Results

Qualitative and quantitative phytochemical Composition of aqueous stem extract of *Calotropis procera*

The results of qualitative phytochemical of aqueous extract of *Calotropis procera* stem are presented in table 1 and 2. The results showed the presence of tannin, alkaloids, flavonoids, steroids glycosides, saponins, terpenoids and phenol at varying concentrations. The quantitative phytochemical screening of the aqueous stem extract of *Calotropis procera* showed the concentrations of alkaloid, tannin, flavonoid, saponin, steroids glycosides, phenol HCN and terpinoid in the extract.

Table 1: Qualitative Phytochemical Composition of *Calotropis procera* stem aqueous extract.

Phytochemical	Remark
Alkaloids	+++
Saponins	+
Tannins	+++
Flavonoids	+++
HCN	+
Steroids	+
Terpenoid	++
Phenol	+++
Glycosides	++

Keys; + means present, ++ means Abundant while +++ means Very Abundant

Table 2: Quantitative Phytochemical Composition of *Calotropis procera* stem aqueous extract

Phytochemicals constituents	Concentrations (mg/ 100 g)
Alkaloids	610.39 ± 6.62^{c}
Saponins	0.30 ± 0.01^{a}
Tannins	912.78 ± 3.47^{d}
Flavonoids	1232.80 ± 3.56^{e}
HCN	0.20 ± 0.01^{a}
Steroids	0.86 ± 0.03^{a}
Terpenoid	330.21 ± 2.95^{b}
Phenol	688.17 ± 7.63^{c}
Glycosides	460.86 ± 2.95^{b}

Results are expressed in Means ± SD (n= 3)
Mean values with different superscripts down the column are considered significantly different at ($P< 0.05$).

Mineral Element Composition of *Calotropis procera* Stem Aqueous Extract

The results in of mineral element contents in *Calotropis procera* stem aqueous extract as presented in Table 3. The results showed varied concentrations of Mg, Ca, Na, K, Zn, Cu, P, Cd, and Fe in the extract with Cu and K having the lowest and highest concentration respectively, in the extract as seen in the Table 3 below.

Table 3 Mineral composition of *Calotropis procera* stem aqueous extract.

Minerals	Concentrations (mg/ 100 g)
Magnesium	15.86 ±1.70[d]
Calcium	6.85±0.17[c]
Sodium	18.30 ±0.62[d]
Potassium	38.74 ±1.50[e]
Zinc	9.50±0.01[c]
Copper	1.86 ±0.01[a]
Phosphorus	3.2 ±1.31[b]
Cadmium	2.25 ±0.11[a]
Iron	2.03±0.01[a]

Results are expressed in Means ± SD (n= 3)
Mean values with different superscripts down the column are considered significantly different at ($p< 0.05$).

Effect of aqueous extract of *Calotropis procera* stem on sexual performance in male albino rats at day 1, 3 and 5 of treatment.

The result on the chat below showed the effect of the oral administration of aqueous extract of *C. procera* stem at day 1, 3 and 5 on the sexual performance of the male albino rats paired with receptive females. It was observed that at day 1 the mounting frequencies of treated groups A, B, C and E respectively were significantly ($p\leq 0.05$) higher when compared with the negative control (group D), however, comparing the result in the extract treatment and positive control (group E), the result also showed significant increase in positive control group compared to extract treatment group and negative control respectively, the same trend of result was obtained with the intromission. The result, also showed dose dependent increase on the latencies among the treated when compared with control groups However, the result of the ejaculatory latency showed a

significant (P≤ 0.05) delayed time among the treated groups A, B, C and E, when compared with group D (untreated).

Also the graph chat showed the effect of the oral administration of aqueous extract of *Calotropis procera* stem at day 3 on the sexual performance of the male albino rats paired with receptive females to have same trends of result but more proactive.

At day 5 of oral administration of aqueous extract of *Calotropis procera* stem on the sexual performance of the male albino rats paired with receptive females. The result showed that the mounting frequencies of treated groups (A, B, C and E); were significantly (P≤ 0.05) higher when compared with control groups (group D). Similarly, the result showed that in the treatment groups, there was a delayed time of ejaculation when compared with control groups. The results of performance latencies showed a significant decrease among the treatment groups (A, B, C and E) when compared with negative control group (Group D).

Table 4. **Effect of aqueous extract of *Calotropis procera* stem on sexual performance in male albino rats at Day 1 of treatment**.

Groups	MF (NO)	IF (NO)	ML (Sec.)	IL (Sec.)	EL (Sec.)
A	13.02 ± 1.33^{a}	8.78 ± 0.83^{b}	24.30 ± 1.0^{c}	27.90 ± 1.93^{c}	164.40 ± 8.91^{b}
B	16.34 ± 2.46^{b}	11.74 ± 0.50^{b}	15.67 ± 0.76^{b}	21.64 ± 2.37^{b}	173.60 ± 5.50^{c}
C	20.60 ± 2.59^{c}	14.71 ± 0.48^{c}	12.34 ± 0.47^{a}	13.08 ± 0.18^{a}	178.20 ± 7.33^{c}
D	11.23 ± 0.37^{a}	7.12 ± 1.15^{a}	29.33 ± 1.28^{d}	30.42 ± 0.72^{d}	159.60 ± 3.46^{a}
E	25.60 ± 3.59^{d}	18.50 ± 2.50^{d}	10.60 ± 0.59^{a}	18.40 ± 1.29^{b}	180.60 ± 9.59^{d}

Keys: Group A: 1 ml of 200 mg/kg extract administration, Group B: 1 ml of 300 mg/kg extract administration, Group C: 1 ml of 400 mg/kg extract administration, Group D: Distilled water and Group E: 1 ml of 100 mg/Kg Sildenafil Citrate. MF= Mounting Frequency, IF= Intromission Frequency, ML= Mounting Latency, IL= Intromission Latency and EL= Ejaculation Latency. No = number, and Sec, = seconds.

Results are expressed in Means ± SD (n =5). Mean values with different superscripts down the column are considered significantly different at ($p < 0.05$).

Table 5 Effect of aqueous extract of *Calotropis procera* stem on sexual performance in male albino rats at day 3 of treatment.

Groups	MF (NO)	IF (N)	ML (Sec.)	IL (Sec)	EL (Sec.)
A	15.62 ± 1.04^{b}	9.40 ± 0.89^{a}	24.30 ± 1.0^{c}	27.90 ± 1.93^{b}	184.00 ± 1.30^{b}
B	20.66 ± 0.85^{c}	13.00 ± 2.12^{b}	15.67 ± 0.76^{b}	21.64 ± 2.37^{b}	187.20 ± 1.30^{b}
C	22.74 ± 2.67^{d}	15.78 ± 1.32^{b}	12.34 ± 0.47^{a}	13.08 ± 0.18^{a}	187.20 ± 1.30^{b}
D	11.66 ± 1.49^{a}	8.40 ± 1.52^{a}	29.33 ± 1.28^{d}	30.42 ± 0.72^{c}	191.60 ± 0.89^{c}
E	24.54 ± 2.87^{e}	22.74 ± 2.87^{c}	12.43 ± 0.67^{a}	12.44 ± 1.67^{a}	178.74 ± 2.67^{a}

Keys: Group A: 1 ml of 200 mg/kg extract administration, Group B: 1 ml of 300 mg/kg extract administration, Group C: 1 ml of 400 mg/kg extract administration, Group D: Distilled water and Group E: 1 ml of 100 mg/Kg Sildenafil Citrate.
MF= Mounting Frequency, IF= Intromission Frequency, ML= Mounting Latency, IL= Intromission Latency and EL= Ejaculation Latency. No = number, and Sec, = seconds.
Results are expressed in Means ± SD (n =5)

Mean values with different superscripts down the column are considered significantly different at ($p < 0.05$)

Table 6: Effect of Aqueous Extract of *Calotropis procera* Stem on Sexual Performance in Male Albino Rats at day 5 treatment.

Group	MF (No)	IF (No)	ML (Sec.)	IL (sec.)	EL (sec)

A	22.20±1.30^{b}	16.80±1.48^{b}	26.82±0.81^{c}	24.20± 2.17^{c}	162.60±9.89^{c}
B	30.84±0.85^{c}	23.40±1.14^{c}	18.20±0.84^{b}	18.40 ± 1.14^{b}	154.60±8.59^{b}
C	32.14±1.40^{c}	22.60±6.12^{c}	12.60±1.52^{a}	13.80 ± 2.49^{a}	144.80±6.65^{a}
D	17.36±1.12$^{\mathbf{a}}$	13.60±1.67^{a}	37.00±1.22^{d}	46.80 ±10.94^{d}	181.60±3.54^{c}
E	38.14±1.85^{d}	27.54±2.85^{d}	10.84±0.85^{a}	12.30 ±1.65^{a}	154.60±8.59^{b}

Keys: Group A: 1 ml of 200 mg/kg extract administration, Group B: 1 ml of 300 mg/kg extract administration, Group C: 1 ml of 400 mg/kg extract administration, Group D: Distilled water and Group E: 1 ml of 100 mg/Kg Sildenafil Citrate.
MF= Mounting Frequency, IF= Intromission Frequency, ML= Mounting Latency, IL= Intromission Latency and EL= Ejaculation Latency. No = number, Sec. Seconds.
Results are expressed in Means ± SD (n = 5).
Mean values with different superscripts down the column are considered significantly different at ($p < 0.05$)

Effect of *Calotropis procera* stem aqueous extract on some Reproductive Markers

Table 7, shows the effect of aqueous extract *C. procera* stem on some reproductive markers. The results showed that the concentration of serum luteinizing hormone (LH) and in the extract treatment groups and positive control (sildenafil citrate) to be significantly ($p > 0.05$) higher (A,B, C and E), respectively when compared to negative control group (D) treated with distilled water also, the concentrations of serum follicle stimulating hormone (FSH)) in the treatment groups and positive control (A, B, C and E) respectively, were observed to be significantly (($p \leq 0.05$) higher when compared to negative control (group D). Similarly, there was significant ($p \leq 0.05$) increase on testosterone serum levels (of the treatment groups and positive control groups (A, B, C and E) respectively, higher when compared to control group (D).

.

Table 7: Effect of *Calotropis procera* stem aqueous extract on some Reproductive Markers

Group	LH (mg/ml)	FSH (mg/ml)	TESTOSTERONE (mg/ml)
A	2.38 ± 0.12^{b}	3.38 ± 0.24^{b}	2.15 ± 0.21^{a}
B	2.39 ± 0.24^{b}	3.52 ± 0.19^{b}	2.23 ± 0.36^{a}
C	2.72 ± 0.19^{b}	3.53 ± 0.37^{b}	2.52 ± 0.17^{a}
D	1.93 ± 0.53^{a}	2.82 ± 0.29^{a}	2.01 ± 0.17^{a}
E	2.98 ± 0.32^{b}	3.98 ± 0.32^{b}	2.88 ± 0.32^{b}

Keys; LH: Luteinizing Hormone, FSH: Follicle Stimulating hormone

Group A: received 1 ml of 200 mg/kg body weight of extract, group B: received 1 ml of 300 mg/kg body weight of extract, group C: received 1 ml of 400 mg/kg body of extract, group D: received equal volume of distilled water and group E: received 1 ml of 100 mg/Kg Sildenafil Citrate (control group).

Results are expressed in Means ± SD (n = 5)

Mean values with different superscripts down the column are considered significantly different at ($p < 0.05$).

Effect of *Calotropis procera* Stem Extract on Penile Nitric oxide level

The effect of *Calotropis procera* aqueous stem extract on the nitric oxide levels of penile tissue homogenate is presented in table 8. The result showed that the concentration of nitric oxide in the treatment groups and positive control were significantly (($p \leq 0.05$) higher than the negative control group (group D), with treated positive control group that received the standard drug having highest concentration followed by the highest dose (400 mg/kg) extract treated group and negative control group having the lowest concentration. The result showed that the increase in nitric oxide concentrations of extract treated groups when compared with control groups were dose-dependent.

Table 8: Effect of *Calotropis procera* Stem Extract on Nitric Oxide (NO) of Rats

Group	Nitric Oxide (mMol/g)
A	35.23 ± 2.66^{a}
B	41.33 ± 4.46^{b}
C	54.37 ± 3.21^{c}
D	32.11 ± 1.85^{a}
E	58.57 ± 2.21^{c}

Group A: received 1 ml of 200 mg/kg body weight of extract, group B: received 1 ml of 300 mg/kg body weight of extract, group C: received 1 ml of 400 mg/kg body of extract, group D: received equal volume of distilled water and group E: received 1 ml of 100 mg/Kg Sildenafil Citrate (control group).

Results are expressed in Means ± SD (n = 5)

Mean values with different superscripts down the column are considered significantly different at ($p < 0.05$).

Discussion

The background for confirming any medicinal plant as having the potential to stimulate and enhance sexual performance was reported by Ratnasooriya and Dharmarsiri (2000). In their opinion they stated that medicinal plant with a tendency to stimulate and enhance sexual behavior should produce a statistically significant increase in mount frequency and intromission frequency and also significant reduction in mount latency and intromission, since these indices are indicators of sexual arousability, motivation, and vigor. Various parts of *C. procera* has been reported to be used in Nigeria and many other countries for the treatment of varieties of diseases, such as muscular spasm, joint pain, constipation, skin diseases and etc. (Mossa *et al*., 1991). The results of the present study indicated that the aqueous extract of stem of *C. procera* has aphrodisiac potential since its ingestion improved the sexual performance of the rats. Also, *C. procera* extract significantly increased serum luteinizing hormones (LH) and follicle stimulating hormones (FSH) with increased testosterone levels that are statistically significant in a dose dependent manner. There was also dose dependent significant ($P \leq 0.05$) increase in penile tissue Nitric

Oxides concentration on the treatment group when compared with the control. These findings indicated that C. *procera* is a potential medicinal drug for increasing sexual performances and management of erectile dysfunction in sexually experienced rats. Several studies reported that aphrodisiac plants are good alternatives for the improvement of sexual behavior (Guoha *et al.,* 2009), probably due to their efficacy and availability. The mounting latency and intromission latency are considered as indicators of sexual motivation Yakubu and Afolanyan, (2009). The significant reduction figure 1 in these parameters observed in the rats treated with *C. procera* aqueous stem extracts imply improvement of sexual motivation and sexual appetite which further justifies the folkloric use of this plant as sexual booster. Moreover, the increase of ejaculation latency after treatment with the plant extracts indicates the persistence of sexual drive Yakubu and Afolanyan, (2009). On the other hand, mounting frequency and intromission frequency are the indicators of vigor, libido, and potency. The increase in the mounting frequency indicates sexual motivation while elevated intromission frequency reflects the efficiency of erection. These findings agree with earlier report by Ratnasooriya and Dharmasiri (2000), Yakubu and Afolayan, (2008); Yakubu and Akanji (2011); Gbankoto *et al*. (2015) on the significant changes in mounting and intromission latencies. Also, the prolonged ejaculatory latency (EL) by the aqueous stem extract of *C. procera* is a strong indication that the sexual function of the male rats was enhanced (prolonged duration of coitus) suggesting an aphrodisiac activity. These findings which is in line with the work of on reproductive health by Fouche *et al*., 2015, the aphrodisiac property of *C. procera* could be attributed to the various active components present in this plant. The phytochemical analysis of *C. procera* revealed the presence of saponin, terpenoid, alkaloid, tannin, phenol glycoside, phenol and flavonoid. It has been reported that flavonoids facilitate male sexual behavior by boosting testosterone production and/or preventing its metabolic degradation (Yakubu and Akanji, 2011).The presence of alkaloid, flavonoid, sterol and saponin in this plant may account for its use as aphrodisiac. Phytochemicals have been reported to enhance erection and prolong ejaculatory latency in male albino rats (Yakubu *et al*., 2005).

Terpenoids have an implication in the triggering of penile erection as well as in the improvement of the sexual performances (Kim and Christianson, 2004). Kim *et al*.,

(2012), demonstrated that saponin facilitated relaxation of the corpus cavernosum muscles by stimulating the L-arginine/nitric oxide pathway.

These bioactive components could have an effect on the central nervous system by activating neurotransmitters or the periphery by stimulating the release of nitric oxide. Micronutrients play essential roles in metabolism and serves as co-factors and co-enzymes for enzymatic reactions. As shown in Table 3 the plant part is very rich in magnesium a co-factor for many biochemical reactions in the body which include synthesis of sex hormones such as androgens, estrogens and neurotransmitters from the brain that modulate sex drive such as dopamine and norepinephrine. The concentration of potassium in stem sample of *Calotropis procera* was significantly high. Potassium is important as the major cation of the intracellular fluid and helps to maintain the electrode potential, regulate the aldosterone concentration and consequently the permeability of the cell membrane. (Kaplan, 1991; Robert et al., 2003). The level of calcium in *Calotropis procera* is low. Calcium performs two categories of physiological functions; one category involves provision of the structural integrity of the skeleton. The second category depends on the calcium ion in cellular and intracellular fluids. Calcium ion serves as the coupling factor linking excitation and contraction in skeletal and cardiac muscles. Intracellular calcium ion is also required in control of key enzymes regulating intermediary metabolism and so may play a role in providing energy for contraction (Breslau, 1991). Calcium also play role in the permeability and excitability of plasma membrane. It has been implicated as an important coupling factor in neurotransmitter release, exocrine secretion (for example, amylase) and endocrine secretion, such as the case of insulin. Zinc in the plant apart from boosting the immune system is also required for the production of testosterone (the male sexual hormone) and help to ward off male infertility. Deficiency in any of these micronutrients could lead to muscle weakness and general fatigue (Davis, 1998; Yakubu *et al*., 2005).

Conclusion

This study suggested that *C. procera* stem aqueous extract possesses aphrodisiac potential particularly at higher dose of 400 mg/kg of the extract, which showed the highest aphrodisiac effects on mounting frequency, intromission frequency, mounting latency, intromission latency and ejaculation latency,. The extract has a functional

capacity to increased FSH, LH, Testosterone and Nitric oxide concentrations which are possible mechanisms of action for its aphrodisiac property and validated its use as an aphrodisiac by local herbalists.

.

References

AOAC, (2005). Official methods of analysis of the association of official analytical chemists, 17th edn. *Journal of the Association of official analytical chemists*, Arlington, Virginia.

Barry, L. L. & Zito, S.W. (1996).Comparism of the West Indian Aphrodisiac□Love stone□ to the Chinese medication□Chan Su□: Bufotenine and related Bufadienolides. *Journal of Forensic Science*; **41**(6), 1068-1073.

Baskin, H. J. (2017). Endocrinologic evaluation of impotence. *Southern Medical Journal*. **82**, 446□451.

Basu, A., & Chaudhuri, A. (1991). Preliminary studies on the anti-inflammatory and analgesic activities of *Calotropis procera* root extract. *Journal of Ethnopharmacology*. **31**(3), 319-24.

Breslau, N., Kilbey, M. M., and Andreski, P. (1991). Nicotine dependence, major depression, and anxiety in young adults. *Archives of General Psychiatry*, 48, 1069□1074

Calixto, J. B (2000). Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). *Brazilian Journal of Medical and Biological Research*. **33,** 179□190.

Chiou, W. F., Huang, Y. L., Chen, C. F & Chen, C.C (2001). Vaso-relaxing effect of coumarins from Cnidiummonnieri on rabbit corpus cavernosum. *Plantamedica*. **67**(03), 282-4.

Claudino, M .A., Priviero, F. B., Teixeira, C.E., De Nucci, G., Antunes, E & Zanesco, A. Improvement in relaxation response in corpus cavernosum from trained rats. *Urology*. **63** (5), 1004-8.

Colpo, L. M (1998). Evaluation, treatment, & management of erectile dysfunction: *an overview*. *Urology*. **18**, 100-106.

Cui, T., Kovell, R. C., Brooks, D. C & Terlecki, R. P (2015). A urologist's guide to ingredients found in top-selling nutraceuticals for men's sexual health. *The journal of sexual medicine*. **12**(11), 2105-17.

Davis T. A. C (1985). Aphrodite: Historical background .*University of Pennsylvania press*, 19, 98-110.

Ejikeme, C. M., Ezeonu, C. S and Eboatu, A (2014). N□Determination of physical and phytochemical constituents of some tropical timbers indigenous to Niger Delta Area of Nigeria, *European Scientific Journal*. **10**(18), 247□270.

El-Olemy, M. M., Al-Muhtadi, F. J & Afifi, A. A (1994). Experimental Phytochemistry.

English, J. A (2002). Natural approach to enhancing sexual libido and performance. *Annals of New York Academy of Sciences*. **962**(15), 372-377.

Evans, W. C (1989). *Trease & Evans Ph armacognosy.*13th edition.BailliereTindall London. 57-770.

Evans, W. C (1996). Trease & Evans‟ Pharmacology. 14th edition. W.B Saunders company Ltd, London. 15-775.

Farnsworth, N. R (1996). Biological and phytochemical screening of plants. *Journal of pharmaceutical Science.* **55**, 225-276.

Fouad, R., Vivien, K., Koussa, T., Andronald, S & Swerdolf, F (2001). Male Sexual Function and Its Disorders: Physiology, Pathophysiology, Clinical Investigation, and Treatment. *Endocrine Reviews*. **22**(3), 342□388.

Fouche G, Afolayan A. J, Wintola O. A, Khorombi T. E, & Senabe J. (2015). Effect of the

Fullick A. (1994). Biology. Heinemann Advanced Science. Oxford. (1994) p. 332

Gauthaman, K., Adaikan, P. G & Prasad, R. N (2002). Aphrodisiac properties of Tribulus terrestris extract (Protodioscin) in normal and castrated rats. *Life Sci*. **71**, 1385-96

Gbankoto, A., Anago, E., Houndjo, P. A., Adjahouinou, D. C & Gbaguidi, F (2015). Effect

Gerstenberg, T. C., Levin, R. J. & Wagner. G. (1990) Erection and Ejaculation in Man.

Gray, P. B & Garcia J. R (2013). Evolution and human sexual behavior. USA: Harvard University Press.

Guay, A. T., Spark, R. F., Bansal, S., Cunningham, G. R., Goodman, N. F., Nankin H. R., Steven M. P & Jesus B. P. (2003). Medical guidelines for clinical practice for the evaluation and treatment of male sexual dysfunction: a couple's problem in *Endocrinological Practice*. **9**(1), 78-95.

Hackett, G., Kirby, M., Wylie, K., Heald, A., Ossei-Gerning, N., Edwards, D and Muneer, A (2018). British Society for Sexual Medicine guidelines on the management of erectile dysfunction in men. *The journal of sexual medicine*. **15** (4), 430-57.

Harborne, J. B (1998). *Phytochemical Methods*, A guide to modern techniques of plant analysis, Chapman and Hall: New York. 1150.

Kaplan N. M (1991). The Adrenal glands in Textbook of of Endocrine Physiology, 2nd edition. James G, Ojeda SR (eds) Dallas Beaverton Ore, pp. 247-275

Kim, H, K., Choi, B. R., Bak, Y. O., Zhao, C., Lee, S. W., Jeon, J. H., So I & Park, J. K (2012). The role of capillarisin from Artemisia capillaris on penile erection. Phytotherapy Research. **26**(6), 800-805.

Kow, L. M & Pfaff, D. W (1998). Mapping of neural and signal transduction pathways for lordosis in the search for oestrogen actions on the central nervous system. *Behavioral Brain Research*. **92**(2), 169-80.

Laumann, E. O., Paik, A & Rosen R.C (1999). Sexual dysfunction in the United States: Prevalence and predictors. *Journal of American Medical Association*. **281** (6):537-44.

Lugg, J. A., Rajfer, J. & Gonzalez-Cadavid, N. F. (1995). Dihydrotestosterone is the active androgen in the maintenance of nitric oxidemediated penile erection in the rat. *Endocrinology*. **136**, 1495□1501.

Miranda, K. M., Espey, M. G. & Wink, D. A., 2001. A Rapid, Simple Spectrophotometric Method for Simultaneous Detection of Nitrate and Nitrite. Nitric Oxide, **5** (1): 62-71.

Mossa, J. S., Tariq, M., Mohsin, A., Ageel, A. M., al-Yahya, M. A., & al-Said, M. S.,, (1991). Pharmacological studies on aerial parts of Calotropis procera. *American Journal of Chinese Medicine.* **19**, 223-31.

NIH (1993). NIH Consensus Development Panel on Impotence. JAMA 270, 83-90.

Ratnasooriya, N. D & Dharmasiri M. G (2000). Effect of Terminalia catappa seeds on the sexual behaviour and fertility of male rats. *Asian Journal of Andrology*. **2**, 213-219.

Sofowora, A. (1993). *Medicinal plants and traditional medicine in Africa*. Spectrum Books Ltd, Ibadan. 1-2, 160-168.

Tietz, N.M., Puukka & Raija Puukka (1976). *Fundamentals of Clinical Chemistry*. W. B. Saunders, Philadelphia, PA

Tilbrook, A. J., Turner, A. I & Clarke I. J (2000). Effects of stress on reproduction in non-rodent mammals: The role of glucocorticoids and sex differences. Review of Reproduction. **5**(2):105-13.

Uotila, M., Ruoslahti, E & Engvali, E. J (1981).Two-site sandwich enzyme immonuassay with monoclonal antibodies to human. *AFP Journal of Immunological Methods.* **42**: 11-15.

Walkden-Brown, S. W., Martin, G. B & Restall, B. J (1999). Role of male-female interaction in regulating reproduction in sheep and goats. *Journal of Reproductive fertility Supplement*. **54**, 243-57.

Walsh, P.C. & Wilson, J. D. (1987). Impotence and infertility in men. In: Braunwald, E., Isselbacher, K. J., Petersdorf, R. S., Wilson, J. D., Martin, J. B. & Fauci, A.S (eds) Harrison□s Principals of Internal Medicine, ed 11.McGraw-Hill Book Co., New York, NY, pp 217□220.

Yakubu, M. T & Afolayan A. J (2008). Effect of aqueous extract of Bulbine natalensis (baker) stem on the sexual behaviour of male rats. International Journal of Andrology. 32, 629□ 636.

Yakubu, M. T & Akanji, M. A (2011). Effect of aqueous extract of Massularia acuminate stem on sexual behaviour of male Wistar rats, Evidence-Based Complementary and alternative Medicine.

Yakubu, M. T., Akanji, M. A. & Oladiji, A. T (2007). Evaluation of antiandrogenic potentials of aqueous extract of Chromolae naodoratum (L.) K.R. leaves in male rats. Andrologia **39**(6), 235-243.

Yakubu, M. T., Akanji, M. A. & Oladiji, A. T. (2005). Aphrodisiac potentials of aqueous extract of Fadogiaagrestis (Schweinf. Ex Hiern) stem in male albino rats. *Asian Journal of Andrology*. **7**(4), 399-404.

Yang, Y., Li, Z., Wu, X., Chen, H., Xu, W., Xiang, Q. & Huang, Y. (2017). Direct reprogramming of mouse fibroblasts toward Leydig-like cells by defined factors. Stem Cell Reports, **8**(1), 39□53