106-110.

Akinpelu, D.A., Olayinka, A., Athony, I.O. (2009). The bioactive potentials of two medicinal plants commonly used as folklore remedies among some tribes in West Africa. Afr. J. Biotechnol., 8, 1660-1664.

Ashafa, A.O.T., Grierson, D.S., Afolayan, A.J. (2006). Antimicrobial activity of extract from *Felicia muricata* Thunb J. Biol. Sci., 8, 1062-1066.

Burkill, H.M. (1994). The useful plants of west tropical Africa. (Families EJ). Royal Botanical Gardens Kew, 90-94.

Cowan, M.M. (1999). Plant products as antimicrobial agents. Clin. Microbiol. Rev., 12, 564-582.

Ekundayo, F.O., Adeboye, C.A., Ekundayo, E.A. (2011). Antimicrobial activities and phytochemical screening of pignut (*J. curcas* Linn.). J. Med. Plants Res., 5, 1261-1264.

Evans, W.C. (2000). Trease and Evans Pharmacognosy.14th Edt, WB Saunders Company Ltd, 224-293.

Grimm, C. (1996). The *Jatropha* project in Nicaragua. Bagani TULU (Mali), 1, 10-14.

Harborne, J.B. (1998). Phytochemical Methods: A guide to Modern Techniques of plants Analysis. Chapman and Hall, London, 182-190.

Heller, J. (1996). Physic nut. *J. curcas* L. In: International Plant Genetic Ressources.

Henning, R. (2000). The *Jatropha* booklet. A guide to the *Jatropha* System and its dissemination in Zambia. GTZ-ASIP support Project southern Province. Bagani GBR.

Hoist Von, C., Hammer, S., Anklam, E. (2001). Factorial design approach for optimization of high performance liquid chromatography conditions of tropane alkaloids determination. Deutsche Lebensmittel-Rund Schan, 97, 1-7.

Igbinosa, O.O., Igbinosa, E.O., Aiyegoro, O.A. (2009). Antimicrobial activity and phytochemical screening of stem bark extracts from *J. curcas* (Linn). Afr.J. Pharmacy, Pharmacology, 3, 58-62.

Iwu, M.W., Duncan, D.R., Okonji, C.O. (1999). New antimicrobials of plant origin in: Perspectiv on new crops and news uses. J. Janick (Ed.) Ashs Press, Alexandria, 107-108.

Kalimuthu, K., Vijayakumar, S., Senthilkumar, R. (2010). Antimicrobial activity of the biodiesel plant, *Jatropha curcas*. Intern. J. Pharm. Bio. Sci., 1, 1-5.

Kambu, K. (1990). Elements de phytothérapie comparée. Plantes Médicinales Africaines Centres de Recherches Pédagogiques Kinshasa, 105.

Koduru, S., Grierson, D.S., Afolayan, A.J. (2006). Antimicrobial activity of *Solanum aculeastrum* (Solanaceae). Pharmacol. Biol., 44, 284-286.

Kowalski R, Kedzia, B. (2007). Antibacterial activity of *Silphium perfoliatum* extracts pharm, Biol., 45, 495-500.

Krishnaiah, D., Devi, T., Bono, A., Sarbatly, R. (2009). Studies on phytochemical constituents of six Malaysian medicinal plants. J. Med. Plants Res., 3, 067-072.

Macheix, J.J., Fleuriet, Jay allemande, C. (2005). Les composes Phénoliques des végétaux: Un exemple de metabolites secondaires d'importance économique, Ed. Presses polytechniques et universitaires romandes, Lausanne, Italie.

Motar, M.L.R., Thomas, G., Barbosa Fillo, J.M. (1985). Effects of *Anarcadium Occidentale* stem bark extract on in vivo inflammatory models. J. Ethnopharm., 95, 139-142.

Namuli, A., Abdullah, N., Sieo, C.C., Zuhainis, S.W., Oskoueian, E. (2011). Phytochemical compounds and antibacterial activity of *J. curcas* Linn. extracts. J. Med. Plants Res., 5, 3982-3990.

Njoku, P.C., Akumefula, M.I. (2007). Phytochemical and nutrient evaluation of *Spondias mombin* leaves. Pak. J. Nutr., 6, 613-615.

Nobori, T., Miurak, K., Wu, D.J., Takabayashik, L.A., Carson, D.A. (1994). Delection of the cyclin-dependent kinase-4 inhibitor gene in multiple Human cancers. Nature, 368, 753-756.

Rabe, T.S.T. (2000). Isolation of antimicrobial sesquiterpenoid from *Warbugie salutarius* J. Ethnopharmacol., 93,171-174.

Shimada, T. (2006). Salivary proteins as a defense against dietary tannins. J. Chem. Ecol., 32, 1149-1163.

Vaquero, M.J.R., Alberto, M.R., Nadra, M.C.M. (2007). Antibacterial effect of phenolic compounds from different wines. Food Control, 18, 93-101.

WHO. (2002). Traditional medicine growing needs and potential. WHO Policy Perspectives on Medicines, World Health Organization, Geneva, 1-6.

Wurochekker, A.U., Anthnony, E.A., Obadiah, W. (2008). Biochemical effect on the liver and kidney of rats administered aqueous stem bark extract of *Xemenia americana*. Afr. J. Biotechnol., 7, 2777-2780.