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The Efficacy and Histopathological effect in Albino Rat of *Abrus precatorius* and *Croton penduliflorus* used for inducement of infertility in humans.

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ABSTRACT

Seeds of two medicinal plant locally used as contraceptives were evaluated to ascertain their efficacy as well as safety to reproductive and some major body organs. These seeds are *Abrus precatorius* and *Croton penduliflorus*. The pulverized seeds at concentration varying from 0.3g to 6.0g and 0.06g to 0.12g (the equivalent weight administered to humans) as well as the ethanolic extract at concentration of 100, 250 and 500mg/ml were separately administered to female albino rats (in groups of five each) that had all delivered just once. Depo-Provera® (Medroxy Progesterone acetate) which is a standard infertility inducer in humans was used as the chemical control while the non-chemical control received unregulated amount of distilled water. Body weight ratio of rats against humans was the major dosage determinant. The pulverized seed at all concentrations used enhanced productivity of litters rather than serving as contraceptives. The phytochemical substances isolated from these seeds include Saponins, Tannins, alkaloids, flavonoid, glycosides, steriods, cardiac glycosides, terpenoids and anthroquinones. Seven of the chemical functional groups, C-H for alkanes, -CH₃ for alkanes, C-H for aromatics, C=C for alkenes, C-O for alcohol ethers esters carboxylic acid, anhydrides, O-H alcohols, phenols and N-H for primary and secondary amines and amides that were detected through infrared spectro photometry had been reported to be active component in antifertility agent. Two hundred and fifty milligrams per milliliter ethanolic extract prove to be the most effective dose of all the two plant extracts administered as contraceptives. None of the concentration of the ethanolic extract induced damage to the uterus and fallopian tube. Apart from the 500mg/ml of *Croton penduliflorus* extract which caused no significant damage to the liver most others resulted in congestion of the central vein of the liver. Congestion of the liver and kidney occurred at 500 and 250mg/ml concentration of *Abrus precatorius* and *Croton penduliflorus* respectively.

Keywords: Medicinal plants, contraceptives, efficacy and infertility

INTRODUCTION

Overpopulation continues to be a significant contributor to environmental degradation and human suffering, hence birth control is now a great concern of the world. It is estimated that half of all conception are unplanned and half of the resulting pregnancies are undesired[1]. In most cases half of the unintended pregnancies are due to failure of use of contraceptives and the other

half to difficulties with contraceptive use or method failure[2]. Despite the numerous benefit of oral contraceptive (synthetic forms of two hormones produced naturally in the body), their side effect is still experienced by women during the first three months of use. Vast majority of women have only minor, undesired effects. Some side effects are uncommon, but may be

dangerous. Examples are; heart attack, high blood pressure, migraines, stroke, blood clot, headache, depression, nausea and vomiting, breast tenderness, breakthrough bleeding or spotting, decreased enjoyment of sex, weight gain, chloasma (spotty darkening of skin of the face), drug interaction, not suitable for everyone, sexual transmitted diseases, daily routine, diarrhea and vomiting [3]. Herbal contraceptives and abortifacient are those plants used for birth

control or in the prevention of pregnancy or premature expulsion of fetus from the womb [4]. Plants that have contraceptive and abortifacient properties may act through rapid expulsion of the fertilized ova from the fallopian tube, inhibition of implantation due to a disturbance in estrogen-progesterone balance, fetal abortion, perhaps due to lack of supply of nutrient to the uterus and the embryo, and also on motility and viability. [5]; [6]; [7].

MATERIALS AND METHODS

Plant Source: *Abrus precatorius* and *Croton penduliflorus* were purchased from Tundu Wada Market (Kasuwa Barchi), Kaduna, Kaduna State. The samples were identified and authenticated in the Department of Biological Sciences, Nigerian Defence Academy. Medroxy progesterone acetate (Depo-Provera) was purchased from a pharmaceutical shop in Kaduna State.

Preparation of the Pulverized Seeds: *Abrus precatorius* and *Croton penduliflorus* were sorted and cleaned. The weight of one of the seeds of each plant was established. The seeds were air-dried and ground to powder with a Micro-Hammer mill in the Department of Biological Sciences, Nigerian Defence Academy. This was stored in a clean plastic specimen container until required.

Experimental Animals/Experimental Design: This investigation was carried out using albino rats. It was conducted in accordance with the internationally accepted principle for laboratory animal's use and care (NIH publication No. 85-23, 1985). Forty five (45) mature female rats and eleven (11) mature male rats with body weight ranging from 200g-160g were obtained from Zaria, Kaduna state

The animals were housed in a metal cage with male separated from the female. The animals were allowed to acclimatize for two months. During the period of acclimatization, the animals were fed to satiation with animal feed twice daily. The cages were cleaned to remove their litters. The male and female rats were first allowed to mate, all the female got pregnant and delivered. This was done to ensure that they were fertile, i.e. they are reproductively satisfactory, thus

attributing any subsequent contraception to test materials administered. The administration of *Abrus precatorius* seeds and *Croton penduliflorus* seeds were done in two ways:

(i). Administration of the Pulverized Seeds: The pulverized seeds of the two plants were administered orally as in humans. The dose administered were relative to the body weight of the albino rats (200g). Concentration for *Abrus precatorius* were 0.3, 0.45 and 0.6mg/ml. while that of *Croton penduliflorus* were 0.06, 0.09 and 0.12mg/ml.

(ii). Administration of the Ethanolic Seed Extract: Doses of the two seed extract administered were 500, 250 and 100mg/ml.

The mature female rats were grouped into three A-C. **Group A:** A total of fifteen (15) female albino rats were assigned to group A. These (15) fifteen rats were further sub-divided to three (3) groups, with each comprising 5 rats. Rats in group A were administered *A. precatorius* at 0.3g/ml, 0.45g/ml, and 0.6g/m. **Group B:** Fifteen matured albino rats were assigned to this group. These were sub-divided into three (3) groups, of five (5) rats each. The group was administered *C. penduliflorus* seed at 0.06g/ml, 0.9g/ml and 0.12g/ml. **Group C₁:** This group had five female adult albino rats which were administered with Depo-Provera® (Medroxy progesterone acetate) which is a standard drug used for inducing infertility in women for three months. The administration was through intramuscular injection using insulin syringe in order to get the actual pharmacological dose of 0.02ml. **Group C₂:** also contained five mature adult female rats which were administered only

water. At the end of the first experiment, the animals were allowed to rest for thirty-one days before the commencement of the second experiment.

Observation of Clinical Signs after Treatment:

The animals were observed for any change such as antagonism towards each other or friendly disposition, feeding habits, changes in their bodily appearance, body weakness, stool, death, etc. The animals were observed twice daily, in the morning and in the evening, for four days. The body weight of the animals was taken prior to administration of treatment and thereafter, it was taken one week after administration and three weeks after administration.

Observation and Confirmation of Pregnancy:

The female rats were observed for signs of pregnancy after administration of the pulverized seeds. These signs include: enlargement of the mammary glands; increase in the size of the abdomen; movement of the pups inside the abdomen, which can be seen when the female rat is close to delivery, during which there is a change in personality. This includes being more aggressive and less interested in play.

Preparation of the Plant Extract: Two hundred gram of each of the pulverized seeds of the test plant (*Abrus precatorius* and *Croton penduliflorus*) were soaked in 350ml of ethanol, each in a separate glass flask. They were all allowed to soak for five days in order to allow for good extraction. Thereafter, it was filtered and concentrated in a rotary evaporator in the Department of Chemistry, Nigerian Defence Academy. The extract was concentrated to dryness in the laboratory, to yield

RESULT/DISCUSSION

The oral administration of the pulverized seeds did not prevent conception as 100% pregnancy was recorded through all treatments except in the chemical control where Depo-Provera was administered. This failure justifies the advice of the herbalist in Tudun Wada market, Kaduna, Kaduna State, who cautioned that the seeds should be swallowed and not chewed, as it will only work

an ethanol extract. *Abrus precatorius* produced a yield of 10.2g, and *Croton penduliflorus* 14.3g.

Preliminary Qualitative Phytochemical Analysis:

This analysis was carried out using the ethanol extract of the two different seeds (*Abrus precatorius* and *Croton penduliflorus*). The aim of this analysis was to determine the presence of secondary plant metabolite such as Saponins, Tannins, Flavonoids, Glycosides, Steroids, Cardiac Glycoside, Terpenoid and Anthroquinone.

Column Chromatography:

Column chromatography is a solid-liquid phase-partitioning technique (Dudley, 1995), in which the absorbent is silica gel -60-mesh. The solvent used were ethylacetate, N-hexane and chloroform. A total of six samples were collected, labelled, and concentrated to dryness in the laboratory. The labelled dry samples were sent for infrared spectro photometry (IR). In the second phase of the experiment, the design still remained the same, except that *Abrus precatorius* and *Croton penduliflorus* were administered at 500, 250 and 100mg/ml.

Histopathological Test: Five rats were sacrificed after being anaesthetized with chloroform. They were then dissected and the internal organs (heart, spleen, liver and kidney) removed. Also their reproductive organs (uterus, fallopian tube, and ovaries) were removed. The organs were stored in a labeled container, containing formalin. The samples were then subjected to histopathological analysis.

when it is swallowed. The ethanolic extract used in the second treatment showed that the seed can be efficacious at 500 and 250mg/ml. However, the 250mg/ml proved to be the most effective dose inducing infertility. This is consistent in medical practice where, every efficacy is at optimum.

Table 1: Pregnancy Test of the Rats before and after First Administration

Treatment	First Mating (%) (Before treatment)		Second Mating (%) (After treatment)	
	Pregnancy (%)			
	+	-	+	-
A ₁	100	0	100	0
A ₂	100	0	100	0
A ₂	100	0	100	0
B ₁	100	0	100	0
B ₂	100	0	100	0
B ₃	100	0	100	0
C ₁	100	0	0	100
C ₂	100	0	100	0

LEGEND

A₁₋₃: *Abrus precatorius* administered at 0.6g/ml, 0.45g/ml and 0.3mg/ml respectively. B₁₋₃: *Croton penduliflorus* administered at 0.12g/ml, 0.09g/ml and 0.06g/ml respectively. C₁: Depo-Provera administered at 0.002mg/ml respectively. C₂: Sterile distilled water at unregulated or Xml per day. **First Mating**: Mating before administration of treatment. **Second Mating**: Mating after administration of first treatment.

Table 2: Pregnancy Test of the Animals after the Second Administration

Treatment	Pregnancy (%)	
	+	-
A ₁	20	80
A ₂	0	100
A ₃	100	0
B ₁	20	80
B ₂	0	100
B ₃	100	0
C ₁	0	100
C ₂	100	0

LEGEND:

A₁₋₃: *Abrus precatorius* administered at 500mg/ml, 250mg/ml and 100mg/ml respectively. B₁₋₃: *Croton penduliflorus* administered at 500mg/ml, 250mg/ml and 100mg/ml respectively. C₁: Chemical Control group, administered Depo-Provera at 0.02ml. C₂: Non-Chemical Control Group which was administered just water, at unregulated doses.

The phytochemical substances isolated from the test seeds include saponins, tanins, alkaloid, flavonoid and anthroquinones. This result is in agreement with the report of [8] and [9] who isolated similar substances from *Abrus precatorius*. The infrared spectro photometric analysis of the six samples obtained from the column chromatography showed the presence of aldehyde which has been reported by [10] to be particularly important for the antifertility activity of gossypol,

carboxylic acid such as 3-indazole carboxylic acid (lonidamine) has been reported by [11] as a male antifertility agent. It also contain alkalyting agent which have been submitted by [12] to be effective antifertility substances that can induce infertility for up to 68 to 97 days (Busulphan).

Table 3: Qualitative phytochemical screening of *Abrus precatorius* and *Croton penduliflorus*

Secondary Metabolite	Plant Extract	
	<i>Abrus precatorius</i>	<i>Croton penduliflorus</i>
Saponins	+	-
Tannin	-	-
Alkaloid	+	+
Flavonoid	-	+
Glycosides	+	-
Steroids	-	-
Cardiac Glycosides	+	+
Terpenoid	+	+
Anthroquinones	-	-

LEGEND:

- Negative to test. + Positive to test.

Table 4: Infra-Red Spectrophotometer Interpretation

Frequency (cm ⁻¹)	Functional Group	A ₁	A ₂	A ₃	B ₁	B ₂	B ₃
3000-2850	C-H for Alkane (stretch)	+	+	+	+	+	+
1450 and 1375	-CH ₃ Alkane (bend)	+	+		+	++	+
1465	-CH ₂ Alkanes (bend)			+			
3100-3000	C-H Alkanes (stretch)						
1000-650	C-H Alkanes(out of plane bend)						
3150-3050	C-H Aromatics (stretch)						
900-690	C-H Aromatic(out of plane of bend)	+	+		+	+	+
Ca. 3300	C-H Alkyne (stretch)						
2900-2800	C-H Aldehyde				+		
2800-2700							
Not Interpretatively useful	C-C Alkanes						
1680-1600	C=C Alkenes	+	++	++	+	++	+
1600 and 1475	C=C Aromatics	+					
2250-2100	C≡C Alkyne	+	+	+		+	
1740-1720	C=O Aldehyde				+		
1725-1705	C=O Ketone	+			+		+
1725-1700	C=O Carboxylic Acid				+		
1750-1730	C=O Ester						
1680-1630	C=O Amide		+		+		
1810 and 1760	C=O Anhydride						
1800	C=O Acid Chloride						
1300-1000	C-O Alcohol, esthers, esters, carboxylic acids, anhydrides	+	++	+	++	+++	++
3650-3600	O-H Alcohol, phenol (free)						
3400-3200	O-H Alcohol, phenol (H-bonded)	+	+	+	+	+	+
3400-2400	O-H Carboxylic acids				++		
3500-3100	N-H Primary and secondary amines and amides (stretch)	+	+	+	+	+	+
1640-1550	N-H Primary and secondary amines and amides (bend)	+		+			
1350-1000	C-N Amines						+
1690-1640	C=N Imines and ozimes				+		
2260-2240	C≡N Nitrides						
2270-1940	X=C=Y Allenes, ketenes isocyanates, isothiocyanates				+	+	+
1550 and 1350	N=O Nitros (R-NO ₂)				+		
2550	S-H Mercaptans						
1050	S=O Sulfoxides						
1375-1300 and 1350-1140	S=O Sulfones, sulfonyl chlorides, sulfates, sulfonamides	++	+				
1400-1000	C-X Fluoride	+			+		+
785-540	C-X Chloride		+				+
667	C-X Bromide iodide	++	+	+	++	+	+

LEGEND:

A₁: *Abrus precatorius* chloroform Extract; **A₂**: *Abrus precatorius* N-hexane Extract; **A₃**: *Abrus precatorius* Ethylacetate Extract; **B₁**: *Croton penduliflorus* Chloroform Extract **B₂**: *Croton penduliflorus* Ethyl acetate Extract; **B₃**: *Croton penduliflorus* N-hexane Extract; +: Present ++ or +: Number of peaks that fall under that functional group.

The histopathological result of the reproductive organs of the test animals showed no significant observable findings. This findings is in agreement with the submission of [13] who reported that *Ricinus communis* seed cause no damage on the

ovary only ballooning of the ethanolic extract has damage on two effective dose (500 and 250mg/ml). *Croton penduliflorus* ethanolic extract cause damage at 250mg/ml but 500mg/ml surprisingly cause no damage to liver.



Fig. 1: Section of the Uterus of rat administered, 250mg/ml showing no significant histopathological finding (Hx400)

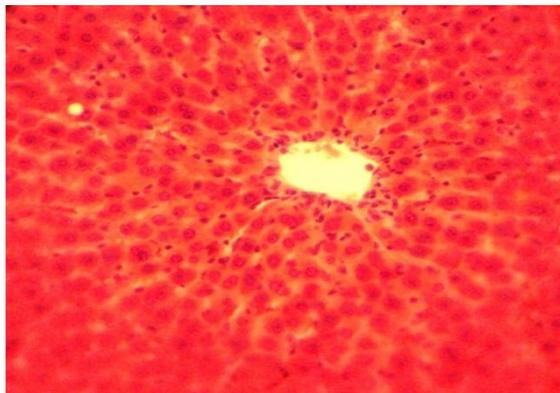


Fig. 2: Section of the liver of rat administered, *Abrus precatorius* 100mg/ml showing no significant histopathological findings

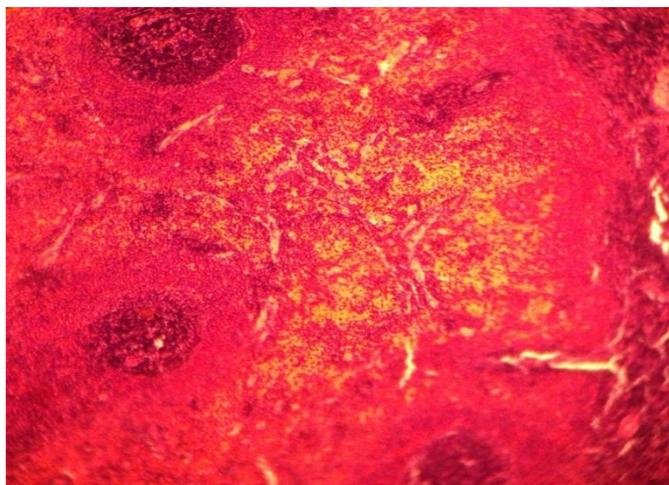


Fig. 3: Section of the spleen of rat administered *Croton penduliflorus* 250mg/ml showing no significant histopathological findings (H and Ex200)

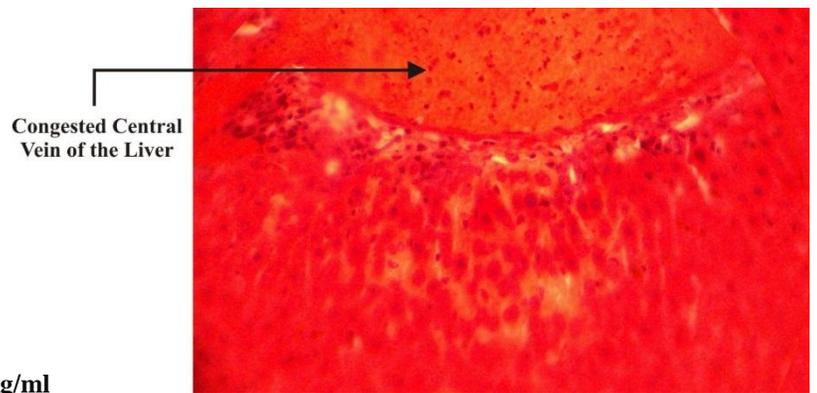


Fig. 5: *Croton penduliflorus* 250mg/ml (H and

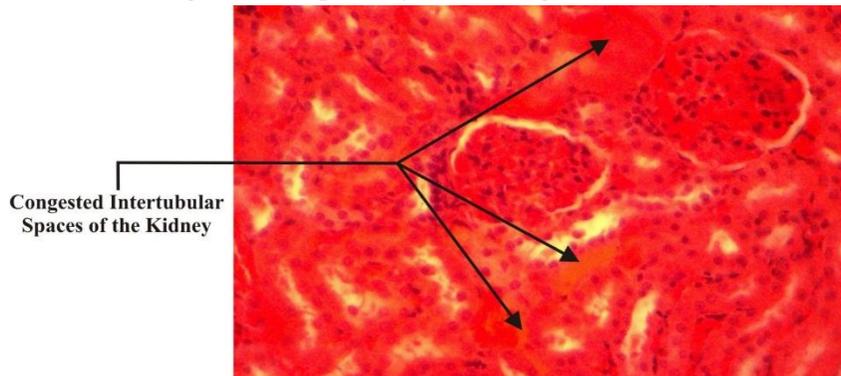


Fig. 6: *Abrus precatorius*, kidney 250mg/ml. (H and



Fig. 4: *Abrus precatorius* (Fallopian Tube 250mg/ml). No significant observable findings

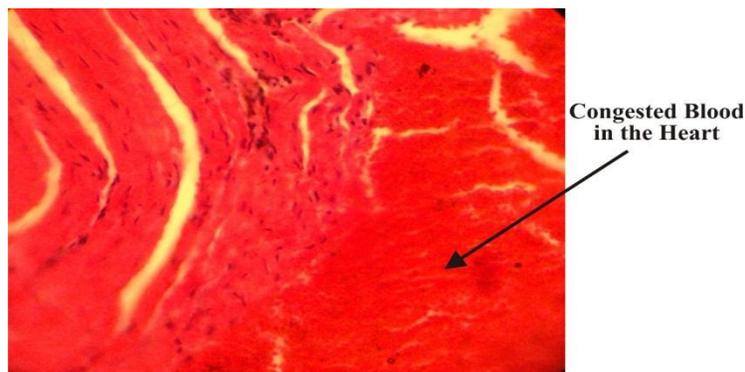


Fig. 7: Section of the heart of rat administered *Croton penduliflorus* 250mg/ml showing congestion (H and Ex400)

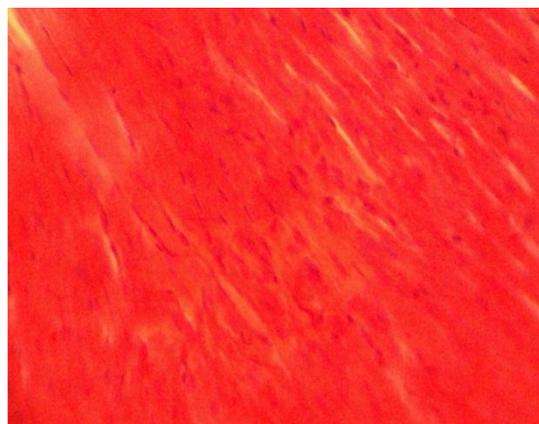


Fig. 8: Non Chemical Control (Heart); No significant Observable Findings (H and Ex400)

CONCLUSION

The pulverized seed were not effective contraceptives at any concentration. Two hundred and fifty milligrams per milliliter (250mg/ml) ethanolic extract proved to be the most effective contraceptive dose of all the two plant extracts evaluated in the albino rats. However, histopathological examination of the body organs indicated that there was congestion in the liver at this effective concentration of 250mg/ml. More so, the histopathological result showed that

500mg/ml of *Croton penduliflorus* was the only dose found to be safe without congestion of the liver and it also induced infertility in the albino rats. All the effective concentrations did not adversely affect the reproductive organs of the albino rats. Further research should be carried out on the drug action to ascertain why a higher dose did not cause damage whereas a lower concentration did.

RECOMMENDATIONS

The functional groups which were found to be abundant in the infra-spectrophotometric analysis of the seeds extract should be investigated for their presence in food and be avoided by child bearing females. The particular active ingredient (substance) that may be responsible for the congestion of the internal organs of the albino rats at the effective concentration that induced

infertility should be further investigated with a view of suppressing the gene responsible for its production, through genetic engineering.

The half-life of each of the test seeds should be determined with a view of determining when its contraceptive effect will be removed from the body system for renewed reproductive activities.

REFERENCES

1. Adedapo A.A., Abatern M.O., Akinloye A.K., Idowu S.O., and Olorunsogo
2. O.O. (2003). Morphometric and Histopathology Studies on the Effect of Some Chromatographic fractions of *Phyllatus amarus* and *Euphorbia hirta* on the Male Reproductive organs of Rats. *Journal of Veterinary Science*. 4:181-185.
3. Adedapo O.A., Omoloye O.A. and Ohores O.G. (2007). Studies on the Toxicity of an Aqueous Extract of the Leaves of *Abrus precatorius* in Rat. *Onderstepro of Journal of Veterinary Research*. 74: 31-36.

4. Agrawal, S.S, Kumar, A., Gullaiya, S. Dubey V. Nagar, A., Tiwari, P., Dhar, P. and Singh V. (2012). *Antifertility Activity of the Methanolic Bark Extract of Aegles Marmelos(l) in Male Wistar Rats. DARU Journal of Pharmaceutical Science.* 10:20-94.
5. Ahmed S., Li, O., Liu L. and, Tsui A.O, (2012). Maternal Death Averted by Contraceptive Use: An Analysis of 172 countries. *The Lancet* 380(9837): 165-171. Doi: 10.1016/50140-6736 (12) 60 827-7. PMID 22784535.
6. Akeem K.B. (2009). An Examination of the Usage of Herbal Contraceptives and Abortifacient in Lagos State, Nigeria. *Ethnobotanical Leaflet.* 13:140-146.
7. Aladesenmi A.J., Iwelewa E.D., Adebajo A.C., Akinkumi E.D., Taiwo B.J., Olorunmola F.O. and Lamikanra A. (2007). Antimicrobial and Antioxidant Activities of Some Nigerian Medicinal Plants. *African Journal of Tradition Contemporary Alternative Medicine,* 4: 173-184.
8. Allais G., Gabellari I.C., Mana O., Schiapparelli P., Terzi Ma, and Benedetto C. (2008). Migraine and Stroke, the Role of Oral Contraceptives. *Neurol Science. Supply*1: 12-16.
9. Alli A. I., Ehinmde J.O. and Ibrahim Y.K.E. (2011). Preliminary Phytochemical Screening and Antimicrobial Activities of Some Medicinal Plants Used in Ebiara Land. *Journal of Pure and Applied Science,* 4 (1) 10 16.
10. Anon 2016; Concentration - Wikipedia, the free Encyclopedia 10th September 2016, at 5:50pm. en.wikipedia.org/wiki/concentration /. IUPAC (1997) *Compendium of Chemical Terminology* 2nd ed. (*The Gold Book*) online corrected version (2006).
11. Arora R., Sighcill N., Kaur S. and Ajay D.J. (2011). Phytopharmacological Evaluation of the Seeds of *Abrus precatorius*. *Journal of Pharmacology and Toxicology.* 6: 580-588.
12. Ashidi J.S., Olaosho E.O., and Ayodele A.E. (2013). Ethno-botanical Survey of Plants Use in the Management of Fertility and Preliminary Phytochemical Evaluation of *Albelmoschus esculentus* (L) moench. *Journal of Pharmacognosy and Phytotherapy.* 5(a): 164-169.
13. Ciganda C. and Laborde A. (2003). Herbal Infusion Used to Induce Abortion. *Journal of Toxicology and Clinical Toxicology.* 41(3): 235-239.