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Potential Effect of Some Local Antimalarial Herbs on Reproductive Functions of Male Albino Rat

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Authors' contributions

This work was carried out in collaboration between all authors. Author EVI designed the study and wrote the protocol. Author UBE wrote the first draft of the manuscript. Author OUU performed the statistical analysis, interpreted and formatted the final manuscript. While author EEE did the bench work and literature search. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

In this study, stem bark extracts of *Cylicodiscus gabunensis, Nauclea latifolia* and *Araliopsis soyauxii* were investigated for possible adverse effects on male reproductive organs and sex hormones of male albino rats of about eleven weeks weighing between 120-180g. The total of twenty eight rats were divided into seven groups (A, B, C, D, E, F and G) with four rats in each group. Two levels of each plant extract 125mg/kg body weight (BW) and 225 mg/kg BW (low and high dose) were administered to the rats by oral intubation. Group A served as the control and were fed with normal commercial feed only, group B and C were fed with 125 and 225mg/kg BW of *C. gabunensis,* group D and E were fed with 125 and 225mg/kg BW of *N. latifolia* while F and G were fed with 125 and 225mg/kg BW of *A. soyauxii.* The results of the phytochemical screening showed significant differences ($p<0.05$) in the bioactive components of the three plants. The results obtained on the reproductive organs showed no significant effect ($p>0.05$) on organ weight (testes and epididymides) semen pH, sperm count and sperm head abnormality among the different groups but there were differences ($p<0.05$) in sperm motility and sperm viability in the different groups of the rat. On the hormonal analysis, the sex hormones under this study were generally decreased ($p<0.05$) as the concentration of each extract

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was increased. Thus, this study has x-rayed the potential reductive effects of the extracts of *C. gabunensis, N. latifolia* and *A. soyauxii* on sex hormones and some sperm parameters.

Keywords: Cylicodiscus gabunensis; Nauclea latifolia; Araliopsis soyauxii; anti-malarial herbs; phytochemistry; reproductive functions and hormones.

1. INTRODUCTION

The role of medicinal plants in pharmaceutical industries cannot be underestimated. Medicinal plants have high therapeutic values and have been employed in traditional medicine for the treatment of various disease conditions. Undoubtedly, the growing use of herbs to treat a wide range of illness have been enhanced by the fact that some diseases do not have any appropriate treatments and that herbal treatments are innocuous [1]. There is a general belief that herbal medicines are safe, available, affordable and therefore superior to synthetic ones [2]. As a result of this, there is a shift from the use of synthetic drugs to the use of various antimalarial herbs to treat malaria and other related diseases. The efficacy of these medicinal herbs depend on the inherent phytochemicals in these plants. A short communication with the local people in northern part of Cross River State during the course of this research revealed that there are some locally used antimalarial herbs including *C. gabunensis, N. latifolia* and *A. soyauxii.* Though they revealed that these plants are very potent for treating malaria, there are fears among researchers regarding the toxic side effects or health effects of these herbal remedies, especially as regards reproductive functions of animal systems.

The potency and dosage of these antimalarial herbs notwithstanding, it is very crucial to assess the toxicity of these acclaimed potent herbs. This is partly on the backdrop of the potential of developing new drugs or vaccines for the treatment, prevention and control of malaria [3,4] and partly because the inherent toxicity will affect the cells of the animal system.

Some of the antimalarial herbs which are of great interest to the local people as a reservoir of antimalarial properties include *Cylicodiscus gabunensis, Nauclea latifolia* and *Araliopsis soyauxii* [5,6]. Though locally used as antimalarial herbs, there is paucity of information about these plants, especially on the reproductive toxicity. This paper is poised at x-raying the phytochemistry and potential toxicological effect of these antimalarial herbs on the reproductive functions of male albino rat.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Materials

The stem barks of *C. gabunensis, N. latifolia* and *A. soyauxii* were collected from Mr. Okoi Otu and certified in the herbarium unit of the Department of Forestry and Wildlife, University of Calabar, Calabar, Nigeria. The plant materials were oven -dried at 25ºC for 6hrs and milled into powder using heavy duty miller (Christison 37 BLIB, model 204C BC). They were extracted using 98% ethanol by soxhlet extraction. The ethanol and water were removed using rotary evaporator (Buchi RE 111) under reduced pressure at 60ºC. The extract paste

obtained was stored at -70ºC until used for bioassays. The dried extract was reconstituted in distilled water for animal oral treatment.

2.2 Experimental Animal and Administration of Extract

Twenty eight healthy matured male albino rats weighing between 120±20g were obtained from the animal House of the Department of Biochemistry, University of Calabar, Calabar. The rats were housed in standard cages under normal laboratory conditions and left to acclimatize for one week with water and feed *ad libitum* before commencement of treatment. Ethical care and handling of experimental animals was observed at all times and the study was approved by the University of Calabar ethical committee. The rats were grouped into three groups with four rats in each group. They were administered with 0, 125 and 225mg/kg of individual plant extract in a 3×3 factorial experimental layout using completely randomized design (CRD). The administration of each extract was via oral gavage for the period of 65 days. At the termination of the experiment, the rats were sacrificed using diethyl ether. The testes and epididymides were surgically removed and weighed. Semen was obtained for sperm analysis while blood was collected via cardiac puncture into sterile tubes for hormonal analysis.

2.3 Phytochemical Screening

2.3.1 Test for saponins (frothing test)

Froth emulsion test was used. 2ml of the aqueous extract was mixed with 6ml of distilled water in a test tube. The mixture was shaken and observed for the presence of stable forth. Forth (foam) showed the presence of saponins in the dry stem sample. To further confirm saponin presence, drops of olive oil was added to the forthing mixture and shaken. The formation of stable emulsion confirmed the presence of saponin [7].

2.3.2 Test for alkaloids

Alcohol extract was obtained by dispensing 2g of sample in 10ml of absolute ethanol and after shaking by hand for 30mins, it was filtered using Watman filter paper and the filtrate was used as the extract. 2ml of the extract from each sample was mixed with drops of Mayer's reagent in the test tube. The formation of orange brown precipitate indicated the presence of alkaloids in the sample [8].

2.3.3 Test for flavonoids

The presence of flavonoids in the test sample was determined by the acid alkaline test. 2ml of aqueous extract was mixed with drops of concentrated ammonia. The formation of yellow colouration was recovered as positive test. To the coloured solution, a drop of concentrated hydrochloric acid was added, the disappearance of the formed yellow colour confirmed the presence of flavonoids [9].

2.3.4 Test for polyphenols

2ml of extract was added to 5ml of distilled water and heated for 30mins in a water bath. This was followed by 1ml of 1% $FeCl_3$ and 1ml of 1% potassium ferrocyanide solution. Formation of purple colouration indicated the presence of polyphenols [10].

2.3.5 Test for carotenoids

Test for vitamin A on Retinol. Blue colour formed by an oil in methanol solution treated with copper sulphate was read at 352 in a spectrophotometer, value obtained multiply by 2 gives percentage carotenoids content [8].

2.3.6 Test for xanthonoids

2ml aliquot of extract was added to 5ml of 5% ammonia solution. The formation of a purple-pink colour precipitate indicated the presence of xanthonoids [9].

2.3.7 Test for limonoids

Alcoholic extract was obtained by dispensing 2g of each sample in 10ml of absolute ethanol and after shaking for 30mins, it was filtered and the filtrate was used as the extract. 2ml of extract from each sample was mixed with drops of Mayer's reagent in test tubes. The formation of yellow colouration precipitate indicated the presence of Limonoids in the sample [8].

2.4 Hormonal Assay

The blood samples collected at termination of treatment were spun at 2500 rpm for 10min using Wisperfuge model 1384 centrifuge (Tamson, Holland) at 10-25ºC. Serum samples were assayed for total levels of testosterone, follicle stimulating hormone (FSH), luteinizing hormone (LH) and prolactin using the commercial kit, Microwell enzyme linked immunoassay (ELISA) technique; using analytical grade reagents (Syntron BioresearchInc., USA) [11]. Values were reported according to the various units.

2.5 Sperm Quality Analysis

2.5.1 Estimation of sperm count

This was carried out according to the method of [12]. The epididymal content was obtained by macerating with fine scissors known weights of the caput and cauda epididymides in a glass petridish containing warmed buffered physiological saline in the ratio of 1:10w/v. After vigorous pipetting, the suspension was separated from tissue fragments by filtering it through an 80µm stainless mesh. A tissue-free aliquot was loaded into the Neubauer haemocytometer (Deep1/10, Labart, Germany). Five different counts were done for each sample, and the mean were taken as the mean count for each male rat. Sperm count was reported as millions of sperm/ml.

2.5.2 Estimation of sperm motility

The sperm suspension was diluted in 2ml of warmed buffered physiological saline and dropped on glass slides. This was viewed under light microscope as to determine the motile and non – motile sperm cells by their movement [13].

2.5.3 Estimation sperm viability

This was estimated using the improved one step eosin-nigrosin staining technique. A fraction of each suspension of the sperm samples was mixed with equal volume of eosin–nigrosin stain and air dried smears were prepared on glass slides for each samples according to [14]. The slides were coded randomly and examined under the microscope for percentage viability. Normal live sperm cells exuded the eosin – nigrosin while dead sperm cells took up the stain. Percentage viability was calculated based on the number of viable (live) sperm cells divided by the number of sperm cells within 30 minutes multiply by 100.

2.5.4 Estimation of sperm head abnormality

A portion of the sperm suspension was mixed with 1% eosin Y solution (10:1) for 30 minutes and air-dried smears were prepared on glass slides for the sperm head abnormality test. The slides were examined for percentage sperm head abnormalities in every 200 spermatozoa observed on each slide and five air-dried smears were prepared on glass slides for each sample. The percentage of sperm head abnormality was calculated according to [12].

2.5.5 Estimation of semen pH

The pH of semen was measured using a specially treated calibrated paper blot that changes colour according to the pH of the semen that it is exposed to [15].

2.5.6 Estimation of weight of organs

The epididymides and testes were dissected out at termination of experiment and excess blood and fat were cleaned off and weighed in a clean weighing balance (Model: Mettle LA×214) to record the weight. (Grand G model: JJ 500ld = 0.01g).

2.6 Statistical Analysis

All data collected on sperm and hormonal assays were subjected to analysis of variance (ANOVA) using Predictive Analytics SoftWare (PASW), version 18.0. Significant means were separated using the least significant difference at 5% probability level.

3. RESULTS

3.1 Phytochemistry of the Plant Extracts

Result on phytochemistry showed that there were significant differences in the bioactive components of the three antimalarial herbs screened in this study. It showed that *A. soyauxii* had the highest levels of flavonoid (9.5%), polyphenols (7.5%), alkaloids (5.54%), carotenoids (3.18%), limonoids (1.99%) and xanthonoids (4.57%) and this was followed by *C. gabunensis* and then *N. latifolia* (*A. soyauxii* > *C. gabunensis* > *N. latifolia*). However, *N. latifolia* had more saponins followed by *C. gabunensis* (Table 1).

Table 1. Phytochemical screening of *C. gabunensis, N. latifolia and A. Soyauxii*

Phytochemicals	*C. gabunensis*	*N. latifolia*	*A. soyauxii*
Saponins	1.65^{b}±0.01	4.59^{c}±0.01	0.77^{a}±0.2
Flavonoids	7.64^{b}±0.03	5.15^{a}±0.04	9.5^{c}±0.04
Polyphenols	5.37^{b}±0.03	3.78^{a}±0.03	7.5^{c}±0.02
Alkaloids	3.29^{b}±0.02	2.28^{a}±0.03	5.54^{c}±0.02
Carotenoids	2.17^{b}±0.02	1.78^{a}±0.01	3.18^{c}±0.02
Limonoids	1.20^{b}±0.02	0.67^{a}±0.01	1.99^{c}±0.03
Xanthonoids	2.17^{a}±0.01	2.23^{b}±0.01	4.57^{c}±0.03

^{abc} *values with different superscript along the horizontal array differ significantly*

3.2 Weight of Testes and Epididymides

Testicular and epididymal weight were not significantly affected (P>0.05) by the plant extracts as compared with the control group (Table 3).

3.3 Semen Analysis

Our result revealed that in all the concentrations tested, there were no significant (P>0.05) difference in the semen pH, sperm count and sperm head abnormality. However there was significant difference (P<0.05) in percentage sperm motility and viability, respectively. There was no significant difference between sperm motility of rats treated with 125mg/kg and 225 mg/kg BW of *A*. *soyauxii.* However, there was a significant difference (P<0.05) in sperm motility of rats treated with 125 and 225 mg/kg BW of extract of both *C. gabunensis* and *N. latifolia* as compared to their control. *N. latifolia* extract at 125 and 225 mg/kg concentrations had no effect on the animals in that group as compared with the control. Extracts of *C. gabunensis* and *A. soyauxii* significantly affected the animals in the two concentrations of 125 and 225 mg/kg BW. The reducing effect was increased as the concentration of the extract increased.

3.4 Hormonal Profile

Extracts of *C*. *gabunensis, N. latifolia* and *A*. *soyauxii* had significant effects (P<0.05) on reproductive hormones. Testosterone was significantly (P< 0.05) reduced in rats treated with 125 and 225 mg/kg BW of the three plant extracts. Similarly, there was significant (p< 0.05) reduction in serum levels of prolactin, luteinizing hormone, and follicle stimulating hormone of rats treated with 125 and 225 mg/kg BW of three plant extracts as compared to the control. Serum testosterone level was lowest in rats treated with 225 mg/kg concentration of *A*. *soyauxii* while serum follicle stimulating hormone (FSH) was lowest in rats administered with 125 mg/kg contraction of *C*. *gabunensis* (Table 2).

Table 2. Effect of *Cylicodiscus gabunensis, Nauclea latifolia and Araliopsis soyauxil* on reproductive hormones of male albino rats

Parameters	Concentrations in mg/kg body weight								
	C. gabunensis			*N. latifolia*			*A. soyauxii*		
	0	125	225	0	125	225	0	125	225
Testosterone (ng/ml)	2.11^{c}±0.04	1.31^{b}±0.03	1.05^{a}±0.03	2.11^{c}±0.04	1.60^{b}±0.01	1.10^{a}±0.06	2.11^{c}±0.04	1.63^{b}±0.03	0.88^{a}±0.05
Prolactin (MIU/L)	1.30^{a}±0.04	1.80^{b}±0.04	1.2^{a}±0.004	1.30^{a}±0.04	2.70^{b}±0.04	1.30^{a}±0.04	1.30^{a}±0.04	2.30^{b}±0.02	1.30^{a}±0.04
Luteinizing hormone(MIU/ML)	3.20^{c}±0.06	2.80^{b}±0.004	2.40^{a}±0.01	3.20^{c}±0.06	3.00^{b}±0.04	2.10^{a}±0.04	3.20^{c}±0.06	2.80^{b}±0.01	2.20^{a}±0. 02
Follicle stimulating hormone (MIU/ML)	2.05^{c}±0.07	1.33^{b}±0.03	0.84^{a}±0.03	2.05^{c}±0.07	1.60^{b}±0.004	1.20^{a}±0.004	2.05^{c}±0.07	1.40^{a}±0.004	1.30^{a}±0.02

^{abc} values along the horizontal array with the same superscript are not significantly different

Table 3. Effect of *Cylicodiscus gabunensis, Nauclea latifolia* and *Araliopsis soyauxil* on sperm parameters of male albino rats

Parameters	Concentrations in mg/kg body weight								
	Cylicodiscus gabunensis			*Nauclea latifolia*			*Araliopsis soyauxii*		
	0	125	225	0	125	225	0	125	225
Weight of Testis(g)	1.33^{a}±0.016	1.32^{a}±0.009	1.31^{a}±0.016	1.33^{a}±0.016	1.34^{a}±0.011	1.33^{a}±0.013	1.33^{a}±0.016	1.33^{a}±0.007	1.32^{a}±0.009
Weight of Epididymes (g)	0.38^{a}±0.02	0.37^{a}±0.02	0.35^{a}±0.02	0.38^{a}±0.02	0.35^{a}±0.01	0.31^{a}±0.02	0.38^{a}±0.02	0.36^{a}±0.02	0.32^{a}±0.02
Semen pH	7.1^{a}±0.13	7.2^{a}±0.08	7.1^{a}±0.13	7.1^{a}±0.13	7.1^{a}±0.13	7.05^{a}±0.13	7.1^{a}±0.13	7.2^{a}±0.13	7.1^{a}±0.10
Motility (%)	67.37^{b}±2.50	63.3^{b}±1.76	55.0^{a}±1.21	67.37^{b}±2.50	67.90^{b}±1.40	55.17^{a}±2.29	67.37^{b}±2.50	58.33^{a}±1.00	55.18^{a}±2.17
Sperm viability (%)	77.08^{b}±3.31	74.31^{a}±3.31	68.76^{a}±0.55	77.08^{b}±3.31	77.71^{a}±1.36	74^{a}±2.84	77.08^{b}±3.31	75.37^{a}±2.69	72.84^{a}±2.69
Sperm count (x10^{6}/ml)	6.47^{a}±0.09	6.18^{a}±0.29	6.06^{a}±0.34	6.47^{a}±0.09	6.30^{a}±0.75	6.07^{a}±0.34	6.47^{a}±0.09	6.52^{a}±0.17	6.30^{a}±0.75
Sperm head abnormality(%)	91.89^{a}±2.42	92.48^{a}±2.30	89.21^{a}±2.08	91.89^{a}±2.42	90.39^{a}±2.40	87.44^{a}±3.90	91.89^{a}±2.42	90.84^{a}±1.79	88.11^{a}±1.33

^{abc} values along the horizontal array with the same superscript are not significantly different

4. DISCUSSION

The consumption and exploitation of medicinal plants in herbal remedies is increasing by the day. As earlier portrayed, a short communication with some local people in Ikom, northern Cross River State revealed that some herbal plants including *C. gabunensis, N. latifolia* and *A. soyauxii* are locally and effectively used as antimalarial options. This people only consume these plants due to its antimalarial potency and other medicinal values without the knowledge and understanding of possible dosage and side effects as regarding sex hormones and reproductive organs. Following the reports on the variant phytochemicals inherent in *C. gabunensis, N. latifolia* and *A. soyauxii* [16,17,18,19] and our phytochemistry result on these plants, it becomes apparent that the synergistic effect of these variant bioactive components could implicate either a positive or negative effects on the body metabolism when these plants are directly taken or incorporated into other drugs. This may provide safety precaution on the consumption of these plants, its antimalarial potential notwithstanding.

Our result on hormonal assay revealed that the extract of the three herbal plants exhibited significant reducing effects on testosterone, prolactin, luteinizing hormone (LH) and follicle stimulating hormone (FSH). The highest effect was observed at 225mg/kg BW on testosterone by the extract of *A. soyauxii.* There are reports that some phytochemicals such as alkaloids and saponins possess toxic effects on cell metabolism [20,21,22]. In consonant with the result of the phytochemical analysis obtained in this study, the drastic reducing effect exhibited by these herbal plants on the male albino rats could be connected to the different phytochemicals in the extracts. It thus become interesting to note that the high reduction in sperm motility and the level of the testosterone by the extract of *A. soyauxii* could be specifically due to the quantity of flavonoids, alkaloids, carotenoids, limonoids and xanthonoids which were highest in its extract compared to the other two extracts (Table 1). This might therefore imply that the amount of bioactive components in the extract could be directly proportional to the toxic effect on sex hormones and sperm quality.

The reducing effect exhibited generally by the extracts of the three plants on hormonal parameters of the rats thus suggest that *C. gabunensis, N. latifolia* and *A. soyauxii* might contain bioactive components with similar effect in hormonal production pathways. This notwithstanding, the reducing effect exhibited by the extract on testosterone was not significant enough to cause reduction in organ weight (testes and epididymes), sperm count and semen pH of the male albino rats although there was a concomitant reduction in sperm motility and viability. At this juncture, it is important to state that this concomitant reduction in the level of testosterone, sperm motility and viability could spell doom as regarding reproductive efficiency of the rats because when the sperm cells are not motile and viable the rate of fusion with the egg is compromised leading to infertility. This immobility may be as the result of the fact that the bioactive compounds in the plants had caused immobilization or weakening effects on the sperm cells [23]. According to [24], chemical action during the spermatogonial phase will probably have greater effect on sperm output than the action during the spermatid formation phase. This presupposes that the bioactive components inherent in these herbal plants might have exerted effect at the spermatogonial phase of spermatogenesis [25] resulting in insignificant deviation in the sperm count of the treated groups and the controls. Obviously, the differential reducing potential of the three extracts on the sex hormones, sperm motility and viability might not be unconnected to the differential quantity of the plants' phytoconstituents.

5. CONCLUSION

This study has explicity shown the potential effects of the extracts of *C. gabunensis, N. latifolia* and *A. soyauxii* at two doses on sex hormones, sperm motility and sperm viability of male albino rats. This therefore suggests that though these plants are medicinal and very potent in treating malaria, they should be taken with caution as the administration of high dose could be fatal to sex hormones and some reproductive organs which might result in infertility.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ang HH, Lee KL. Education of mercury contamination in smilax myosotiflora herbal preparations. International Journal of Toxicology. 2007;26:433-439.
2. Calixto JB. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicine (phytotherapeutic agents). Brazilian Journal of Medicinal and Biological Research. 2000;33:179-189.
3. Kilama WL. Ethical perspective on malaria research for Africa. Acta Tropica. 2005;95:276-284.
4. Waako PJ, Gumede B, Smith P, Folb PI. The *in vitro* and *in vivo* antimalarial activity of *Cardiospermum halicacabum L.* and *Momordica foetida.* Journal of Enthopharmacology. 2005;99:137-143.
5. Okokon JE, Ita BN, Udokpoh AE. Antiplasmodial activity of Cyclodiscus gabunensis. Journal of Enthopharmacology. 2006;107(2):175-178.
6. Emeje MO, Isimi CY, Oqua DA, Kunle OO. Some compaction characteristics of the hot water leaf extract of Nauclea latifolia: a potential malarial agent. Journal of Herbal Pharmaccotherapy. 2005;5(4):23-30.
7. Harbone JB. Phytochemical methods: a guide to modern techniques of plant analysis. Charpman and Hall, London; 1999.
8. Harbone JB. Phytochemical methods. Charpman and Hall, 1973: 2nd ed. New York.
9. Trease GE, Evans WC. A textbook of pharmacolognosy:12th ed. Tindall, London; 1983.
10. Boham BA, Kocipai-Abyazan R. Flavonoids and condensed tannins from leaves of *Hawaiian vaccinium vaticulatum* and *V. calycinium.* Pacific Science. 1974;48:458-463.
11. Ekaluo UB, Ikpeme EV, Udensi O, Markson AA, Madunagu BE, Omoun G, Umana EJ. Effect of aqueous leaf extract of neem (*Azadirachta indica*) on the hormonal milieu of male rats. International Journal of Current Research. 2010;4:1-3.
12. Ekaluo UB, Ikpeme EV, Udokpoh AE. Sperm head abnormality and mutagenic effects of aspirin, paracetamol and caffeine containing analgesics in rats. The Internet Journal of Toxicology. 2009;7(1).
13. World Health Organization. WHO Laboratory Manual for the Examination of Human Semen and sperm cervical mucus interaction. Cambridge: Cambridge University Press; 1992.
14. Bjórndahl L, Sódourlund I, Kvist U. Evaluation of the one-step eosin-nigrosin staining technique for human sperm vitality assessment. Human Reproduction. 2003;18:813-816.
15. Comhaire F, Vermeulen L. Human semen analysis. Human Reproductive Updates. 1995;1(4):343-362

16. Tchivounda HP, Koudogbo BC, Besace Y, Casadevall E. Triterpene saponins from Cylicodiscus gabunensis. Phytochemistry. 1991;30:2711–2716.
17. Tane P, Bergquist KE, Tene M, Ngadjui BT, Ayafor JF, Sterner O. Cyclodione, an unsymmetrical dimeric diterpene from Cylicodiscus gabunensis. Tetrahedron. 1995:51;11595–11600.
18. Nchancho K, Kouam J, Tane P, Kuete V, Watchueng J, Fomum ZT. Coumestan glycosides of the stem bark of Cylicodiscus gabunensis. Natural Product communication. 2009;4:931–934.
19. Khan ME, Maiters ON, James TF. Phytochemical analysis and chemotherapeutics of leaves and stem-bark of *Nuclea latifolia* grown in Hong, Anambra State Nigeria. Asian Journal of Plant Science and Research. 2011;1(3): 16-22.
20. Dewick JM. Medicinal natural products: a biosynthetic approach. 2nd ed. John Wiley and Sons Limited, Chichester; 2002.
21. Francis G, Kerem Z, Makkar HP, Becker K. The biological action of saponins in animal system: a review. British Journal of Nutrition. 2002;88:587-605.
22. Robert MF, Wink M. Alkaloids: Biochemistry, ecology and medicinal applications. Plenum Press, New York and London; 1998.
23. Ikpeme EV, Udoh PB, Ekaluo UB, Udensi O, Asuquo BO. Spermicidal and hormonal response of wistar rats treated with ethanol seed, leaf and pulp extracts of *Carica papaya* (Linn). International Journal of Recent Scientific Research. 2010;7:155-159.
24. Sharpe RM. Are environmental chemicals threat to male fertility. Chemistry and Industry. 1992;3:88-94.
25. Ikpeme EV, Udensi O. Ekaluo UB, Uyoh EA. Enthobotanical relevance of spice plants (*Parkia biglobossa and Gongronema latifolium*): Indices for conservation and domestication. Journal of Basic and Applied scientific Research. 2012;2(3):3094-3102.

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