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# The Effect of the Chromatographic Fractions of Bares Precatorius Leaf on the Reproductive Hormones Using Female Westar Rats

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***Abstract:***
***AIM:*** *To determine the effect of chromatographic fractions of Abrus precatorius extract on female reproductive Hormones in Wistar Rats.*
***STUDY DESIGN****: Female wistar rats were treated with chromatographic fractions of A. precatorius, F1, F2,F3 and F4 (30mg/kg, 60mg/kg,90mg/kg, 120mg/kg and 150mg/kg) for 4 estrus cycles. The hormones studied are Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Progesterone (P4), Estrogen (E2) and Prolactin (PRL). On the day 16 (4 estrous cycle) the animals were sacrificed, dissected and the uterus and ovaries obtained for histology. Some Liver enzymes such as Alanine Transferase (ALT) , Alanine Phosphate (ALP) and Aspartame Transferase (AST) were also studied to evaluate the effect of Abrus precatorius fractions on liver functions.*

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## I. Introduction

Phytomedicines have been used as a means of valuable medication in cultures worldwide due to presence of important phytoconstituents . (Sharma et al., 2014). 60% - 85% of the people in the world rely on plants or plant related products for their various general health issues and countering several diseases/disorder (Tiwari et al., 2012). They come in the form of medicinal teas, decoctions and crude tablets used in traditional medicine to concentrated, standardized extracts produced in modern pharmaceutical facilities.

Chromatographic fractionation according to Sharma B.K (2007) is an analytical technique commonly employed for, purification and identification of constituents of a mixture. There are many types of chromatographic fractionation e.g. liquid chromatography, gas chromatography, thin layer chromatography, column chromatography, ion-exchange chromatography, affinity chromatography, but all these employ the same basic principle. Chromatography technique for separating the components or solute of a mixture on the basis of the relative amount of each solute distributed between a moving fluid stream, called the mobile phase and a contiguous stationary phase. The mobile phase may be either a liquid or a gas, while the stationary phase is either a solid or a liquid (Calvin and Keller, 2004). Kinetic molecular motion continuously exchanges solute molecules between the two phases. Fractionation of plant extract and purification of active principle optimizes their potencies and also found to extend the activity of the plant extracts (Okoli, 2005).

A couple is classified as infertile if they have failed to conceive after 12 months of regular sexual intercourse without the use of contraception. (Nordquist, 2016). It is the inability of a sexually active, non-contraceptive couple to achieve pregnancy in one year. In Africa, its prevalence is particularly high in sub-sahara African ranging from 20% to 60% (Ogunniyi, 1999). Herbal materials have been used in preparing remedies for infertility and few examples are: *Carica papaya* Linn. (Cultivated) Caricaceae (Kamatenesi-Mugisha and Oryem-Origa,2005), *Jatropha curcas* L. (Wild) Euphorbiaceace (Diame,2010) and *Polygonum hydropiper* (Polygonace).

Occasionally, in bioassay-guided plant purification, crude extracts are seen to exhibit more biologic activity than the active purified compound from the same extract (Rasoanaivo *et al*., 2011). At other times, though, fractionation of plant extracts and purification of the active principles, optimize their potencies (Nwodo *et al*., 2011). Crude *Abrus precatorius extract* has been reported to boost female reproductive hormone especially FSH and LH levels in wistar rats (Ogbuehi *et al*, 2015).

This study seeks to determine whether further purified fractions of *the Abrus precatorius leaf* crude extract will produce a diminished, enhanced or sustained hormonal activity in female rats.

## II. Materials and Method

**2.1 Plant Samples**

**2.1.1 Collection**

The leaves of *A. precatorius* were collected at Orlu, Imo state and were authenticated by Dr. I. Ekeke of the Department of Plant Science and Biotechnology, University of Port Harcourt. A voucher specimen was then deposited in the Herbarium of the Department of Plant Science and Biotechnology with voucher specimen number UPH/P/120.

**2.1.2 Extraction**

375g of pulverized *A. precatorius* leaf were successively macerated with 70% methanol in a jar for 72 hours. The extract obtained were then concentrated in a rotary evaporator to a very small volume which was dried on a water bath. Dried methanol extract was selected and stored in an air-tight container in a cool place.

**2.1.3 Qualitative phytochemical screening**

The pulverized samples of the leaves of *A.* precatorius were analyzed for the presence of alkaloids, anthraquinones, cardenolides, tannins, flavonoids and saponins. The methods were adopted from Sofowora, (1993), Trease and Evans, (2002) and Harborne (1973).

**2.1.4 ALKALOID TEST**

About 0.5g 0f the powdered plant was extracted by boiling with 5% HCl on a steam bath and then filtered. 1ml of the filtrate was treated with few drops of Mayer's reagent, another 1ml with Dragendorff's reagent, another with Hager's reagent and finally the last 1ml was treated with few drops of Picric acid.

**2.1.5TESTS FOR ANTHRAQUINONE**

**1 . Borntrager's test for free Hydroxyanthraquinones**

About 3g of each powdered plant was shaken with chloroform and filtered. To the filtrate were added 10% ammonia solution. The presence of pink, red or violet colour in the ammonia phase indicates a positive result.

**2.Test for Anthraquinone O-Glycosides**

About 3g of each powdered plant was boiled with 10% HCL and filtered. The filtrate was shaken with chloroform and 10% ammonia solution was added to partition. A pink red or violet colouration in the ammonia solution indicated the presence of anthraquinone O-Glycoside.

**3.Test for Anthraquinone C-Glycosides**

About 3g of each powdered plant was boiled with 10% HCl and 15% $FeCl_3$ solution and filtered. The filtrate was shaken with chloroform and 10% ammonia solution was added to partition. A pink red or violet colouration in the ammonia solution indicated the presence of anthraquinone C-Glycoside.

**2.1.6 TANNINS TEST**

**Ferric Chloride Test.** A small quantity of each powdered plant was extracted by boiling with distilled water and filtered. 5% Ferric Chloride reagent was added. A blue-black, green or blue green precipitate is taken as evidence for the presence of Tannins.

**2.1.7 FLAVONOIDS TEST**

**Sodium hydroxide Test.** Few quantity of each of the powdered plant was extracted with distilled water by boiling and then filtered. To the filtrate equal volume of 10% aqueous sodium hydroxide was added to produce a yellow colouration. A change in colour from yellow to colourless on addition of equal volume dilute hydrochloric acid is indicative of the presence of flavonoids. (Trease and Evans, 2002).

**2.1.8 FROTHING TEST FOR SAPONINS**

About 1g of each of the powdered plant was boiled with 5ml of distilled water and filtered. To the filtrate about 3ml of distilled water was further added and shaken vigorously for about 5minutes. Frothing which persisted on warming was taken as an evidence for the presence of saponins. (Sofowora, 1993).

**2.1.9 TEST FOR CARDIAC GLYCOSIDES**

**1. Lieberman-Burchard's Test for steroid.**

About 5g of each powdered plant was extracted with 10% HCl by boiling on the water bath and then filtered. The extract was reduced to dryness and about 0.5g was dissolved in 2ml acetic anhydride, the solution was cooled well in ice. Concentrated sulphuric acid was carefully poured down the side of the test tube using

Pasteur pipette to form a layer. A colour change from violet to blue to green indicated the presence of steroidal nucleus (the aglycone portion of a cardiac glycoside).

**2.Salkowski's Test**

About 0.5g of the extract was dissolved in 2ml chloroform and concentrated sulphuric acid was added carefully to form a layer. A reddish brown colour at the interface was indicative of the presence of a steroidal nucleus.

**3.Kedde's test for Lactone ring.**

1ml of an 8% solution of the extract in Methanol was mixed with 1ml of a 2% solution of 3, 5-dinitrobenzoic acid in methanol and 1ml of a 5% solution of sodium hydroxide. An immediate violet colour indicated the presence of cardenolide..

**2.2 CHROMATOGRAPHIC FRACTIONATION:**

A clean glass column was plugged with cotton wool at the base to support the packing material. Silica gel was then packed into the column length with gentle tapping at intervals to compact the silica gel particles tightly. This was stopped when the packing length reached the required height. N-hexane was then run through the column to fill it. The plant extract was extract then mixed, with dry silica gel and the mixture evenly transferred to the column head. Gradient elution then commenced, powered by vacuum pump using n-hexane, n-hexane + ethylacetate, ethylacetate, ethylacetate + methanol, and methanol, in that sequence, as elution solvents. Fractions were collected serially during elution and were monitored by Thin Layer Chromatography (TLC). Eluates with similar chromatographic patterns were pooled together, concentrated and dried. This process yielded four fractions which were used for the study.

**ORDER OF ELUTION:**

| HEXANE | ETHYLACETATE | METHANOL |
|---|---|---|
| 100 | - | - |
| 75 | 25 | - |
| 50 | 50 | - |
| 25 | 75 | - |
| - | 100 | - |
| | 75 | 25 |
| | 50 | 50 |
| | 25 | 75 |
| | - | 100 |

**2.3 ACUTE TOXICITY DETERMINATION**

**LORKES METHOD** (Akhila et al.,2007)

This assay was performed in two phases:

**PHASE 1**

Animals were grouped into three groups with three mice per group, each receiving a dose intraperitoneally. The mice were monitored for 24 hours for mortality and general behavior.

**PHASE 2**

Twenty four hours after Phase 1 study, three animal groups each having one mouse were given doses of extracts intraperitoneally. The mice were again monitored for 24 hours.

The geometric mean of the lowest dose ($D_1$) that killed mice and the highest dose ($D_2$) that did not kill the mice was taken as median lethal dose, $LD_{50.}$

$LD_{50} = \sqrt{(D_1 \times D_2)}$.

**2.4 ANIMAL EXPERIMENTS**

One hundred and ten (110) female wistar rats in the same estrus cycle stage were divided into twenty-two(22) groups of five rats each. All the rats in the groups were weighed every 3 days and the weight recorded. (Ogbuehi *et al*, 2015). Group 1 (Control) received 0.5mls, Phosphate Buffer Solution (PBS); Group 2 (Standard) received Clomiphene citrate, 10mg; Group 3-7. Received 30mg/kg, 60mg/kg, 90mg/kg, 120mg/kg and 150mg/kg of F1 fraction of A. *precatorius* extract. Group 8-12 received 30mg/kg, 60mg/kg, 90mg/kg, 120mg/kg and 150mg/kg of F2 fraction of Abrus *precatorius* extract. Group 13-17 received 30mg/kg,60mg/kg,90mg/kg,120mg/kg and 150mg/kg of F3 fraction of A. *precatorius* extract and Group 18-22

received 30mg/kg, 60mg/kg,90mg/kg,120mg/kg and 150mg/kg of F4 fraction of A. *precatorius* extract respectively. The hormones studied are Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Progesterone (P4), Estrogen (E2) and Prolactin (PRL). On the Day 16 of treatment, animals were fasted and opened up under anaesthesia. Blood samples taken from abdominal aorta for hormonal profile and biochemical analysis.

**2.5 Statistical Analysis**

Data were analyzed using Student T-test for paired sample and One Way Analysis of Variance (ANOVA). The results were considered significant at $p<0.05$. All results are expressed as Mean ± Standard Error of Mean (S.E.M).

**2.6 Ethical Approval**

Ethical approval was obtained from the Research Ethics Committee, of the College of Health Sciences, University of Port Harcourt. Also, animal handling and test procedures were in accordance to the guiding principles on animal use in experiments**.**

## III. Results and Discussion

**3.1 PERCENTAGE YIELD OF EXTRACT.**

Weight of the extract that was dried = 47g

Total weight of the plant material = 375g

% Yield = (Weight Of Dried Extract / Weight Of Plant Material) X 100

= (47/375) x 100

= 12.5%

**3.2 ACUTE TOXICITY DETERMINATION ($LD_{50}$)**

$LD_{50} = \sqrt{(D_1 \times D_2)}$

D1= the lowest dose that did killed =5000mg

D2= the highest dose that did not kill = 3500mg

$LD_{50} = \sqrt{5000x3500} = \sqrt{17,500,000}$

Median lethal dose ($LD_{50}$) =4183.3mg

**Table 4.1:** Phytochemical screening result of *A. precatorius* Extract

| | METABOLITE CLASS | TEST | RESULT |
|---|---|---|---|
| 1 | Alkaloid | Dragendorf's test | +ve |
| 2 | | Mayer's test | +ve |
| 3 | | Hager's test | +ve |
| 4 | Flavonoid | Shinoda test | +ve |
| 5 | | Alkali test | +ve |
| 6 | Borntrager's | Free anthraquinone | -ve |
| 7 | | Combined anthraquinone | -ve |
| 8 | Triterpenoids | Liebermann-Burchard | +ve |
| 9 | | Salkowski's | +ve |
| 10 | Oils | Fixed oils | +ve |
| 11 | Carbohydrates | Molisch | +ve |
| 12 | | Fehlings | +ve |
| 13 | Cardenolides | Keller-Kiliani | +ve |
| 14 | | Kedde | +ve |
| 15 | Cyanogenic | | |
| 16 | Saponins | Frothing | +ve |
| | | | |

**TABLE 4.2: CHARACTERISTICS OF THE FRACTIONS**

| Methanol Extract of *Abrus precatorius* Leaf | Products of fractionation | Rf Value = Distance spot travels/distance solvent travels | TLC coloration at 254nm Wavelength |
|---|---|---|---|
| | **F1** | 0.58 | Yellow |
| | **F2** | 0.10 | Grey |
| | **F3** | 0.24 | Yellow |
| | **F4** | 0.24 | Brown |

**TABLE: 4.3: EFFECT OF *A. precatorius* FRACTION 1 ON REPRODUCTIVE HORMONES**

| | FSH (MIU/ML) | LA (MIU/ML) | $E_2$ (ng/ml) | PG (ng/ml) | PRL (ng/ml) |
|---|---|---|---|---|---|
| **CONTROL** | 0.19±0.01 | 1.94±0.16 | 89.00±0.01 | 2.60±0.03 | 15.00±0.01 |
| **30mg/kg** | 3.20±0.01* | 2. 36±0.01* | 85.00±0.01 | 4.20±0.01 | 12.00±0.01 |
| **60mg/kg** | 4.70±0.01 | 2. 48±0.03 | 86.00±0.01 | 4.50±0.01 | 11.00±0.01 |
| **90mg/kg** | 5.20±0.01 | 2. 72±0.01 | 84.00±0.01 | 4.30±0.01 | 13.00±0.03 |
| **120mg/kg** | 6.20±0.06 | 2.93±0.06 | 83.00±0.01 | 4.30±0.01 | 11.00±0.01 |
| **150mg/kg** | 6.80±0.01 | 3. 08±0.01 | 83.00±0.01 | 4.40±0.01 | 10.00±0.01 |
| **Clomiphene citrate 10kg** | 7.20±0.01 | 4. 23±0.01 | 80.00±0.01 | 5.60±0.03 | 12.00±0.01 |

Data represents the mean **± SEM for each group of rats n = 5**

***** = significance different at P< 0.05 with respect to control FSH: Follicle Stimulating Hormone, LH: Luteinizing Hormones, $E_2$ Estradiol, PG: Progesterone, PRL: Prolactin.

**Table 4.4:** EFFECT OF *A. precatorius* FRACTION 2 ON REPRODUCTIVE HORMONES

| | FSH (MIU/ML) | LH (MIU/ML) | E2 (ng/ml) | PG (ng/ml) | PRL (ng/ml) |
|---|---|---|---|---|---|
| **Control** | 0.90±**0.01** | 1.94±**0.01** | 82.00±**0.07** | 3.50±**0.07** | 1.42±**0.03** |
| 30mg/kg | 1.67±0.01* | 2.08±0.01* | 84.00±0.07* | 4.10±0.03* | 1.40±0.01 |
| 60mg/kg | 1.78±0.01 | 3.20 ± 0.06 | 86.00±0.01 | 4.20±0.01 | 1.40±0.01 |
| 90mg/kg | 1.89±0.01 | 4.32±0.01 | 86.00±0.07 | 4.30±0.07 | 1.40±0.01 |
| 120 mg/kg | 2.23±0.01 | 5.51±0.01 | 90.00±0.07 | 5.00±0.07 | 1.43±0.01 |
| 150mg/kg | 2.58±0.01 | 6.70 ± 0.01 | 95.00±0.01 | 5.60±0.01 | 1.39±0.01 |
| Clomiphene citrate10kg | 3.32±0.01 | 7.20±0. 06 | 93.00±0.07 | 4.30±0.07 | 1.20±0.01* |

Data represents the mean ± SEM for each group of rats n = 5

***** = significance different at P< 0.05 with respect to control FSH: Follicle Stimulating Hormone, LH: Luteinizing Hormones, $E_2$ : Estradiol, PG: Progesterone, PRL: Prolactin

**TABLE 4.5: EFFECT OF *A. precatorius* FRACTION 3 ON REPRODUCTIVE HORMONES**

| | FSH (MIU/ML) | LH (MIU/ML) | $E_2$ (ng/ml) | PG (ng/ml) | PRL (ng/ml) |
|---|---|---|---|---|---|
| Control | 0.90 ± 0.01 | 1.94 ± 0.01 | 89.00 ± 0.07 | 6.60 ± 0.20 | 1.42 ± 0.03 |
| 30mg/kg | 1.61 ± 0.01* | 3.75 ± 0.01* | 80.00 ± 0.7* | 4.10±0.20* | 1.75 ± 0.01 |
| 60mg/kg | 1.54 ± 0.01 | 3.51 ± 0.07 | 81.00 ± 0.07 | 4.20±0.07 | 1.66 ±0.07 |
| 90mg/kg | 1.29 ±0.01 | 2.35 ± 0.01 | 83.00 ± 0.01 | 4.50 ±0.07 | 1.33 ± 0.01 |
| 120mg/kg | 1.96 ±0.01 | 1.77±0.01 | 86.00±0.06 | 4.30±0.07 | 1.00±0. 10 |
| 150mg/kg | 0.96±0.01 | 1.75±0.01 | 88.00±0.07 | 4.40±0.07 | 1.17±0.02 |
| Clomiphene citrate 10mg | 1.32±0.01 | 2.58±0.08 | 80.00±0.07 | 4.30±0.07 | 2.90±0.10 |

Data represents the mean **± SEM for each group of rats n = 5**

***** = significance different at P< 0.05 with respect to control FSH: Follicle Stimulating Hormone, LH: Luteinizing Hormones, : **$E_2$** Estradiol, PG: Progesterone, PRL: Prolactin

**TABLE 4.6: EFFECT OF A. precatorius FRACTION 4 ON REPRODUCTIVE HORMONES**

| | FSH (MIU/ML) | LH (MIU/ML) | $E_2$ (ng/ml) | PG (ng/ml) | PRL (ng/ml) |
|---|---|---|---|---|---|
| Control | 0.92±0.01 | 1.94±0.01 | 89.00±0.70 | 2.10±0.01 | 21.00± 0.01 |
| 30mg/kg | 1.25±0.01* | 2.36±0.01* | 81.00±0.70* | 3.90±0.10* | 18.00±0.01* |
| 60mg/kg | 1.32±0.01 | 2.53±0.01 | 80.00±0.01 | 4.20±0.10 | 15.00±0.01 |
| 90mg/kg | 1.37±0.01 | 2.64±0.01 | 80.00±0.01 | 4.10±0.01 | 16.00±0.01 |
| 120mg/kg | 1.39±0.01 | 2.73±0.01 | 82.00±0.01 | 4.30±0.01 | 16.00±0.01 |
| 150mg/kg | 1.43±0.01 | 2.82±0.01 | 83.00±0.01 | 4.50±0.01 | 15.00±0.01 |
| Clomiphene citrate 10mg | 3.24±0.01 | 3.73±0.03 | 79.00±0.01 | 2.00±0.01 | 13.00±0.01 |

Data represents the mean **± SEM for each group of rats n = 5**

***** = significance different at P< 0.05 with respect to control FSH: Follicle Stimulating Hormone, LH: Luteinizing Hormones, $E_2$ : Estradiol, PG: Progesterone, PRL: Prolactin.

## IV. Discussion and Conclusion

The Preliminary phytochemical investigation of *Abrus precatorius* extract revealed that the leaf contains alkaloids, Triterpenes, glycosides, tannins, flavonoids, carbohydrates, steroids, oils and saponin. Upon fractionation, four fractions were realized: FI, F2, F3 and F4 with Retention factor (Rf) value of 0.58, 0.10, 0.24 and 0.24 respectively.

Median Lethal dose, LD50 for a particular substance is the amount that can be expected to cause death of half (50%) of a group of some particular animal species, usually rats or mice when administered by a particular route ( Randhawa et al.,2009). Acute toxicity test yielded an $LD_{50}$ value of 4183mg/kg. The lower the value, the more toxic the drug and *vice versa*. *Abrus precatorius* $LD_{50}$ value that is so high indicates its relative safety compared to specimens with $LD_{50}$ pegged on microgram`and nanogram levels per kilogram body weight (Fleming and Hunt, 2000).

Administration of the Fraction 1(FI), Fraction 2(F2) and Fraction 4(F4) caused a significant increase ($p<0.05$) in the serum levels of Luteinizing hormone (LH) and Follicle Stimulating Hormone (FSH) from table 1,2 and 4 in a dose dependent manner which is comparable to Clomiphene citrate. There was no increase in the level of estrogen and progesterone with the same Fraction 1, 2 and 4. Prolactin serum level was also reduced with Fraction 1,2 and 4 respectively. Ironically, Fraction 3 caused a decrease in the serum level of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) with an increase in the estrogen serum level. Also, the serum level of progesterone decreased significantly ($p<0.05$). FSH activates the growth and maturation of ovarian follicles (folliculogenesis) directly on the receptors located on granulosa cells (Simoni, M et al., 1995). Luteinizing hormones (LH) stimulates growth of corpus luteum and progesterone release (Miller, et al 2002; O' Rahilly, 1973). LA surge is necessary for Ovulation and also for final follicular growth. Hence, high level of these hormones (LH & FSH) as observed with Fraction F1, F2 and F4 may also mean that higher numbers of follicles will develop and there will be Graffian follicle formation. Therefore, the *Abrus precatorius* Fraction F1,F2, and F4 may augment female reproductive hormone thereby boosting fertility. Normally as follicles develop, estrogen level rise which helps to stimulate the endometrium (Mishra et al; 2002). The experiment also showed that F1, F2 and F4 did not increase the level of prolactin .High level of prolactin is one of the causes of infertility (Avasthi Kumkum, et al. 2006). Progesterone initiates endometrial proliferation towards successful implantation. Reasonable and significant level of estrogen ($P<0.05$) was observed with the F1, F2, and F4.Estrogen when combined with FSH will activate granulosa cell production and this potentiates folliculogenesis and ovulation respectively. (Telefo, PB et al; 1998). Progesterone prepares the wall of the uterus so that the lining is able to accept fertilized egg and also that the egg can be implanted (Montaserti, et al., 2007). If the egg is fertilized progesterone and estrogen are influential in the prevention of further egg release via feedback mechanism (Montaserti; et al 2007). LH stimulates ovulation growth of corpus luteum and progesterone release (Miller, *et al*; 2002; O 'Rahilly, 1973). LH acts to augment progesterone secretion by granulosa cells, which stimulates FSH release at midcycle (Bowman and Rand, 1980). Fraction 3 administration induces reduction in serum LH & FSH level. The effect of this reduction in the serum level of FSH may be due to increase serum levels of estrogen observed in the table. Therefore it could be that Fraction 3, F3 of purified A. *precatorius* leaf extract does not augument ovulation to promote female reproductive hormone.

Conclusively, this study suggests that the fractionation of crude extract of the leaf of Abrus *precatorius* is a phytoestrogen and could be formulated as a female reproductive hormone booster to augment female reproductive hormones in cases of female infertility caused by hormonal imbalance and anovultio. *Abrus precatorius* methanol extract has demonstrated a significant hormone boosting effect and could offer candidate drugs or lead compounds of African biodiversity in the management of infertility arising from hormonal deficiency in women.

## References


[1]. Adetoro, O.O, Ebomoyi, E.W (1997) "The Prevalence of Infertility in a rural Nigerian community. African Journal of Medicine and Medical Sciences''. 20:23-27 (Pubmed).

[2]. Akhila, J.S., Deepa,S and Alwar, M.C (2007). "Acute toxicity studies and determination of median lethal dose". Current Science. **93**(7) 917-920 Albino Mice.

[3]. Allan, A.L.,William, J.W., Bettina, H (2017) "Centre for Occupational and Environmental Medicine''.vol 3.

[4]. Alvaro Viera, R. Subsidio Para O Estudo (1959). "Da Flora Medicinal Da Guinea Portuguesa Agencia-Geral Do Ultramar Lisboa''. Am. J. Pharmacol. Toxicol. **10**(2): 40.45

[5]. Anbu, J., Ravichandiran, V., Sumithra M., Chowdary, S.B., Swaroop Kumar,S. Kannadhasan, R. and Kumar, R.S (2011). "Anticancer activity of petroleum ether extract of

[6]. Antioxidant and Free Radical Scavenging Capacity of Seed and Shell Essential Oils Extracted from Abrus precatorius L''. Antioxidants 3, 278-287

[7]. Arthur J. Vander, James H. Sherman, Dorathy S. Luciano (2001) "Human Physiology:The Mechanism of body function" 8th Ed.

[8]. Asha, A. (2013) "Anti Oogenic Evaluation of Seed Extract of Abrus precatorius T. in Swiss Albino Mice''. Int. Res. J. Biological Sci; 2(6):27-30 Asian J. Pharm. Clin. Res. (**7**)1, 57-62

[9]. Attal, A.R., Otan, K.V., Shete, R.V., Upasani, C.D., Nandgude, T.D (2010) "Abrus precatorius Linnaeus: a phytopharmacological review''. J. Pharm1 Res. 3 (11):2585-2587.
[10]. Avasthi Kimkum (2006). "The Journal of Obstertrics And Gynecology of India''. Vol 56, 68 -71.
[11]. Barve, K.H and Ojha, N (2013) "Effective detoxification of Abrus precatorius Linn. seeds by Shodhana".
[12]. Bates,R and Riddle, O (1935). "The preparation of prolactin''Journal of Pharmacology and Experimental Therapeutics. 55 (3): 365–371.
[13]. Beverly G Reed., Bruce R. Carr.,(2015) "The Normal Menstrual Cycle and the Control of Ovulation". University of Texas South Western Medical Center, Dallas. 16-114.
[14]. Bhatia, M. NAS., Gupta, S. (2013) "Abrus precatorius L: An Evaluation of Traditional Herb" . Indo American Journal of Pharmaceutical Research. ISSN No:2231-6876
[15]. Bobbarala, V and Vadlapudi V. (2009) "Abrus precatorius L seed extracts' antimicrobial properties against clinically important bacteria"
[16]. Burkhill, I. H (1966). "Dictionary of the economic products of the Malay peninsula".
[17]. Calvin, J. and Roy, Keller (2017) "A. Dynamics of Chromatography". University Of Utah, Salt Lake City, UT, United States., Encyclopedia Britannica.
[18]. Canella, C. F. C., C. H. Tokarnia and J. Dobereiner (1966). "Experiments with plants supposedly toxic to cattle in Northeastern Brazil, with negative results. Cerebral Venous Thrombosis Due to Abrin Toxicity". A Case Report Chem. Pharm. Bull. **14**: 890.
[19]. Chaudhari, S.K., Sharma, R., Pawar, S.P., Kashikar, A.V (2012) "Pharmacological activities of Abrus precatorius Linn – A Review" . International Journal of Ayurvedic and Herbal medicine; 2:336-348.
[20]. Chaudhary, G., Goyal, S., Poonia, P., Lawsonia Linnaeus (2010) "A phytopharmacological review". Int. J Pharm Sc. Drug Res. 2(2):91-98.
[21]. Chimsamy, M., Finnie, J.F., Van Staden, J (2011). "The ethnobotany of South African medicinal orchids" . South African Journal of Botany.vol 77; pp.2-9.
[22]. Das, S. K (1955). "Medicinal, Economic and Useful Plants of India". Bally Seed Store, West Bengal.
[23]. De Wet, H., Nciki, S., Va Vauren, S.F (2013) "Medicinal plants used for the treatment of various skin disorders by a rural community in northern Maputaland, South Africa" Journal of Ethnobiology and Ethnomedicine, 9: P.51.
[24]. De Wet, H., Nikwanyana, M.N., Van Vuuren, S.F (2010) "Medicinal plants used for the treatment of diarrhea in northern Maputaland, Kwazulu -Natal Province, South Africa" Journal of Ethnopharmacology, 130: pp. 284-289.
[25]. De Wet, H., Nzama, V.N., Van Vuiren, S.F (2012) "Medicinal plants used for the treatment of sexual transmitted infections by lay people in northern Maputaland, Kwazulu-Natal province, South Africa" South African Journal of Botany, 178; pp-12-20
[26]. Dhawan, B. N., Patnaik G.K., Rastogi, R.P.,Singh,K.K and J. S. Tandon (1977) Der Pharma Chemica, **7**(8):112-11
[27]. Diame, G.L.A (2010) " Ethnobotany and ecological studies of plants used for reproductive health: A case study at BiaBiosphere reserve in the Western region of Ghana. Accra, Ghana" UNESCO.
[28]. Dimetry, N.Z., El-Gengaihi S., Reda A.S and Amer SAA (1990) "Toxicity of some compounds Isolated from Abms Precatirous L. seeds towards the two spotted spider mik tetranychus urtical Koch" J. Research gate 65 (s): 99 – 101.
[29]. Duke, J. A (1992). "Handbook of phytochemical constituents of GRAS herbs and other economic plants. Boca Raton, FL" CRC Press. Egyptian Dermatology Online Journal **7** (2): 4
[30]. Elisabetsky, E., Figueiro, W. and G. Oliveria. (1992) "Traditional Amazonian nerve tonics as antidepressant agents, Chaunochiton kappleri. A case study"
[31]. Evans, A.M., Petersen, J.W., Sekhon, G.S., DeMars, R (1989). "Mapping of prolactin and tumor necrosis factor-beta genes on human chromosome 6p using lymphoblastoid cell deletion mutants". Somatic Cell and Molecular Genetics. Fitoterapia**61**(1): 61–63.
[32]. Fluhmann, C.F (1938). "Estrogenic Hormones: Their Clinical Usage". Cal West Med. **49** (5): 362–6. PMC 1659459 . PMID 18744783.
[33]. Friesen, H., Guyda, H and Hardy, J (1970). "The biosynthesis of human growth hormone and prolactin" The Journal of Clinical Endocrinology and Metabolism. **31** (6): 611–24. doi:10.1210/jcem.316-611. PMID 5483096
[34]. Fritz F. Parl (2000). "Estrogens, Estrogen Receptor and Breast Cancer" IOS Press. pp. 4–. ISBN 978-0-9673355-4-4.
[35]. Ganesan, R and Ettiyan, R (2015). "Abrus precatorius Induced Hemorrhagic Colitis" American Journal of Pharmacology and Toxicology. 10(2): 40, 45.
[36]. Georgewill, O.A., Georgewill, U.O. (2009) "Evaluation of the anti-inflammatory activity of extract of Abrus precatorius" East .J. Med. 2009; 14:23-25.
[37]. Gupta, J and Gupta, A (2016) "Isolation and characterization of flavonoid glycoside from leaves of Abrus precatorius" Int. J. Chem. Studies 2016; **4**(1): 14-17.
[38]. Guyton A.C: (1984) "Physiology of the Human Body" 6th ed. Philadelphia: Saunder College publishing.
[39]. Guyton AC and Hall JE (2006) " Textbook of Medical PhysiologyW" 11th Edition.Elservier Saunders. Philadelphia. pp1011-1025
[40]. Hair, W.M, Gubbay O, Jabbour, H.N., Lincoln, G.A (2002). "Prolactin receptor expression in human testis and accessory tissues localization and function. Molecular Human Reproduction" **8** (7): 606–11. doi:10.1093/molehr/8.7.606. PMID 12087074.
[41]. Hedberg, I. O., Hedberg, P. J., Madati, K. E., Mshigeni, E. N., Mshiu and G. Samuelsson (1983). "Inventory of plants used in traditional medicine in Tanzania. Part III. Plants of the families"
[42]. Jain, S. P. and D. M. Verma.(2010) "Medicinal plants in the Folk-lore of Northern Circle Dehradun Up India"
[43]. Kamatensis – Mugisha, M., Oryem Origa, H., Olwa-Odyek.(2007) "Medicinal plants used in some gynecological morbidity ailments in Western Uganda" African Journal of Ecology. 45(1):34-40.
[44]. Kametenesis-Mugisha, H. Oryem-Origa (2007) "Medicinal plants used to induce labour during childbirth in Western Uganda" Journal of Ethno pharmacology, 109 : pp. 1-9.
[45]. Kartika, R, Rao, CV, Pushpangadan P, Trivedi SP, Reddy GD (2010). "Exploring the Protective Effects of Abrus precatorius in HepG2 and N-Nitrosodiethylamine-Induced Hepatocellular Carcinoma in Swiss Albino Rats" . Kerba Polonica. **56** No. 3, 63-80
[46]. King, T.L and Brucker, M.C (2010). "Pharmacology for Women's Health" Jones & Bartlett Publishers. pp. 372–373. ISBN 978-1-4496-5800-7.
[47]. Kokwaro, J. O (1976) "Medicinal Plants 4of East Africa" East Afr. Literature Bureau, Nairobi.
[48]. Larsen, C.M and Grattan, D.R (2012). "Prolactin, neurogenesis, and maternal behaviors. Brain, Behavior, and Immunity." **26** (2): 201–9. doi:10.1016/j.bbi.2011.07.233. PMID 21820505.
[49]. Lébri, M., Tilaoui, M., Bahi, C., Achibat, H., Akhramez ,S., Fofié, YBN., Gnahoué, G., Lagou, SM., Zirihi, GN., Coulibaly A, Zyad A, Hafid Aand Khouili M. (2015) " Biotechnology and Production of Anti- Cancer Compounds" Mol Pharmacol 51:193-200.
[50]. Malhi, B. S. and V. P. Trivedi. (1972). "Vegetable antifertility drugs of India" Q. J. Crude Drug Res **12**: 19–22.

[51]. Malpani, Aniruddha. Malpani, Anjali. Malpani Infertility Clinic(2015) "Cervical Mucus And Fertility: The Cervical Factor" chapter 16.

[52]. Maregesi, S.M,, Mwakigonja, A.R and Urio,P (2016). "Toxicity evaluation of Abrus precatorius seeds collected from Bunda District,Tanzania"

[53]. Marieb, Elaine (2013) "Anatomy &Physiology of the body" Benjamin-Cummings. p. 903. ISBN 9780321887603.

[54]. Martin-Herrera D, Abduala S, Benjumea D, Gutierrez-Luis J (2008) "Diuretic activity of some Withania aristata Ail fraction" J. Ethnopharmcol. 117:496-499 (Pubmed)

[55]. Mascarenhas MN, FlaxmanSR, Boerma T, Vanderpoel S, Stevens GA, (2012). "National, regional, and global trends in infertility: a systematic analysis of health surveys" Trends in prevalence. WHO, GENEVA.

[56]. Mensah AY, Bonsu AS, FleischerTC (2011). "Investigation of the Bronchodilator Activity of Abrus precatorius"

[57]. Mensah, YA., Bonsu, SA., Fleischer, CT.(2011) "Investigation of the Bronchodilator Activity of Abrus precatorius" Volume 6, issue 2 : Article 003.

[58]. Midrarullah, H., Sher Attaullah., Samiullah Sikandah and M.S. Ali.(2014) "Traditional uses of medicinal plants for the treatment of livestock ailment in Udigram Swat. Kyhber Pakhtunkhwa Pakistan" Res. Opin. Anim-Vet.Sci. 4:138-141

[59]. Miller, W.G.,Waymack PP, Anderson FP. (2002) "Performance of four homogenous direct methods for LDL – Cholesterol" . Clinical Chemistry, 48(3), 489 – 498. Ministry of Agriculture and Cooperatives, Kuala Lumpur, Malaysia. Vol. 1.

[60]. Mishra, R., Baraja, V., Gupta, J. (2002) Journal of Obstertrics Gynecology India, 2002, 52: 40 -43

[61]. Monago, C.C and Alumanah, E.O (2005) "Antidiabetic Effect of Chloroform -Methanol Extract of Abrus precatorius Linn Seed in Alloxan Diabetic Rabbit" J. Appl.Sci. Environ. Manag.9(1):85-8. Monobind Inc. (2012). Estradiol test system. Monobind Inc. Lake Forest, CA 92630,

[62]. Montaserti, A., Pourheydar, M., Khazaci, M., Ggorbani, R (2007) "Antinfertitlity effects of physalis alke alcohol extract in female rats" Iran J Reprod Med – 2007, 5: 13 -16

[63]. More, R.A and Khandagle, A. J (2011) "Antifertility potential of Abrus precatorius against male albino mice" Indian Str. Res. J. Vol. 1 issue 0ne.Nat Acad. Sci. Lett. (India) 1981; **4**(7): 269–271.

[64]. Moshi, M.J., Kagashe, G.A.B., Mbwambo, Z.H (2005) "Plants used to treat epilepsy by Tanzanian traditional healer" J. Ethnopharmacol. 97:327-336

[65]. Nordquist, Christian (2016) "Infertility: Causes, Diagnosis,Risks, Treatments". Medical News Today. Medileticon, Intl., 21.Web.

[66]. O' Rahilly R. (1973). "The Embroyology and Anatomy of the uterus" In: Norris H.J; Hertig, A.T; Abell M.R. (Eds). The uterus, PP.87 -98 – Baltmore: Williams and Wilkins.

[67]. Obi. E., Oriaskwe, O.E.I., Asomiyha, L.A and Udemezue, O.O (2004). "The hepatotoxic effect of halo fantirne in guirea pigs" Indian Journal of pharmacentical sciences, 3615: 303 -305; the stimulation of the liver enzymes were not control

[68]. Ogbuehi, I.H., Ebong, O.O., Obianime, A.W (2015). "A preliminary study on the effects of Abrus precatorius Linn. on reproductive parameters in female Rattus norvegicus Wistar strain" Eur. J. Med. Plants **7**(3) 156-166.

[69]. Ogunniyi, S.O., Makinde, O.O. and Dare, F.O (1999) " Abortion related deaths in Ile-Ife, Nigeria" African Journal of Medicine and Medical Science 19(4):271-274.

[70]. Okoh, S.O., Asekun,O .T., Familoni, O.B and Afolayan, AJ (2014) " Antioxidant and Free Radical Scavenging Capacity of Seed and Shell Essential Oils Extracted from Abrus precatorius (L) .pub, MDPI ; 3,278-287

[71]. Okoko, I.E and Yama, O.E (2011). "Cytoarchitectural Variations in the Ovary, Oviduct and Uterus Following Intra-Gastric Gavages of Abrus precatorius Linn in Albino Rats" Int. J. Morphol., **29**(4):1408-1413, 2011.

[72]. Olanipekun M.K.,Arowosegbe S., Kayode J.O and OluwoleT.R (2016) "Ethnobotanical survey of medicinal plants used in the treatment of women related diseases in AkokoRegion of Ondo-State, Nigeria"

[73]. Palvai, V.P., Mahalingu, S and Urooj, A (2014). "Abrus precatorius Leaves: Antioxidant Activity in Food and Biological Systems, pH, and Temperature Stability" Hindawi Publishing Corporation, International Journal of Medicinal Chemistry, Volume 2014, Article ID 748549, 7 pages, http://dx.doi.org/10.1155/2014/748549 Papilionaceae-Vitaceae. Pesqui Agropecu Brasil Ser Vet **1**: 345–352.

[74]. Popli, S. P (1977) "Screening of Indian indigenous plants for antifertility activity. Progress report on project" 74219 (WHO), Dec. 20, 1977.

[75]. Prabha, P.M., Perumal, P.C., Kumar, P. S.S., Sampath, K.R (2015) "Pharmacological activities of Abrus precatorius (L.)" International Journal of Pharmaceutical and Medicinal Research, 2015:3(2):195-200

[76]. Prakash, A. O. and R. Mathur (2008) "Screening of Indian plants for antifertility activity". Indian J Exp Biol **14**: 623–626.

[77]. Premanand, R and Ganesh, T (2010). "Neuroprotective effects of Abrus precatorius Linn. aerial extract on hypoxic neurotoxicity induced rats"

[78]. Rajalakshmi, K.V., Shivkumar G, Karthikeyan S, Punitha V, Sangeetha K (2013) " cerebral venous thrombosis Due to Abrin Toxicity: Acase Report" Indian Journal of Clinical Practice. Vol 24, No 6.

[79]. Randhawa, M.A (2009) "The Median Lethal Dose" Jayub Med Coll Abbottabad;21(3)

[80]. Raymond, P. and Scott, W (2003) "Principles and Practice of Chromatography" Book1. Library for Science, LLC. 2003

[81]. Reilly, W.A (1941) "Estrogens: Their Use in Pediatrics". Cal West Med. **55** (5): 237–9. PMC 1634235 .PMID 18746057.

[82]. Ross, I.A (2003) " Medicinal Plants of the World, Vol. 1 Chemical Constituents, Traditional and Modern Medicinal Uses" 2nd ed. Pp. 13-30.

[83]. Saganuwan, S.A, and Onyeyili, P.A (2010) "Biochemical effects of aequous leaf extract of Abrus precatorius (Jecquirity bean) in Swiss albino mice"

[84]. Salah Ahmed, M., G. Honda and W. Miki. (1979) "Herb drugs and herbalists in the Middle East. Institute for the study of languages and cultures of Asia and Africa"

[85]. Salman Khan., (2011) "Principles of Chromatography" Faculty of Khan Academy Mountain View; Sc.

[86]. Sethi, N., D. Nath and R. K. Singh. (1990). "Teratological aspects of Abrus precatorius seeds in rats"

[87]. Sharma, B.K (2007) "The Chromatography" Department of Chemistry N.A.S. College, Meerut. GOEL publishing House: Krishna Prakashan Media (P) Ltd. 5th Ed, Bk Code: 270-5 pp 3-20

[88]. Sharma, A.K., A. Kumar, S.K. Yadar and A. Rahal ( 2014) "Studies on antimicrobial and modulatory effects of hot aqueous extract of Acacia nilotica L. leaves against common Vertinary pathogen" Vet. Med. Int. 10.1155/2014/747042.

[89]. Shingo, T., Gregg, C., Enwere, E., Fujikawa, H., Hassam, R., Geary, C., Cross, J.C and Weiss, S (2003) "Pregnancy-stimulated neurogenesis in the adult female forebrain mediated by prolactin Science" **299** (5603): 117–20. doi:10.1126/science.1076647. PMID 12511652.

[90]. Simoni, M., Nieschlay, E(1995) "FSH in therapy: Physiological basis, new preparations and clinical use" Reprod. Med Rev. 1995; 4:163 -177.

[91]. Sivakumar, R., Alagesabor pathi, C (2008) "Studies on cytotoxicity and antitumor screening of red and white forms of Abrus precatorius L" African Journal of Biotechnology. 7(22):3984-3988
[92]. Society for Endocrinology (2015).www.yourhormones.info/Hormones/Oestradiol.aspx accessed
[93]. Society for Endocrinology (2017) Journal of Endocrinology: BioScientifica
[94]. Suralkar, A. and Gund, K.A (2013) "Gastroprotective effect of Abrus precatorius on ethanol-induced and aspirin + pylorus ligation-induced peptic ulcer in rats"
[95]. Telefo,P.B., Moundipa, P.F., Tichana, A.N (1998) "Effects of aqueoris extract of Aloc buettreri; Justicia insularis, Hibiscens Macranthus, Dicliptera verticillata on some physiological and biochemical parameters of reproduction in immature female rats" J Ethnopharmaol 1998; 63: 193 -200
[96]. Tilwari, A., Shukla, N.P., Pathirissery, U.D (2011) "Immunomodulatory Activity of the Aqueous Extract of Seeds of Abrusprecatorius Linn (Jequirity) in Mice"
[97]. Tiwari, R., A. Latchumikanthan, R.K. Yadav and K. Dhama (2012) "Herbal and traditional botanical medicines for safeguarding animal and human health" Livestock Line, 2012. 6:29-33. Toxicity of some compounds isolated from Abrus precatorius L. seeds towards the two-spotted spider mite Tetranychus urticae Koch.
[98]. Umadevi, M., Sampath Kumar, P.K, Bhowmik, D., Duraivel, S(2013)J. of Med. Plants Studies. 1(1)26-33
[99]. Upadhyay, S., Ghosh ,A.K and Singh,V (2011). "Anti-lice activity of Abrus precatorius L. Fabacae seed oil"
[100]. Van Wyk, B.E. Gericke, N (2000) "People's Plants: A Guide to useful plants of Southern Africa" Briza publications, Pretoria, South Africa (2000)
[101]. Vander, A.J., James H. Sherman., Dorathy ,S. Luciono (2001) "Human Physiology:The Mechanism of Body Function" 8th Ed.
[102]. Watt, J. M. and M. G. Breyer-Brandwijk (1962). "The medicinal and poisonous plants of Southern and Eastern Africa" 2nd Ed, E. S. Livingstone, Ltd., London.
[103]. Weniger, B., M. Rouzier, R. Daguilh, D. Henrys, J. H. Henrys and R. Anthon. (1986) "Popular medicine of the Plateau of Haiti 2" . Ethnopharmacological inventory.
[104]. York, H., De Wet, H., Va Vuuren S.F.(2011) "Plants used for treating respiratory infections in rural Maputaland, Kwazulu-Natal, South Africa" Journal of Ethnopharmacology, 135 (2011), pp. 696-710.
[105]. Zegers-Hochschil,F., Adamson,G.D., de Mouzon,J, Ishihara O., Mansour R., Nygren K., Sullivan E., and Vanderpoel S (2009) " International Committee for Monitoring Assissted Technology(ICMART) and the World Health Organization (WHO) revised glossary of ART terminology. Pubmed .gov

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