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# Effects of Extracts of *Anchomanes difformis* on Female Sex Hormones: Preliminary Results

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***Authors' contributions***

*This research was carried out in collaboration between all the authors. Authors JNE, AN, MCO and KCU conceived the work, designed the study, wrote the protocol and interpreted the data. Authors JNE, BCC, MCE, RCU and KCU anchored the field study, gathered the initial data, managed the literature search, performed preliminary data analysis and produced the initial draft. All the authors read and approved the final manuscript.*

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## ABSTRACT

This study assessed the effects of extracts of *Anchomanes difformis* on female sex hormones in albino wistar rats. Forty female albino wistar rats weighing between 150-200 g divided into five groups of eight rats each were used for the work that lasted for four weeks. Group 1, the control group received normal saline and normal rat chow, while the test groups 11,111,1V and V received

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100, 200, 300 and 400 mg per kilogram body weight of the extract respectively throughout the duration of the experiment, in addition to normal rat feed and water *ad libitum*. Blood samples were collected via cardiac puncture after chloroform anaesthesia every two weeks from four rats per group and assayed for the following sex hormones: Oestradiol, FSH, prolactin, LH and progesterone. The results showed statistically dose dependent decrease in the serum levels of oestradiol and progesterone in the test groups after two weeks of treatment when compared with the control ($p<0.05$). Furthermore, after four weeks of treatment, there were statistically significant dose dependent decrease in the serum concentrations of oestradiol and progesterone in the test groups when compared with the control ($p<0.05$). The statistically significant decrease in LH was not dose dependent. In conclusion, extracts of *Anchomanes difformis* reduced the serum levels of female sex hormones in albino wistar rats. This may explain the use of this extract by alternative medicine practitioners in the treatment of uterine fibroids.

*Keywords: Anchomanes difformis; albino wistar rat; female sex hormones; uterine fibroid.*

## 1. INTRODUCTION

*Anchomanes difformis*, a member of the family, Aracea [1] is a large herbaceous plant indigenous to Nigeria and Cameroon. It has stout prickly stem (leaf petiole) that may be up to 2 m high, bearing a huge much divided leaf, spathe 20-25cm long, both the stem and spathe arise from a horizontal tuber [1]. *Anchomanes difformis* flourishes well in moist shady areas, as occurs in Southern parts of Nigeria [2]. It is known as “abrisoko” in South West of Nigeria [3]; “olumahi” by the Igbos, “ebaenan” by the Efik, “chakara” by the Hausas, “boubekeodu” by the Ijaws [1] and “Olikhoror” by the Bini tribe of Edo state [4].

*Anchomanes difformis* contains carbohydrates, fats, minerals, proteins and amino acids [5]. It also contains sodium, potassium, magnesium, manganese, iron, lead, calcium and zinc [6].

*Anchomanes difformis* has varied medical applications. These include antibacterial [3,6]; anti-inflammation [2,7]; analgesic and hypothermic effects [2,8]; diuretic, antidiabetic [9]; antifilariasis [10]; antidiarrheal [11]. It also has insecticidal properties [12].

Uterine fibroids, also called leiomyomas, leioma, fibroma, fibromyomas, hysteromyoma or myomas are the commonest pelvic tumour in women of reproductive age [13,14,15], with a prevalence of 70-80% in women who have reached the age of 50 [16]. The prevalence increases with age, peaking in women in their 40s [17]. They are benign, monoclonal tumours of the smooth muscle cells of the myometrium and consist of large amounts of extracellular matrix containing collagen, fibronectin and proteoglycan. The collagen fibrils are abnormally formed and in disarray, similar to that seen in keloids [18,19].

The exact cause is unknown, but most cases have increased levels of oestrogen and progesterone [14,20], the fibroids rarely appear before menarche [21] and often regresses after menopause [14,22]. Myomas express higher proliferative index than normal myometrium throughout the menstrual cycle [23]. Lee and co-workers [24] have also reported the existence of more than 100 genes in myoma cells, these genes, including the sex-steroid associated genes, estrogen receptor α, estrogen receptor β, progesterone receptor A, Progesterone receptor B, growth hormone receptor, prolactin receptor, extracellular matrix genes, and collagen genes, have been found to be up-regulated or down-regulated in the fibroid cells. Other possible risk factors for the development of myomas are early age of menarche [25], familial predisposition [26] and overweight [27,28].

Several approaches are available for the management of uterine fibroids. These options include watchful waiting, especially for myomas that are mildly or moderately symptomatic and for women approaching menopause [14,29,30]; pharmacologic, such as hormonal therapies and gonadotropin-releasing hormone agonists; surgical approaches, such as myolisis, myomectomy, hysterectomy, laparoscopic uterine artery occlusion, magnetic resonance imaging-guided focused ultra sound surgery, uterine artery embolization [15,30,31]. According to Carranza-Mamane et al. [31], current medical management for myomas is associated with suppression of ovulation, reduction of estrogen production, or disruption of the target action of estrogen or progesterone at the receptor level and it may interfere with endometrial development and implantation. The choice of approach may depend on the patient’s desire to become pregnant in the future, the need for

uterine preservation, symptom severity, and tumor characteristics [15]. Availability of funds can also determine the chosen approach.

Uterine fibroids cause increased morbidity and affect quality of life [30,32] especially after surgical treatment [33]. Traditional medicine practitioners in South-Eastern Nigeria use extracts of the rhizomes of *Anchomanes difformis* to treat uterine myomas. The essence of this study is therefore to assess the effects of the hydromethanol extracts of the rhizomes of *Anchomanes difformis* on female sex hormones, uterine fibroids, seeking for possible mechanism of the extract that may help in the management of uterine fibroids.

## 2. MATERIALS AND METHODS

### 2.1 Collection and Processing of Sample

The rhizomes of the mature plants were harvested from Owerre-Ebeiri, Nigeria. The rhizomes were washed, in Orlu Local Government Area of Imo State, sliced, cooked and sun dried for three weeks. They were further pulverized using electrical blender (Binatone, China) and stored in air-tight bottles.

### 2.2 Extraction with Solvents

A methanol-water mixture of 80% v.v of methanol and 20% of water was constituted to form hydro-methanol. A 300 g of the dried powder was extracted with 400 ml of methanol and 100 ml of distilled water using soxhlet extractor. The hydro-methanol extract was then concentrated using a rotary evaporator. Thereafter, 1 g of aqueous methanol extract of *Anchomanes difformis* was mixed with 10 ml of distilled water, thus 0.1 ml of the extract being equivalent to 100 mg for ease of administration.

### 2.3 Phytochemical Screening

The hydromethanol extract of *Anchomanes difformis* was screened for the presence phytochemicals: alkaloids, flavonoids, saponins, tannins, cardiac glycosides, anthraquinones [34,35,36].

#### 2.3.1 Test for alkaloids

About 0.2 g of the extract was warmed with 2% of $H_2SO_4$ for two minutes, it was then filtered and few drops of Dragendoff's reagent were added. Orange red precipitate indicated the presence of alkaloids.

#### 2.3.2 Test for anthroquinones

One milliliter of the extract was shaken with 10 ml of benzene; the mixture was filtered and 5 ml of 10% (v/v) ammonia were added, then shaken and observed. A pinkish solution indicates a positive test.

#### 2.3.3 Test for cardiac glycosides

The Legal test (the killer-killiani) were adopted as follows: 0.5 g of the extract were added to 2 ml of acetic anhydrous plus $H_2SO_4$ (Trease and evans).

#### 2.3.4 Test for flavonoids

One milliliter of the plant extract was mixed with 2 ml of 10% lead acetate, a brownish precipitate indicated a positive test for the phenolic flavonoids. For the other flavonoids, I ml of the plant extract were mixed with 2 ml of dilute NaOH, a golden yellow colour indicated the presence of flavonoids.

#### 2.3.5 Test for saponins

One milliliter of the plant filtrate was diluted with 2 ml of distilled water; the mixture were vigorously shaken, and left to stand for 10 minute during which time the development of foam on the surface of the mixture lasting for more than 10 minutes, indicates the presence of saponins.

#### 2.3.6 Test for tannins

One milliliter of the extracts were mixed with 2 ml of FeCl, a dark green colour indicated positive test for tannins.

### 2.4 Experimental Animals

Forty female albino wistar rats used for this work were sourced from the Animal House of University of Nigeria, Nsukka. The animals were allowed to acclimatize for two weeks and their health status was properly monitored before and during the study that lasted for 28 days. The rats were grouped according to their weights into 5 of 8 rats each. Group 1, the control group, received 0.3 ml of normal saline, normal rat chow and water *ad libitum*. The test groups II, III, IV and V received 100, 200, 300 and 400 mg per kilogram body weight of the hydro-methanol extract of *Anchomanes difformis* daily between the hours of 8 and 10 am in addition to normal rat feed (Pfizer Plc) and water *ad libitum.* The extracts were

administered orally using 2 ml syringe without the needles.

#### 2.4.1 Ethical consideration

The research was conducted in accordance with the U.K. Animals (Scientific Procedures) Act (1986) and associated guidelines, the European Communities Council Directive of 24 November 1986 (86/609/EEC) [37,38] and the National Institutes of Health guide for the care and use of Laboratory animals [39]. Ethical Committee approval was not needed for animal studies in Imo State University, Owerri, when this study was done in 2012.

### 2.5 Collection of Blood Samples and Analyses

Blood samples were collected by cardiac puncture after chloroform anaesthesia from 4 rats per group every two weeks into plain universal containers, allowed to clot and retract properly, centrifuged at 5000 rpm for 5 minutes. The supernatant was then stored frozen at -20℃ until analyzed for Luteinizing hormone (LH), Follicle stimulating hormone (FSH), oestradiol, prolactin (PRL) and progesterone by ELISA method, using Microplate reader and Microplate Washer by Accurex, U.S.A.

### 2.6 Statistical Analysis

The data was cleaned up using SPSS Version 17. Student t-test, ANOVA, and post-hoc tests were used for statistical analysis and $p<0.05$ was deemed statistically significant. Quadruplet results were presented as Mean±SEM.

## 3. RESULTS

The following physical findings were observed in the test group rats following administration of the extracts when compared with the controls: sleeping few minutes after ingesting the extract; increased urination and frequent loose stools.

Table1 below shows the qualitative analysis of the phytochemical constituents of *Anchomanes difformis.* It shows that it contains alkaloids, tannins, saponins, steroids, flavonoids. Cardiac glycosides, anthraquinones and phlobatannins are absent in the rhizome.

**Table 1. Qualitative analysis of the phytochemical constituents of *Anchomanes difformis***

| Phytochemicals | Rhizome |
|---|---|
| Alkaloids | + |
| Tannins | + |
| Saponins | + |
| Flavonoids | + |
| Steroids | + |
| Anthraquinones | - |
| Cardiac glycosides | - |
| Phlobatannins | - |

*+: Present; -: Absent*

Table 2 and Fig. 1 show that the hydro-methanol extracts of *Anchomanes difformis* statistically and significantly reduced the serum concentrations of oestradiol and progesterone ($p <0.001$) when compared with the control in a dose dependent manner. No statistically significant differences were observed in the serum levels of LH, FSH and Prolactin when compared with the control ($p>0.005$) after two weeks of administration.

Table 3 and Fig. 2 show that after 4 weeks administration of hydro-methanol extracts of *Anchomanes difformis*, there is statistically significant dose dependent reduction in the serum concentrations of progesterone and oestradiol ($P<0.001$ ) when compared with the control. Furthermore, the observed reduction in LH was statistically significant but not in a dose dependent manner. There is however, statistically significant increase in serum level of FSH in group II only when compared with the control ($p<0.001$). No statistically significant difference was observed in the serum concentration of prolactin in the test groups when compared with the control ($p>0.05$).

**Table 2. Effects of hydro-methanol extracts on female sex hormones after 2 weeks**

| Group | LH | FSH | PROL | EST | PROG |
|---|---|---|---|---|---|
| 1 | 0.58±0.005 | 1.1±0.100 | 1.9±0.000 | 9.75±1.250 | 8.45±0. 450 |
| 2 | 0.59±0.000ns | 0.35±0.050ns | 2.05±0.050ns | 6.6±0.100** | 5.85±0.150** |
| 3 | 0.58±0.000ns | 0.5±0.000ns | 0.75±0.050ns | 5.10±0.000** | 5.25±0.050** |
| 4 | 0.61±0.0005ns | 0.65±0.050ns | 1.05±0.050ns | 4.15±0.050** | 2.05±0.050** |
| 5 | 0.59±0.005ns | 0.65±0.050ns | 0.75±0.050ns | 3.1±0.000** | 2.35±0.050** |

*Ns: not significant.*
*** : p<0.001 * : p<0.05*

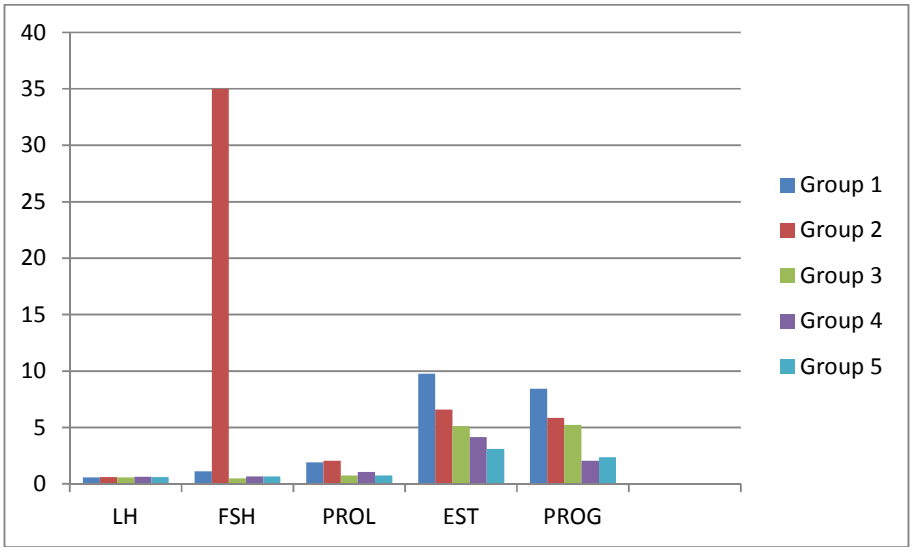


**Fig. 1. Effects of *Anchomanes difformis* on female sex hormones in albino wistar rats after two weeks of treatment**

**Table 3. The effects of hydro-methanol extracts on female sex hormones after 4 weeks**

| Group | LH | FSH | PROL | EST | PROG |
|---|---|---|---|---|---|
| 1 | 1.85±0.050 | 0.55±0.050 | 0.9±0.000 | 6.55±0.050 | 15.2±0 .20 |
| 2 | 2.05±0.050ns | 1.35±0.050** | 0.55±0.050ns | 6.05±0.050* | 11.5±0.300** |
| 3 | 1.25±0.050** | 0.25±0.050ns | 0.55±0.050ns | 5.5±0.100* | 10.6±0.100** |
| 4 | 1.05±0.050** | 0.65±0.050ns | 0.75±0.050ns | 4.05±0.05** | 7.6±0.100** |
| 5 | 1.05±0.050** | 0.65±0.00ns | 5.2±0.050ns | 3.8±0.100** | 2.65±0.15** |

*ns =not significant; ** statistically significant*

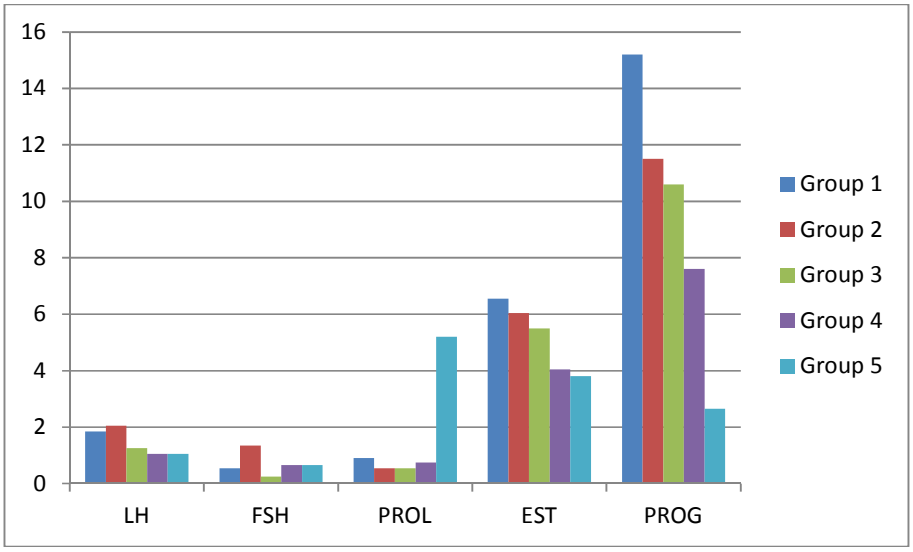


**Fig. 2. Effects of *Anchomanes difformis* on female sex hormones on albino wistar rats after four weeks of treatment**

The results also show that animals in group 5 that received 400 mg per kilogram body weight of the plant extracts had marked reduction in the serum concentrations of oestrogen and progesterone when compared to other groups.

## 4. DISCUSSION

Unorthodox health practitioners (Alternative medicine) in the South-Eastern Nigeria use extracts of some plants such as *Anchomanes difformis* in the treatment of uterine fibroids. Uterine fibroid, a common benign tumor, has become one of the major Public Health problems [40] of our time, with the attendant high morbidity and reduced quality of life. There is renewed and increased interest in natural vegetal medicines [41]. We therefore set out in this research to assess the effects of hydro-methanol extracts of *Anchomanes difformis* on female sex hormones, some of which have been implicated in the pathogenesis of uterine fibroids and possibly postulate mechanism of action.

The observed physical findings such as excessive sleeping, few minutes after ingesting the extract may be due to the usual postprandial sleep. It may also be due to the extract, which collaborates with the work of Akah and Njike [2]. The rats were also observed to have increased diuresis and loose stools when compared with the controls. These findings were also in keeping with Bouquet and Debray [42], as well as Akah and Njike [2], that *Anchomanes difformis* extracts have been used as diuretics and purgatives. The diuretic activity of plant extracts containing flavonoids have also been reported by other workers [43,44,45,46].

The etiology of uterine leiomyomata is complex and the exact cause is unknown. However, most cases have reduced concentrations of some sex hormones especially oestrogen and progesterone [14,20].

The results showed statistically significant dose dependent decrease in the serum levels of oesrtadiol and progesterone in the test groups when compared with the control. ($p<0.05$). Also the serum concentration of LH reduced significantly in week two of the treatment but not in a dose dependent manner.

Flavonoids have estrogenic properties and the most potent phytoestrogens are members of the flavonoid family [47]. There are two main forms of estrogen receptors: Estrogen receptors alpha and estrogen receptor beta. Estrogen receptor alpha(ER-α) is mainly found in the uterus, vagina, mammary gland, liver and pituitary glands [48]. Flavonoids such as quercetin, chrysin and 3-hydroxylflavone significantly inhibited cell proliferation induced by 17-β-estradiol, indicating that these compounds can act as estrogen receptor antagonists [47]. Antiestrogen compounds can act in a variety of ways including competing with 17-β-estradiol for ER binding, resulting in functionally inactive, ligand bound complex; depleting endogenous estrogen via the inhibition of estradiol biosynthesis (aromatase activity) and stimulation of estradiol catabolism [49]. Furthermore, flavonoids also function as selective estrogen receptor modulators (SERMs) [50,51], tend to change endogenous activities of estrogen receptors, which can slow down or prevent tumorigenesis.

The other antitumor biologic effects of flavonoids include inhibition of cell growth, inhibition of protein kinase activity, induction of apoptosis, inhibition of tumor cell invasion, anti-angiogenic properties and inhibition of adhesion/spreading of cells [52,53,54].

## 5. CONCLUSION

In conclusion, hydro-methanol extracts of *Anchomanes difformis* reduced serum levels estrogen, progesterone and luteinizing hormone ($p<0.05$) in albino wistar rats. This may in part, explain why alternative medicine practitioners in South-Eastern Nigeria use it to manage uterine fibroids. However, further researches are needed to fully separate and identify the active ingredients of the rhizome and possibly establish the exact mechanism(s) of action.

## CONSENT

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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