



RESEARCH PAPER

Antibacterial and Toxicological Studies of *Erythrophleum suaveolens* Crude Extracts on Some Pathogenic Bacteria

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ABSTRACT

The phytochemical screening, antibacterial activity and toxicological studies of leaf and stem- bark extract of *Erythrophleum suaveolens* was carried out using a standard method. The preliminary phytochemical analysis revealed the presence of secondary metabolites. The acetone, aqueous and methanolic leaf extract of *E. suaveolens* indicated the presence of alkaloid, glycoside, steroid, flavonoids, while tannin, saponin and terpenes were absent. The stem-bark extracts revealed the presence of alkaloid, glycoside, tannin, saponin, while steroid and flavonoids were found to be absent. The antibacterial activities of the *E. suaveolens* extracts were assayed using agar well diffusion method. The bacterial isolates used were *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *S. aureus*. The leaf extract had appreciable inhibitory effect on all the test isolates at 10, 5.0, 2.5 and 1.25mg/ml, while stem-bark extract exert greater effect on all test isolates except *K. pneumoniae* at various concentration (10, 5.0, 2.5, and 1.25mg/ml). The antibacterial activity of the plant extracts are less effective compared to the control (ciprofloxacin). The toxicological study (LD₅₀) was conducted using fixed dose method. The toxic effect of both leaf and stem-bark extracts showed that 66.67% of the test animal died and only 33.33% survived at 3000mg/kg concentration. The activity of the plant extracts indicates that the plant can be used to treat some ailment associated with the test isolates. This study has also shown that the plant is not safe due to its toxic effect.

Keywords: Antibacterial, Extract, Phytochemical, Toxicity

INTRODUCTION

Medicinal plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals (Steep, 2004). Plants for long had formed the basis for complicated traditional medicine systems of natural products, and higher plants have been used for centuries as remedies for human disease (Saadabi, 2007).

Approximately 80% of the world's inhabitants rely on traditional plant medicine for their primary health care solutions (Cragg *et al.*, 1999) and over 60% of people in Nigeria rural areas depend on traditional medicine to cure their ailment (Ghani, 1986). Medicinal plants produce a variety of compounds of known therapeutic properties (Iyenger, 1985; Harborne,

1999). Traditional medicine practice is a prominent aspect of the primary healthcare systems in most developing countries. It is estimated that about 80% of the world's population especially people from developing countries depend on traditional medicines of plant origin (Nath *et al.*, 2011).

Erythrophleum suaveolens is a tree belonging to the Caesalpiniaceae Family (Neuwinger, 1998). Aside from the therapeutic values of some of the compounds from plants, *Erythrophleum* species are extremely toxic to

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livestock all over the world especially to goats, sheep and cows. Species of *Erythrophluem* include *E. lasicanthum*, *E. guineense*, *E. cholostachys*, *E. invarense* are known to be poisonous (Koita, 1998; Tuuliki, 2003; Adeoye and Oyedapo, 2004; Agaie *et al.*, 2007). The plant is called Obo/erun (Yoruba), Inyi (Igbo), Baska (Hausa), Kor (Tiv), Sacchi (Nupe), Aba (Akan-Asante, Ghana). The plant (*E. suaveolens*) is often referred to in English as sassy, sasswood, red water tree and ordeal tree (Nwudu and Chineme, 1981).

The crushed bark is applied to swellings caused by filarial worm. In DR Congo, the dried powdered bark is taken as a snuff to cure headache. In Kenya a diluted decoction of the roots is used as an anthelmintic, especially against tapeworm. In Malawi a decoction of the roots and bark is applied to soothe general body pain. The bark has been used in arrow poisons, and the bark and leaves as fish poison (Marshall, 1998). The bark extract has excellent local anesthetic activity on eyes and skin, although its use on the eye was disputed because of its irritant effect on the conjunctiva.

METHODOLOGY

Collection and identification of plant samples.

The leaves and stem-bark of *Erythrophleum suaveolens* were obtained from Batagi village along Bida- Minna road, Niger state. The plant samples obtained was identified and authenticated by a Botanist Mal. Yunusa Audu from the Department of Biology, Ibrahim Badamasi Babangida University Lapai, Niger state.

Sources of test organisms:

The test organisms were collected from Department of Microbiology laboratory, Ibrahim Badamasi Babangida University Lapai, Niger state. These organisms include *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus*.

Preparation of plant material

The leaf and stem-bark of *Erythrophleum suaveolens* were allowed to air dry for about two weeks at ambient temperature. The plant material were pulverized with the aid of a sterile mortar and pestle, and then sieved with the help of a mesh sieve (0.50mm) to obtain fine powder of both leaf and stem-bark of *E. suaveolens*. This

was further packed in a sterile container and store for further use.

Sterilization of materials.

Equipment such as glassware was washed using detergent, rinsed and air dry. Then each of the glass ware and other materials were rapped with aluminum foil paper before sterilization (using hot air oven at 170°C for 60 seconds). All media were sterilized using autoclave for 15minutes at 121°C. Cork borer and glass rod were also sterilized by dipping into 70% ethanol and flame. Using 70% alcohol, the laminar flow hood was sterilized and the UV light was switched on for 15-30 minutes.

Extraction of plant materials

Forty gram (40g) of the plant material each was weighed into three different 250ml conical flask for extraction. Methanol and acetone were used for extraction alongside with distilled water which the traditional healers use for extraction. Four hundred milliliter (400ml) of each solvent including distilled water was measured and poured into the powder of each sample, covered with foil paper and kept for 48 hours with constant stirring. Whatman's No1. filter paper was used to filter in order to obtain the filtrate and the residue was discarded. The methanol and acetone extracts were concentrated into semi-solid paste in water bath at 65°C. However, the extract containing water was allowed to evaporate to dryness in water bath since water is a non-volatile solvent.

Experimental animal (Albino rats)

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

Preparation of media

Twenty eight gram (28g) of Nutrient agar was weighed into one liter of distilled water according to manufacturer instruction. The media was put into a water bath to homogenize

the mixture. With the aid of an autoclave the media was sterilized at 121°C for 15 minutes.

Standardization of inoculums

One gram of Barium chloride was weighed in 99ml of sterile distilled water; this was followed by measuring 1ml of concentrated sulphuric acid in 99ml of sterile water. To prepare 10ml of 0.5 McFarland Nephelometre. 0.2ml of 1% Barium chloride was added to 9.8mlml of concentrated sulphuric acid. Turbidity corresponds to 6×10^8 /ml bacterial cells (McFarland, 1999).

Reconstitution of extracts.

One (1) gram of each leaf and stem-bark of *E. sauveolens* extract was weighed into a McCartney bottle and dissolved with 10ml of glycerol or dimethylsulphide to give a stock of 10mg/ml respectively. The serial dilution of each was carried out to give a concentration of 10mg/ml, 5mg/ml, 2.5mg/ml and 1.25mg/ml respectively.

Preparation of inoculums

All test organisms were prepared separately by sub-culturing from the pure isolates into nutrient agar and incubate for 37°C for 24hours.

Phytochemical analysis.

This analysis was carried out to determine the secondary metabolite present in the plant that is responsible for its therapeutic effect. These secondary metabolites include alkaloids, glycosides, tannins, flavonoids, steroids, saponin and terpene. The crude extracts were mixed with chemicals to detect the metabolite and standard qualitative method described by Harborne, (1998), Sofowora, (2003) and Hassan *et al.* (2005) was adopted or used

Determination of antibacterial assay

Susceptibilities of the test organism against the plant extract were assayed using agar well diffusion method as described by Kirby and Bauer, (2000) The workbench (laminar flow hood) was swabbed using 70% ethanol and also UV light was switched on for 15minutes to attain aseptic condition. The sterilized media was poured into petri dishes and allowed to solidify. A sterile spreader was used to spread the standardized suspension inoculums after inoculating on the surface of agar plate to attain an even distribution.

A standard 6mm cork borer was flamed, allowed to air dry and 5 holes were bored on the agar

plate. Zero point two (0.2) ml of the extracts with concentration of 10mg/ml, 5mg/ml, 2.5mg/ml, 1.25mg/ml and 0.625mg/ml was dispensed into the holes respectively. Commercial antibiotic (Ciprofloxacin) used as control was dispensed into the remaining center hole of each agar plate. The plates were labeled appropriately to avoid mix-up. The plates were incubated at 37°C for 24 hours to observe the inhibited zone.

Determination of acute toxicity of the plant extracts (LD₅₀)

A fixed dose method described by OECD, (2001) was adopted for LD₅₀. Aqueous leaf extract and aqueous stem-bark extract (3000mg/kg) was administered to 2 groups (four rats per group) one after the other at a grace period of 48 hours) in a single oral dose by using a feeding needle, while control group received distilled water. Observation of toxic symptoms were made and recorded at one, three, six and twelve hours after administration. The number of mortality was recorded after 48 hours. The toxicological effect was assessed on the basis of toxicity sign: slow movement, restlessness and mortality, which were expressed as LD₅₀ (OECD, 2001).

RESULTS

The data in Table 1 reveals the amount and percentage of crude extracts obtained from the plant sample using acetone, aqueous and methanol as extractants. From the extraction, it indicates that methanol stem-bark has the highest yield extract 6.7g (16.75%), followed by acetone stem-bark with 5.0g (12.5%) and aqueous leaf extract having 4.9g (12.5%). Others include methanol leaf with 4.7g (11.75%), aqueous stem-bark which yielded 4.6g (11.5%) and acetone leaf have the least with 4.0g (10%). The color of all extracts appears to be dark brown except for acetone leaf extract and acetone stem-bark extract have black and red color respectively.

The result of preliminary phytochemical screening of crude extract of acetone, aqueous and methanol of *E. sauveolens* are represented in Table 2. The analysis of acetone, aqueous and methanol leaf extract revealed the presence of alkaloid, glycoside and flavonoids, while terpene, tannin and saponin were found to be absent. The acetone, aqueous and methanol stem-bark extract of *E. sauveolens* contained

100% glycoside, alkaloid, tannin, saponin, while steroids and flavonoids were found be absent.

Antibacterial activities of acetone, aqueous and methanol extract of *E. sauveolens* against *P. aeruginosa*, *E. coli*, *K. pneumoniae* and *S. aureus* are shown in Table 3. In Table 3 the acetone, aqueous and methanol leaf extracts had an appreciable inhibitory effect on all test isolates at different concentration except for the lowest concentration (1.25mg/ml) which had no effect on *K. pneumoniae*, *E. coli* and *P. aeruginosa*.

Table 4 shows that acetone, aqueous and methanolic stem-bark extract exert a greater inhibitory effect on all test isolates at all concentration except *S. aureus* and *P. aeruginosa* that were resistant at 1.25mg/ml concentration. The aqueous stem-bark extract shows significant effect on *P. aeruginosa*, *E. coli*, *K. pneumoniae* and *S. aureus* at

concentration of 10mg/ml, 5mg/ml, 2.5mg/ml, with exception of *P. aeruginosa* and *E coli* that were resistant at 1.25mg/ml.

The Ciprofloxacin used as a standard commercial antibiotic (control) had highest inhibitory effect compare with *E. sauveolens* extracts on the entire test isolates which give average zone of inhibition of 17mm at 10mg/ml concentration.

Table 5 and 6 shows acute toxicity of aqueous leaf and stem-bark extract of *E. sauveolens* at 3000mg/kg. The oral administration of the extract shows some physical changes like clumping of the animal at the edge of the cage, restlessness and slow movement after 2-5minute of administration.

Table1: The weight of *E. sauveolens* leaf and stem-bark extracts obtained after extraction.

Plant parts	Solvents	Weight of Samples (g)	Weight of extracts (g)	Percentage yield (%)	Color of extracts	Texture of extracts
Leaf	Acetone	40	4.0	10.00	Blackish	Gummy
	Aqueous	40	4.9	12.25	Dark brown	Gummy
	Methanol	40	4.7	11.75	Dark brown	Gummy
Stem-bark	Acetone	40	5.0	12.50	Reddish	Smooth
	Aqueous	40	4.6	11.50	Dark brown	Gummy
	Methanol	40	6.7	16.75	Dark brown	Smooth

Key: g = Gram, % = percent

Table2: The phytochemical properties of acetone, aqueous and methanolic leaf and stem-bark extracts of *Erythrophluem sauveolens*

Phytochemical Constituents	ALE	QLE	MLE	ASBE	QSBE	MSBE
Alkaloids	+	+	+	+	+	+
Flavonoids	+	+	+	--	--	--
Glycosides	+	+	+	+	+	+
Saponin	+	--	--	+	+	--
Steroids	+	--	+	--	--	--
Tannin	--	+	+	+	+	+
Terpenes	--	--	--	+	--	+

Key: ALE= Acetone leaf extract, QLE= Aqueous leaf extract, MLE= Methanol leaf extract ASBE= Acetone stem- bark extract, QSBE= Aqueous stem-bark extract, MSBE= Methanol stem-bark extract, + = Present and -- = absent

Table 3: Antibacterial activity of acetone, aqueous and methanolic leaf extract of *Erythrophluem suaveolens*.

Test isolates	Concentration of extracts (mg/ml)												
	Acetone extract				Aqueous extract				Methanolic extract		CPX		
	10.0	5.0	2.5	1.25	10.0	5.0	2.5	1.25	10.0	5.0	2.5	1.25	10.0
Zone of inhibition (mm)													
<i>E. coli</i>	6.0	4.0	0.0	0.0	7.0	4.0	0.0	0.0	12	8.0	6.0	0.0	19
<i>K. pneumoniae</i>	6.0	4.0	2.0	0.0	6.0	4.0	0.0	0.0	10	8.0	7.0	4.0	18
<i>P. aeruginosa</i>	6.0	4.0	2.0	0.0	8.0	6.0	0.0	0.0	10	6.0	4.0	0.0	17
<i>S. aureus</i>	8.0	6.0	4.0	0.0	10	6.0	4.0	2.0	10	8.0	6.0	2.0	17

Key: CPX= Ciprofloxacin

Table 4: Antibacterial activity of acetone, aqueous and methanolic stem-bark extract of *Erythrophluem suaveolens*.

Test isolates	Concentration of extracts (mg/ml)												
	Acetone extract				Aqueous extract				Methanolic extract		CPX		
	10.0	5.0	2.5	1.25	10.0	5.0	2.5	1.25	10.0	5.0	2.5	1.25	10.0
Zone of inhibition (mm)													
<i>E. coli</i>	12.0	8.0	6.0	0.0	10.0	6.0	4.0	2.0	8.0	7.0	6.0	0.0	19
<i>K. pneumoniae</i>	10.0	8.0	7.0	4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	18
<i>P. aeruginosa</i>	10.0	6.0	4.0	0.0	8.0	6.0	2.0	0.0	10.0	6.0	4.0	0.0	17
<i>S. aureus</i>	8.0	6.0	4.0	2.0	8.0	6.0	2.0	2.0	10	8.0	6.0	2.0	17

Key: CPX= Ciprofloxacin

Table 5: Determination of acute toxicity (LD₅₀) of aqueous leaf extracts of *Erythrophluem suaveolens*.

Weight of animal (g)	Dosage (mg/kg)	Vol. of extract (g/ml)	Observation period	Behavioral changes
			Period (hrs)	
101.40	3000	0.30	24	Mortality
120.40	3000	0.36	24	No mortality
122.60	3000	0.37	24	Mortality
129.40 (Control)	0.0	0.1(distilled water)	24	No mortality

Table 6: Determination of acute toxicity (LD₅₀) of aqueous stem-bark of *Erythrophluem suaveolens*.

Weight of animal (g)	Dosage (mg/kg)	Vol. of extract (g/ml)	Observation	Behavioral changes
			Period (hrs)	
90.00	3000	0.27	24	No mortality
92.30	3000	0.28	24	Mortality
100.10	3000	0.30	24	Mortality
129.40(Control)	0.0	0.1ml (distilled water)	24	No mortality

DISCUSSION

The investigation done on different extract of *E. suaveolens* revealed that leaf extracts contain significant amount of tannins, alkaloid, flavonoids and glycoside, with little presence of saponin, while terpenes was found to be absent. Steroid and tannins were found to be absent in the stem-bark extracts but indicates the presence

of glycoside, tannin, terpenes and alkaloids. This result goes in line with the finding of Hassan *et al.* (2007) who reported the presences of glycoside, saponin and alkaloids in *Erythrophluem suaveolens*.

Presence of these compounds is known to be responsible for the antibacterial potentials, which could also serve as a defense mechanism for the plant against fungi and grazing animals. The presence of tannin which is used in treating intestinal disorder such as diarrhea and dysentery (Dhannanada, 2003). The presence of alkaloids and terpenes reveals that the plant could be used as herbal medicine (Hayashi *et al.*, 1993).

The antibacterial activity of acetone, aqueous and methanol leaf extract of *E. suaveolens* shows varying zone of inhibition against the test organisms at different concentrations of 10, 5, 2.5, 1.25mg/ml indicating antibacterial activities. Comparing the extracts activity with the control (Ciprofloxacin), the control had the highest zone of inhibition ranging from 14-19mm, followed by methanol leaf extract ranging from 2-12mm and then aqueous and acetone extract. The highest zone of inhibition was observed against *E. coli* while *P. aureginosa* had the least zone of inhibition in acetone leaf extract.

The antibacterial activity of acetone, aqueous and methanol stem bark extracts of *E. suaveolens* showed varying zone of inhibition against all test organisms used at different concentrations of 10, 5, 2.5, 1.25mg/ml indicating antibacterial activity. The highest zone among all was the control antibiotics (Ciprofloxacin) at concentration of 10mg/ml. At 10mg/ml the control zone of inhibition ranges from 14-17mm, followed by methanolic stem bark extract ranging from 2-12mm, then acetone and aqueous. At 10mg/ml of the methanol stem bark extract *E. coli* has the highest zone but having less significant zones in 5, 2.5 and 1.25mg/ml. Although the aqueous stem bark against *K. pneumoniae* had no zone of inhibition at all concentration. This finding is contrary to with the findings of Aiyegoro *et al.* (2007) in which *K. pneumoniae* was inhibited at different concentrations ranging from 100-250mg/ml. This is due to the fact that *K. pneumoniae* was subjected to higher concentration compared to the concentration used in this study.

Furthermore, stem bark extracts of *E. suaveolens* shows greater inhibitory effect than leaf extracts with zone of inhibition ranging (8-12mm) and (6-8mm) respectively. The leaf and stem bark extract inhibited both Gram positive

and Gram negative, therefore could be called broad spectrum extract.

The crude aqueous leaf and stem-bark extracts of *E. suaveolens* at 3000mg/kg on albino rat indicates the toxicity potentials of the plant extract. This finding is contrary to the findings of Idyu *et al.* (2014) who reported that the toxic effect of *E. suaveolens* at a concentration of 300mg/kg recorded mortality of 100%.

The physical manifestation of toxicity of *E. suaveolens* within just 2 – 3 minutes of administration at various concentrations on the albino rat is an indication that it could penetrate blood brain barriers within a short time. Loss of locomotor coordination in the albino rat may be due to the interference of crude extract with the skeletal muscle, especially the neuromuscular transmission by blocking the neurotransmitter acetylcholine (Singh, 2003).

Acetylcholine is responsible for the termination of cholinergic impulses by the hydrolysis of acetylcholine released during synaptic transmission; inhibition of Acetyl cholinesterase thus permits accumulation of the synapses when concentration raises several folds in comparison to the normal levels, leading to first paralysis and then eventually death (Singh and Singh, 2003).

Conclusion

This study has allowed us to know the phytochemical compounds present in the crude extract, antibacterial potential and toxicity activity of *E. suaveolens*. The phytochemical compounds such as tannin, alkaloid and saponin are known as defense mechanism of the plant. Also the plant has antibacterial potential against *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. These microbes are known to cause various infections. For example *Klebsiella pneumoniae* causes pneumonia and urinary tract infection, *E. coli* causes intestinal disorder, while *Pseudomonas aeruginosa* are known to cause urinary tract infection and septic shocks. The plant is known traditionally to be poisonous and toxicity studies carried out has shown that the plant is moderately toxic.

Recommendations

Further phytochemical analysis, characterization and isolation of pure bio-active

should be carried out so as to determine its toxic constituent and identify the exact constituent responsible for its toxic effect. The bioactive compound responsible for inhibiting the growth of test organism should be compounded in a dosage form. The toxic constituent when isolated and characterized should also be compounded and packed into suitable form for biological control of pest. The unpurified medicines made from *E. suaveolens* should not be used internally due to its toxic effect. Pharmaceutical industries should incorporate the use of the plant parts in production of drug for the treatment of disease condition associated with these pathogens.

REFERENCES

- Adeoye, B. A. and Oyedapo, O. O. (2004). Toxicity of *Erythrophleum guineense* stem-bark: Role of alkaloid fraction. *African Journal Traditional*, 1:45-54.
- Ageaie, B. M., Salisu A. and Ebbo, A. A. (2007). A survey of common toxic plants of livestock in Sokoto state. *Nigeria Sci. Res. Essay*, 2: 40-42.
- Aiyegoro, O. A., Akinpelu, D. A. and Okoh, A. I. (2007). In-vitro antibacterial potentials of the stem-bark of Red water tree (*Erythrophleum suaveolens*). *Journal of Biological Science* 7(7): 1233-1238.
- Cragg, G.M., Boyd, M.R., Khanna, R., Kneller, R., Mays, T. D., Mazan, K. D., Newman, D.J. and Sausville, E.A. (1999). International Collaboration in Drug Discovery and Development. The NCL experience. *Pure Applied Chemistry*. 71 (9): 1619-1633.
- Dharmananda, S. (2003). Gallnuts and the uses of Tannins in Chinese Medicine. Proceedings of Institute for Traditional medicine
- Ghani, A. (1986). Medicinal plants and traditional medicine portions: Problems and prospects of their Standardization. The state of medicinal plant research in Nigeria. 65.
- Harborne, J. B. (1998). Phytochemical methods. Chapman and Hall Publications, London, Pp 7-8.
- Hassan, M.M., Oyewale, A.O., Amupitan, J.O., Abdullahi, M.S. and Okonkwo, E.M. (2005). Preliminary phytochemical and antimicrobial investigation of crude extract of the root-bark of *Detarium microcarpum*, *J. Chem.Sci. Nig.*, 29(1):26-29.
- Kirby, W.M., Bauer, A.W., Sharris, J. C. and Tutch, M.C. (1966). Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clin. Pathol.* 45:493-496
- Hassan, S.W., Ladan, W. J., Dogondaji, R. A., Umar, R. A., Bilbis, L. S., Hassan, L. G., Ebbo, A. A. and Matazu, I. K. (2007). Phytochemical and Toxicological studies of aqueous leaves extract of *Erythrophleum africanum*. *Pakistan Journal of Biological Sciences*; 10 (21): 3815-3821.
- Idyu, I. I., Kele, S.L., Idyu, V.C., Akinyede, A., Builders, M.I., Ogbole, E.A., Ramyil, M.S. and Ogundeko, T.O. (2014). Acute toxicity studies of *E. Suaveolens* in Albino mice (*Mus musculus*). *International Journal of Science and research*, 3 (4):366-371.
- Kamanyi, A., Dongmo, A.B., Salah, M.A., Jatsa, H., Wagner, H., Vierling, W. and Bopelet, M. (2003). Endothelium mediated aortic relaxation and blood pressure lowering effect of a procyanidin rich fraction of the stem bark extracts of *Erythrophleum suaveolens*. *Journal of Pharmaceutical Biology*; 41 (1): 62-67.
- Marshall, N.T. (1998). Searching for a cure: Conservation of medicinal wildlife resources in East and southern Africa. *Traffic International*, Cambridge, United Kingdom. Pp.112.
- Nath, S., Choudhury, M.D., Rouchoudhury, S., Talukae, D., Sirokin, A.V., Bokova, Z., Kadasi, A., Marumiakova, N. and Kolesarova, A. (2011). Restorative aspect of castor plant on mammalian physiology. *Journal of Microbiology. Biotechnology Food Science*. 1 (2): 23.
- Nwude, N. and Chineme, C.N. (1981). Toxic effects of the leaves of *E. africanum* (Harms) in sheep. *Bull. Anim. Health Product Afr.*, 229: 3499-3500.

- Organization for Economic and Cultural Development (OECD) (2001). Guidelines for the Testing of Chemicals. Acute Oral Toxicity. Test Guideline, 42: 1-26.
- Saadabi, A. M. (2007). An evaluation of *Lawsonia inermis* Linn (Sudanese henna) leaf extracts as antimicrobial agent. *Research Journal of Biological Sciences*.2 (4): 419-423.
- Singh, S. K., and Singh, A. (2003). Molluscicidal and anticholinesterase activity of *Alstonia scholaris* plant against fresh water snail *Lymnaea acuminata*. *Pak. Journal of Biological Science*, 6:1442-1446.
- Sofowora, A. (1982). Medicinal Plants and traditional Medicine in Africa, Spectrum book Ltd, Ibadan, Nigeria, Pp 289.
- Stepp, J. R. (2004). The role of weeds as source of pharmaceuticals. *Journal of Ethnopharmacology*, 93 (2-3): 163-166.
- Tuulikki, R., (2003). Defences and responses: Woody species and large herbivores in African Savannah. Swedish University of Agriculture Sciences. *Silverstria Journal*, Pp.276.