

## Original Research Article

### Effects of Hydro-Ethanolic Leaf Extract of *Sida acuta* on Reproductive Hormones, Weight and Pregnancy Status of Female Albino Rats.

#### Abstract:

**Background:** Human fertility control (family planning) is among the major approaches through which unwanted pregnancy can be curbed. Hence, utilization of medicinal herbs in the development and formulation of products, as an alternative to conventional drugs, is one of the method options to achieve this.

**Aim:** This study was designed to assess the contraceptive potentials of hydro-ethanolic leaf extract of *Sida acuta*, as well as evaluating its effects on fertility hormones, pregnancy status, lipid profile, liver, kidney and kidney and body weight of female albino rats.

**Method:** This experiment was conducted using thirty five (35) sexually matured albino rats. Using a CRD, the rats were randomly divided into five (5) groups of seven (7) rats each, with five (5) female and two (2) male replicate assigned to each group. The Control group (Group 1) did not receive any treatment. The females in the standard group (Group 2) were administered with levonorgestrel-etinyl estradiol (standard drug) 7.5/0.75mg/kg b.w./day p.o. The females in the test groups (Groups 3, 4 and 5) were administered the plant extract at different doses of 100mg/kg b.w./day (Low dose), 200mg/kg b.w./day (Medium dose) and 400mg/kg b.w./day (High dose) p.o. respectively, for 28days. The initial and final body weights of the animals were measured. The qualitative and quantitative phytochemical analyses of hydro-ethanolic leaf extract of *Sida acuta*, as well as the GC-MS analysis were also performed using standard methods. Furthermore, the effects of the extract were checked on the reproductive hormones (FSH, LH, Progesterone and Estrogen), pregnancy status as well as on kidney, liver and the lipid profile of the animals.

**Results:** The results revealed the presence of bioactive compounds in the hydro-ethanolic leaf extract of *Sida acuta*, decreased level of FSH across the groups, no pregnancy outcome in the high-dose group, and a significant weight reduction in the high-dose group.

**Conclusion:** The result suggested that hydro-ethanolic leaf extract of *Sida acuta* has suppressive effects on the reproductive hormones (especially the gonadotropins) at higher dose, thereby establishing its contraceptive potentials as confirmed by the pregnancy outcome. Also, the extract is capable of significantly decreasing body weight at a high dose, with no negative effects on the cardiovascular, kidney and liver functions.

**Keywords:** *Sida acuta*, Contraceptives, Hydro-Ethanolic Extract, Hormones, Phytochemicals, Gas Chromatography-Mass Spectroscopy, Estrogenic substance.

#### 1. INTRODUCTION

Currently, there is a considerable significant view of medicinal herbs due to their special characteristics as a great source of therapeutic phytochemical compounds that can lead to the invention of novel drugs. Most of the phytochemicals from plant sources such as phenolics and flavonoids have been reported to have positive impact on health and disease prevention.

[1] [2]. High content of phenols and flavonoids in medicinal plants have been associated with their antioxidant activities that play a role in the prevention of the development of age-related disease, particularly caused by oxidative stress. Interest in utilizing natural sources in the development and formulation of medicinal products, as an alternative to conventional drugs and synthetic products, contribute to paradigm shift to research and industrial application of medicinal plants. [3]. [2] linked high content of phenolic compounds in herbs with their antioxidant activities that play a role in the prevention of the development of geriatric diseases, particularly caused by oxidative stress. With regards to the beneficial phytochemicals in medicinal plants and the shift towards natural products in pharmaceuticals and cosmetology industry, the research on medicinal plants particularly is as important as that on conventional drugs [3]. [4] ~~opined~~Opined that it is estimated that 80% of many developing countries population still use plant-based traditional medicines. These natural products and their derivatives represent almost half of the drugs approved in USA since 1994.

*Sida acuta*, the common wire weed, is specie of flowering plant in the mallow family, *Malvaceae*, believed to have originated in Central America, but recently has a pantropical distribution and is considered a weed in some areas. It is a tropical weed of pastures, road sides and waste areas [5]. Research has revealed that juice from leaf of *Sida acuta* has schistosomicidal activity [6] and antihelminthic activity for intestinal worms [4]. The root inhibits embryo implantation or growth in the mice. Some herbalists have claimed the traditional use of this plant to cure infections such as malaria, ulcer, fever, ~~gonorrhoea~~gonorrhoea, breast cancer, following inflammation, wound infection and for abortion. And some of these claims have also been experimentally proven [4], [5].

[3] ~~have~~Have also reported that leaf extract of *Sida acuta* is not liked by cattle due to the presence of different chemicals including alkaloids. It is mainly known for its healing effects, making it one of the most sought herbs in traditional therapeutics for wide range of ailments such as malaria, ulcer, gonorrhoea, wound infection, asthma, headache, cold, fever, cough, skin disease and facial paralysis. *Sida acuta* has also been reported to have contraceptive property, hepatoprotective, and antimicrobial activities [4].

Unwanted pregnancy is a common global problem which could lead to other numerous problems such as maternal and child ill-health, maternal and child death, pregnancy-related complications, over-population and malnutrition. According to [7], over 40 million gestations are terminated through abortion on yearly basis. However, the standard approach to this menace of unwanted pregnancies has been pre-conception prevention (contraception), backed up by induced abortion [8].

## 2. MATERIALS AND METHODS

### 2.1 Equipments

Equipments used for this research include Soxhlet apparatus, Rotatory evaporator, ~~GC~~and GC-MS machine, Fluorescent Immunoassay (FIA) analyzer (Finecaré™), Pipette, Centrifuge and Analytical weighing balance.

### 2.2 Plants ~~C~~ollection, ~~i~~dentification and ~~P~~reparation

The healthy whole *Sida acuta* plant was harvested in Keffi, Nasarawa state environs, and taken to the herbarium, Department of Plant Science and Biotechnology of Nasarawa State University, Keffi, for identification. The leaves were removed from the stalk and thoroughly

washed with clean water to remove the adherent impurities. They were shade-dried under laboratory condition.

### 2.3 Animals Procurement and Preparation

Thirty five (35) adult (10 male and 25 female) albino rats weighing between 160 – 220g each were used for the experiment. They were procured from the animal house of Benue State University, Markudi, Nigeria. The rats were allowed to acclimatize for two (2) weeks with no experimental treatment, under standard condition of humidity and temperature in animal house of the Department of Biochemistry, Nasarawa state university, Keffi, Nigeria. They were housed in clean still cages lined with wood chip beddings. They were allowed access to commercial feed and water *ad libitum*. All procedures performed in the study were in tandem with the ethical standards of the institutional research committee. This study complied with the ethical and human principles of research and was approved by the Ethical Committee of Nasarawa State University, Keffi, Nigeria.

### 2.4 Plant Extraction

The plant extract was prepared according to the procedure developed by the Institute of Medical Research (IMPM). The dried leave of *Sida acuta* were pulverised to pass a 0.5 mm sieve and weighed. The dried powder was subjected to soxhlet extractoion using hydro-alcohol of 40-60% ratio as solvent [9] for 12 hours at 70°C, followed by filtration through a Whatman No. 1 filter paper. The hydro-ethanolic extracts obtained were concentrated to dryness at 70°C using a rotary evaporator under reduced pressure and the extracts were weighed and then stored at 4°C for further use as described by [10]. The percentage yield was calculated as follows:

$$\text{Percentage Yield (\%)} = \frac{\text{Dry weight of extract}}{\text{Dry weight of plant material}} \times 100$$

The prepared extract was reconstituted in normal saline to give the equivalent dose of 100 mg/kg body weight (low dose), 200 mg/kg body weight (medium dose) and 400 mg/kg body weight (high dose). The reconstituted aqueous extracts of different concentrations were administered orally (oral gavage) using plastic syringes to all female animals in the three different test groups accordingly, via oral intubation method.

### 2.5 Qualitative and Quantitative Phytochemical Screening of Hydro-ethanolic Leaf Extract of *Sida acuta*

The procedures described by [11], [12] and [13], with some modifications, were used for the qualitative phytochemical screening of tannin, flavonoid, alkaloid, saponin, hydrogen cyanide (HCN), phenol, terpenoid, steroid and glycoside.

### 2.6 Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

Gas chromatography–mass spectrometry (GC-MS) of the plant extract was analyzed according to [14]. The chromatography was performed on a capillary column and stationary phase of 5% diphenyl 95% dimethylpolysiloxane. The injection volume of the sample was 1 µl with a 0:1 split ratio. Helium (99.999%) was used as carrier gas throughout the column. The injector and ion source temperature was kept at 280°C and 180°C, respectively. The initial temperature in the oven was programmed from 110 °C for 3 min [15], ramp 5 °C/min

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to 200 °C and hold for 3 min ~~and again~~ and again increased for 5 °C/min to 300 °C and held for 10 min [16]. The sample was scanned from 40 to 600 Da. The peaks were analyzed using the data analysis software NIST-2008. The National Institute of Standards and Technology (NIST) is a mass spectral search database for comparisons of the acquired and unknown spectrum with NIST/EPA (Environmental protection Agency)/NIH (National Institute of Health) databases. The components were identified based on standards employed by the National Institute Standards and Technology (NIST) Library [16].

## 2.7 Experimental Protocol

A Completely Randomized Design (CRD) was used following two weeks (14 days) of acclimatization, the male and female rats were randomly divided into five (5) groups of 7 rats each, with five (5) female and two (2) male replicate assigned to each group. The female received 0.9 ml of normal saline solution (0.9% NaCl) per oral (p.o) as vehicle and each of the tested female rat was given 0.9 ml of normal saline solution that contains levonorgestrel-etinyl estradiol 7.5/0.75mg/kg b.w./day (standard drug) and plant extract respectively, at different doses for 28days

The hydro-ethanolic leaf extract of the *S. acuta* was dissolved in normal saline and administered orally to each of the tested rat at different doses of 100, 200 and 400 mg/kg using oral gavage method of administration.

The animals were grouped as follows:

- Group I: Control group (0.9% NaCl) p.o.
- Group II: (0.9% NaCl + levonorgestrel-etinyl estradiol) p.o.
- Group III: (0.9% NaCl + 100mg/kg extract) p.o
- Group IV: (0.9% NaCl + 200mg/kg extract) p.o
- Group V: (0.9% NaCl + 400mg/kg extract) p.o

After the first administration of the extract to the females, the males were introduced to the groups for mating to occur. At the end of the 28days test period, the females in each group were observed for pregnancy status.

## 2.8 Determination of Body Weight

Initial body weight of all the animals were measured at the beginning of the experiment, and continued weekly throughout the period of the study, using digital analytical weighing balance as illustrated by [17].

## 2.9 Collection of Blood Sample

At the end of 28 days, the female rats were sacrificed under anesthetic condition using chloroform and blood was collected in a plain tube and centrifuged at 3000 rpm for 5 min to obtain serum for hormonal assay, lipid profile, liver and kidney function panels.

## 2.10 Pregnancy Status

The animals were dissected and uterus examined for pregnancy according to standard procedures as described by [18].

## 2.11 Quantitative Determination of Reproductive Hormones

Assays for Follicle Stimulating Hormone (FSH), Lieutinizing Hormone (LH), Estrogen (E2) and Progesterone (Prog.) were carried out using fluorescent immunoassay (FIA) technique that uses sandwich immunodetection method as outlined in Finecar<sup>®</sup>™ Kit. [19].

## 2.12 Statistical Analysis

All results were analyzed using Statistical Package for Social Sciences (SPSS) Version 23, and were expressed as mean  $\pm$  SEM. Multiple group comparisons were performed using one-way analysis of variance (ANOVA) followed by Dunnett's test in order to detect intergroup differences. A significant difference was determined when  $P < 0.05$ .

## 3. RESULTS AND DISCUSSION

### 3.1 Results

#### 3.1.1 Qualitative and Quantitative Phytochemical Composition of Hydro-ethanolic Leaf Extract of *Sida acuta*

The result showed the presence of phenols ( $120.28 \pm 0.96$ ) representing 2.09%; tannins ( $91.40 \pm 0.16$ ) 1.59%; saponins ( $530.36 \pm 0.70$ ), 9.21%; alkaloids ( $1403.17 \pm 2.26$ ), 24.36%; terpenoids ( $114.26 \pm 1.30$ ), 1.98%; cardiac glycosides ( $869.23 \pm 2.11$ ), 15.09%; steroids ( $1440.27 \pm 1.85$ ), 25.01% and flavonoids ( $1190.25 \pm 7.95$ ), 20.67%; while glycosides, phlobatannins, anthracyanins and anthraquinones were absent (Table 1).

Table 1: Phytochemical Composition of Hydro-ethanolic Leaf Extract of *Sida acuta*

Phytochemical Percentage	Qualitative Analysis	Quantitative Analysis (mg/100g)	(%)
Phenols	+	$120.28 \pm 0.96^a$	2.09
Tannins	+	$91.40 \pm 0.16^a$	1.59
Saponins	+	$530.36 \pm 0.70^b$	9.21
Alkaloids	+	$1403.17 \pm 2.26^c$	24.36
Terpenoids	+	$114.26 \pm 1.30^a$	1.98
Cardiac glycosides	+	$869.23 \pm 2.11^d$	15.09
Steroids	+	$1440.27 \pm 1.85^c$	25.01
Flavonoids	+	$1190.25 \pm 7.95^e$	20.67
Glycosides	-		
Phlobatannins	-		
Anthracyanines	-		
Anthraquinones	-		

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Key: + = present, - = absent. Mean values with different letters as superscript are significant at  $p < 0.05$ .

### 3.1.2 Constituent Compounds from GC-MS Analysis of Hydro-ethanolic Leaf Extract of *Sida acuta*

The captured features in the GC-MS result include CAS, name of the compound, RT and peak area (%). The major compounds identified include Hexadecanoic acid, methyl ester, 15-Hydropentadecanoic acid, Nonanoic acid, Stigman-3, 5-diol, 9-oxo-, methyl ester, Glycerol 1-palmitate, cis-10-Pentadecenoic acid, butyl ester, 3-deoxyestradiol, 13-Octadecenal, (Z)-, cis-Vaccenic acid, 14-Pentadecenoic acid, Oleic Acid, Erucic acid, Pyrrole-2-carboxylic acid, 4-(1-chlorodec-1-enyl)-3,5-dimethyl-, ethyl ester, Fumaric acid, hexyl 2,3,4,6-tetrachlorophenyl ester and Stigmasterol (Table 2).

Table 2: GC-MS Compositions of Hydro-ethanolic Leaf Extract of *Sida acuta*.

S/No	Name of Compound	RT	Peak (%)
1	Hexadecanoic acid, methyl ester	13.801	-0.95
2	15-Hydropentadecanoic acid	21.979	-3.33
3	Stigman-3, 5-diol	18.60	0.27
4	Nonanoic acid, 9-oxo-, methyl ester	22.707	-2.56
5	Glycerol 1-palmitate	25.973	-1.77
6	cis-10-Pentadecenoic acid, butyl ester	26.099	-5.39
7	3-deoxyestradiol	18.841	0.36
8	13-Octadecenal, (Z)-	27.337	-0.81
9	cis-Vaccenic acid	29.820	-5.37
10	14-Pentadecenoic acid	30.028	-0.20
11	Oleic acid	30.085	-0.20
12	Erucic acid	31.733	-0.36
13	Pyrrole-2-carboxylic acid, 4-(1-chlorodec-1-enyl)-3,5-dimethyl-, ethyl ester	32.781	-3.37
14	Fumaric acid, hexyl-2,3,4,6-tetrachlorophenyl ester	38.429	127.11
15	Stigmasterol	18.60	0.27

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### 3.1.3 Effects of hydro-ethanolic Leaf Extract of *Sida acuta* on Reproductive Hormones in Female Albino Rats

As shown in Figure 1, the FSH concentration was significantly ( $p < 0.05$ ) lower in Groups 2, 3 and 4 compared to Group 1. Progesterone concentration was significantly ( $p < 0.05$ ) lower in groups 2 and 5 compared to the group 1. Estrogen/Oestrogen levels significantly ( $p < 0.05$ )

decreased across all the test groups (groups 2, 3, 4 and 5) compared to the group 1. LH was not detected across all the groups.

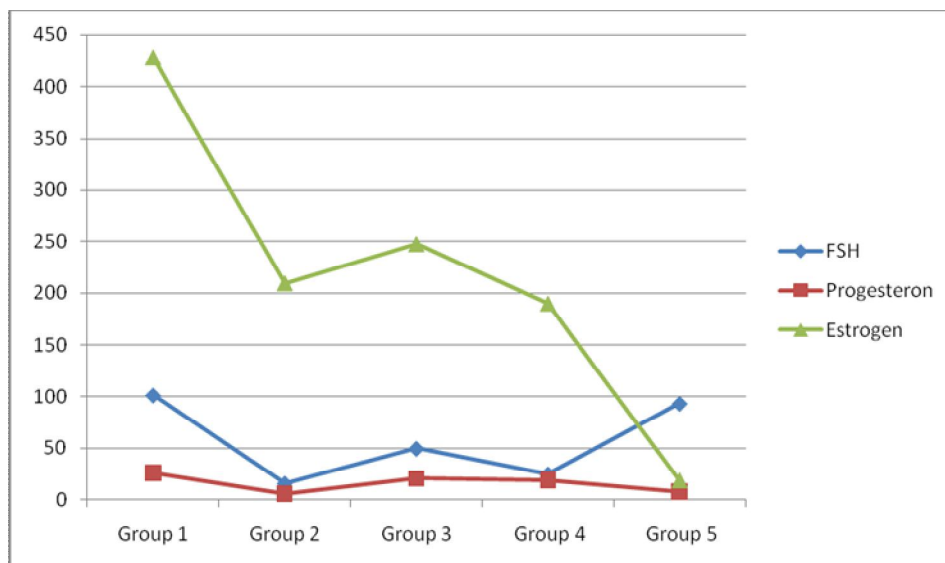


Figure 1. A Graph representing the effects of hydro-ethanolic leaf extract on the reproductive hormones of female albino rats.

### 3.1.4 Effects of hydro-ethanolic Leaf Extract of *Sida acuta* on Body Weights of Female Albino Rats

Figure 2 is a presentation of the results of the body weight readings of the rats before and after (initial and final) treatments. The results show non-significant ( $p > 0.05$ ) alterations in the body weight of rats in groups 2, 3 and 4 when compared to the control group. But there is a significant decrease in body weight in group 5.

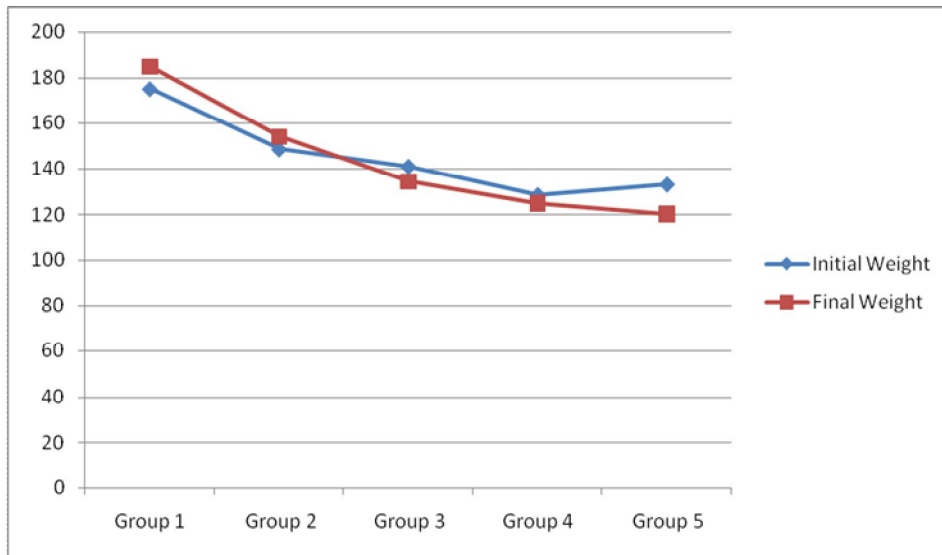


Figure 2. A Graph representing the effects of hydro-ethanolic leaf extract on the weight of Female Albino Rats.

### 3.1.5 Effects of Hydro-ethanolic Leaf Extract of *Sida acuta* on the Pregnancy Status of Female Albino Rats

Figure 3 represents the outcome of pregnancy among female albino rats treated with varied doses of hydro-ethanolic leaf extract of *Sida acuta*, as well as that of control and standard. There is 80% pregnancy occurrence in the Group 1, 20% pregnancy occurrence in both Group 3 and Group 4 respectively, and 0% pregnancy occurrence in Group 2 and Group 5 respectively.

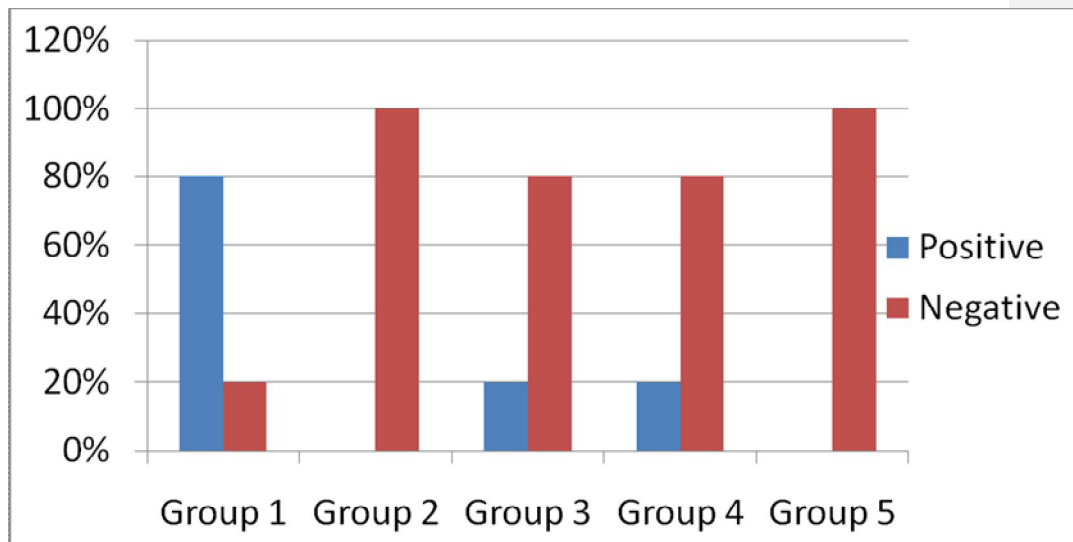


Figure 3. Effects of Hydro-Ethanolic Leaf Extract of *Sida acuta* on the pregnancy status of Female Albino Rats.

### 3.2 Discussion

The use of indigenous plants as contraceptives is currently growing owing to some advantages they may have over orthodox medicines. The effectiveness of plants as contraceptive may stem from the fact that they possess some bioactive compounds accounting for their potency. Therefore, in scrutinizing plants for medicinal properties, it is often necessary to do a preliminary phytochemical analysis so as to have an idea of the kind of phytochemicals present in the plant and possibly attribute whatever effects observed to the phytocompounds present in the plant.

In this study, the presence of alkaloids, flavonoids, phenols, steroids, terpenoids and cardiac glycosides agrees with previous findings by [20] who also detected those phytochemicals in *S. acuta* extract. The absence of phlobatannins also agreed with his findings. However, the presence of saponins in this research contradicts [20] who also reported that tannins were absent in the extract.

Flavonoids, alkaloids and steroids are the most abundant secondary metabolites in this study, therefore concurs with the findings of [21] who also opined that flavonoids are the most abundant phenol group in nature, present in a wide range of conifers. In the same vein, [22], who established *Azadirachta indica* as an effective contraceptive agent, also identified high concentrations of flavonoids and alkaloids in the plant, a clear indication that these phytochemicals may be responsible for the contraceptive efficacy of the tested extract. Furthermore, findings in this study are supported by that of [23] who observed that phenolic compounds are potent contraceptive agents.

The GC-MS spectrum confirmed the presence of various components with different retention times as illustrated in Table 2. The mass spectrometer analyzes the compounds eluted at

different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library. The GC-MS study of the hydro-ethanolic leaf extract of *S. acuta* had shown the presence of lots of phytochemicals which strength contributes to the medicinal bioactivity of the plant, especially hexadecanoic acid, methyl ester; stigmaterol; stigmatan-3, 5-diol; 3-deoxyestradiol; which has one of the highest peak areas (%). The presence of these bioactive compounds may be responsible for the observed decrease in the concentration of hormones observed in this study, which in turn can explain the contraceptive efficacy of this herb. Although there was no literature found to support the contraceptive efficacy of hexadecanoic acid, methyl ester; [24] established the antifungal efficacy of methyl ester of hexadecanoic acid identified in *Annona muricata* leaves.

The homeostasis of sex hormones is directly regulated by the hypothalamic–pituitary–gonadal system; peripheral sex hormones provoke the release of gonadotropin-releasing hormone (GnRH) in the hypothalamus. GnRH influences the secretion of the gonadotropins (LH and FSH) in the pituitary that stimulate the release of sex hormones (oestrogen and ~~progesteron~~progesterone) from the gonads [25]. Since the maturation of pre-ovulatory follicles and release of eggs are under the combined and balanced influences of ovarian and extra ovarian hormonal secretions, imbalances or alterations in the secretion of these hormones often lead to irregularity in the ovarian functions and duration of estrous cycle [26]. These hormonal imbalances may be caused by numerous chemical agents contained in plant extracts. Phytochemical screening has revealed many bioactive as well as toxic chemical substances of plant extracts that can adversely affect the regulation of menstrual/estrous cycle, conception and reproduction of kinds in both humans and animals respectively [27]; [28]. Alkaloids and flavonoids have been shown to have effect on plasma concentrations of LH, estradiol and FSH [29]; [30], because they interact with the hormones and alter their activities in various ways. Therefore, the presence of these phytochemicals may account for the alterations in the levels of the circulating hormone observed in this study.

FSH and LH are the central hormones of mammalian reproduction. While FSH is essential for gonadal development and maturation, the development of secondary reproductive organs at puberty, as well as gamete production during the fertile phase of life [31]; LH stimulates secretion of sex steroids from the gonads and induces ovulation with a large surge [32]. Many researchers have demonstrated that the released LH which increases at the pro-estrous stage of sexual development in mammals is responsible for ovulation [33]. Therefore, any substance capable of enhancing the release of these gonadotropins could accelerate ovulation in females by increasing the number of mature follicles and facilitating their rupture [28]. The decrease in the levels of FSH by the extract in this study, supported by the pregnancy outcome across the test groups (0% in group 5) may be an indication that the extract does not improve folliculogenesis and maturation of the follicle in the pre-ovulatory phase at high dose. It is possible that the extract might have exerted its effects on the anterior pituitary; which secretes the hormone or the hypothalamus since the secretion of FSH is regulated by the gonadotropic releasing hormone secreted by the hypothalamus of the brain. Furthermore, phytochemical tests in this study identified the presence of isoflavanoids, while GC-MS analysis identified the presence of stigmaterol, stigmatan-3, 5-diol and 3-deoxyestradiol, among others, in the extract. These compounds have been reported to be estrogenic (phytoestrogens) [34]; [35]. Estrogenic compounds mimic estradiol activities and can interact with nearly all biological functions [34]. They exert their effects on pregnancy maintenance by affecting the balance of reproductive hormones that regulate the hypothalamic-hypophyseal-gonadal axis [36]; [32]. [37] ~~opined~~Opined that estrogenic compound

(stigmasterol) has an affinity for estrogen receptors and thus leads to infertility in animals. Plants with estrogenic property can directly influence pituitary action by peripheral of LH and FSH, decreasing secretion of these hormones and blocking ovulation [27]. Thus, the decrease in the serum concentration of estradiol may be attributed to a process initiated by aromatase activity or substrate supplementation during estrogen synthesis [38]. On the other hand, any increase in estradiol levels will influence zygote implantation, facilitates ovulation and sustain pregnancy [39]. The observed decrease in the levels of the hormone will therefore suppress conception in the female animals (as evident in pregnancy outcome), making hydro-ethanolic leaf extract of *Sida acuta* a potential contraceptive agent. On the other hand, any substance capable of enhancing the release of the gonadotropins could accelerate ovulation in females by increasing the number of mature follicles [28]. However, the non-detection of LH in the serum as observed in this study may be explained by a non-excitatory effect or suppressive of the extract on the release of LH which may enhance ovulation. Insensitivity of a protein is one factor that may prevent it from being detected; this may also be due to lack or absence of the receptors for the binding of the protein at the expected site of action. This may result in non-enhancement of estrous cycle; and may not promote conception and normal reproduction in the females. But because the test, standard and control groups did not have a detectable level of LH, therefore it is more probably not the effect of the extract, but due to the fact that the amount secreted by the animals was so minute and insensitive that it falls below the analytical range of the machine used.

Furthermore, progesterone is known to be responsible for induction of endometrial transition from a proliferative to the secretory stage to facilitate blastocyst nesting and is essential for the maintenance of pregnancy. These attributes of the hormone explain the etymology of the hormone's name, which comes from the Latin word *pro gesta-tionem*. Progesterone also plays an important role in several tissues that are not part of the reproductive system, such as the mammary gland in preparation for breastfeeding, the cardiovascular system, central nervous system and bones [40]. Progesterone is primarily synthesized from maternal low density lipoproteins (LDL) cholesterol by the placenta through a network of enzyme system and only a small fraction comes from fetal steroidogenesis. Progesterone affects tubal motility by interacting on specific receptors and acts on endometrial maturation and on uterine vascularization in the pre-implantation phase [41]. In this study, Progesterone concentration was significantly ( $p < 0.05$ ) lower in groups 2 and 5, indicating that both levonorgestrel-ethyl estradiol standard drug and high doses of hydro-ethanolic leaf extract of *Sida acuta* can suppress its secretion hence, causing reduced endometrial maturation and uterine vascularization in the pre-implantation phase of the animal's reproductive cycle. These attributes of the extract potentiate it to be an active contraceptive agent. However, plant products as fertility suppression agents will be more acceptable for economic reasons and side effects that are less than chemical agents.

As supported by the work of [23] who observed no significant weight loss in animals treated with herbal contraceptives, this study showed non-significant ( $p > 0.05$ ) alterations in the body weight of rats in groups 2, 3 and 4, when measured before and after the administrations, indicating that the extract had no significant effect on the body mass of the rats administered with a low dose. However, there is a significant decrease in weight of test group 5, which conforms to the findings of [42], who observed a significant decrease in body weights of albino rats administered with *Sida acuta* extract.

#### **4. Conclusion**

From this study, it is established that hydro-ethanolic leaf extract of *Sida acuta* has a dose-dependent contraceptive potentials, owing to fact that it is rich in estrogenic phytochemical compounds, the secondary metabolites of plants, some of which are flavonoids, phenolic compounds, alkaloids, steroids, methyl ester of hexadecanoic acid, stigmasterol, stigmatan-3, 5-diol and 3-deoxyestradiol. It is therefore concluded that hydro-ethanolic leaf extract of *Sida acuta* has a contraceptive effect, and is capable of significantly reducing body weight at a high dose. Due to limited resources and technology, treated animals were not subjected to histological studies in order to establish the effects of the extract on the intra-uterine walls. Further studies on the biological effects of hydro-ethanolic leaf extract of *Sida acuta* on metabolic organs, as well as histological studies of the intra-uterine wall are advocated.

#### Data Availability

All relevant data are within the paper.

#### Informed Consent Statement

Not applicable.

#### References

1. Edeoga H. O., Okwu D. E. & Mbaebrie B. O. (2005) Phytochemical constituents of some Nigerian medicinal plants. *Afri. J. Biotechnol.*; (4): 685-688.
2. Altemimi A., Naoufal Lakhssassi N., Baharlouei A., Watson D. G. & Lightfoot D. A. (2017). Phytochemicals: Extraction, Isolation and Identification of Bioactive Compounds from Plant Extracts. *Plants*. 6:42
3. Kuniata L. & Rapp G. (2001). ~~Biocontrol~~ Bio control of *Sida rhombifolia* in Papua New Guinea 2001. <http://www.nt.gov.au/PDF/publicat/agnotes/542.htm>.
4. Londonkar R. C., Patil S. J. & Patil S. B. (2009). Phytochemical and contraceptive property of *Sida acuta* in albino rats. *Int. Journ. PharmTech Res.* 1 (4): 1260-1266.
5. Karou D., Savadogo A., Canini A., Yameogo S., Montesano C. & Simpore J. (2006) Antibacterial activity of alkaloids from *Sida acuta*. *African. J. Biotechnol.* 106: 425-428.
6. Membe F. U., Kadji F. J.B., Boukeng J. H, Tchoffo Y.L., Amvame N. D. C., Kamdoum B. C., Njonte W. S. C., Toussie T. B., Tchaleu N. B., Sewald N, Ndjakou L. B., Tchuem T. L. A. & Dimo T. (2022). *In Vitro* Assessment of the Cercaricidal Activity of *Sida acuta* Burm. F. and *Sida rhombifolia* Linn. (Malvaceae) Hydroethanolic Extracts, Cytotoxicity, and Phytochemical Studies. *Evid Based Complement Alternat Med.* 2022 Jan;10;2022:7281144.
7. Sedgh, G., Singh, S., Shan, I. H., Ahman, E., Henshaw S. K. and Bankole A. (2012). Induced Abortion: incidence and trends worldwide from 1995 to 2008. *Lancet*; 379(9816):625-32.
8. Sedgh, G., Singh, S. & Hussain, R. (2014). Intended and unintended pregnancies worldwide in 2012 and recent trends. *Family Planning*; 45(3)301-14.
9. Mukilarasi, V., Pavithera, S., Suchitra, V., Nayak, B. K. & Anima, N. (2015). Comparative antibacterial analysis of hydro-alcoholic leaf extract of three medicinal plants by soxhlet extraction process. *Journal of Chemical and Pharmaceutical Research*, 7(4):131-135.
10. Omoregie, E. S. & Osagie, A. U. (2012). Antioxidant Properties of Methanolic Extracts of some Nigerian Plants on Nutritionally-Stressed Rats. *Nigerian Journal of Basic and Applied Science*, 20(1):7-20.

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11. El- Oley, M. M., Farid J. A. & Abdel- Fattah A. A. (1994). Ethanol Extract of *P. stratiotes*; *NISEB Journal* 1(1):51-59.
12. Sofowora, A. (1993). Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria, pp. 191-289.
13. Sasidharan S., Chen Y., Saravanan D., Sundram K. M. & Latha Y. L. (2011). Extraction, Isolation, and Characterization of Bioactive Compounds from Plant's Extract. *Afr J Tradit Complement Altern Med*, 8(1): 1-10
14. Hakani, D. S. (2021). Estimation of drug-likeness properties of GC-MS separated bioactive compounds in rare medicinal *Pleione maculata* using molecular docking technique and Swiss ADME *in silico* tools. *Network Modeling Analysis in Health Informatics and Bioinformatics*, 10:14. <https://doi.org/10.1007/s13721-020-00276-1>
15. Thomas, E., Aneesh, T. P., Thomas, D. G. & Anandan, R. (2013) GC-MS analysis of phytochemical compounds present in the rhizomes of *Nervilia aragoana* Gaud. *Asian Journal of Pharmaceutical and Clinical Research*, 6(3):68-74.
16. Darmasiwi, S., Indriani, V., Innata, D. & Semiarti, E. (2015). The potential production of Aromatic Compounds in Flowers of *Vanda tricolor*. In: The 5th International conference on Mathematics and Natural Sciences 090006- 1- 090006-4. <https://doi.org/10.1063/1.4930751>.
17. Wright K. M., Deighan A., Di Francesco A., Freund A., Jojic V., Churchill G. & Raj A. (2020). Age and diet shape the genetic architecture of body weight in diversity outbred mice. Figshare. [CrossRef].
18. Abdolmaleki A., Jalili C., Monsouri K. & Bakhtiari M. (2021). New rat to mouse xenograft transplantation of endometrium as a model of human endometriosis. *Animal Model Exp Med*, 4(3): 268-277.
19. Shah, P.K., Santella, A., Jacobo, A., Siletti, K., Hudspeth, A. J. & Bao, Z. (2017). An Intoto Approach to Dissecting Cellular Interactions in Complex Tissues. *Developmental Cell*, 43:530-540.
20. Senthilkumar, Bhuvaneshwari V, Malayaman V. Ranjithkumar & Sathiyavima S. (2018). Phytochemical Screening of Aqueous Leaf Extract of *Sida acuta* Burm. F. and its Antibacterial Activity. ETIR August 2018, Volume 5, Issue 8 [www.jetir.org](http://www.jetir.org) (ISSN-2349-5162)
21. Metsämuuronen S. & Sirén H. (2019). Bioactive phenolic compounds, metabolism and properties: A review on valuable chemical compounds in Scots pine and Norway spruce. *Phytochem. Rev.* 2019;18:623-664.
22. Patil S. M., Shirahatti P. S., Ramu R. & Prasad N. (2021), *Azadirachta indica* A. Juss (neem) as a contraceptive: An evidence-based review on its pharmacological efficiency. *Phytomedicine*. 2021;~~88:153596~~; [88:153596](https://doi.org/10.1016/j.phymed.2021.153596).
23. Moroole M. A., Materechera S. A., Otang-Mbeng W., Hayeshi R., Bester C. & Aremu A. O. (2022). Phytochemical Profile, Safety and Efficacy of a Herbal Mixture Used for Contraception by Traditional Health Practitioners in Ngaka Modiri Molema District Municipality, South Africa. *Plants*. 2022, 11, 193
24. Abubacker M. N. & Deepalakshmi T. (2013). *In vitro* antifungal potentials of bioactive compound methyl ester of hexadecanoic acid isolated from *Annona muricata* Linn. (Annonaceae) leaves. *Biosci., Biotech. Res. Asia*, ~~Vol.10~~([Vol.10](https://doi.org/10.1007/s13721-020-00276-1)) (2), 879-884.
25. Moraga-Amaro R., Van Waarde A., Doorduyn J. & De Vries E. F. J. (2018). Sex steroid hormones and brain function: PET imaging as a tool for research. *J. Neuroendocr.* 2018;~~30:e12565~~; [30:e12565](https://doi.org/10.1093/ajph/111.10.193).

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26. Shivalingappa, H., Satyanarayan, N. D., Purohit, M. G., Sahranabasappa, A., & Patil, S. B. (2002). "Effect of Athanol Extract of *Rivea hypocraterifomis* on the Estrous Cycle of the Rat." *J. Ethnopharmacol* 82: 11-7.
27. Yakubu, M. T., Akanji, M. A., and Oladiji, A. T. (2005). "Aphrodisiac Potentials of the Aqueous Extract of *Fadogia agrestis* (Schweinf. Ex Hiern) Stem in Male Albino Rats." *Asian J. Androl*, 7: 399-404.
28. Benie, T., Duval, J. & Thieulant, M. L. (2003). "Effects of Some Traditional Plant Extracts on Rat Oestrous Cycle Compared with Clomid." *Phytother Res.* 17: 748-55.
29. Bianco, F., Basini, G. & Grasselli, F. (2006). "The Plant Alkaloid Sanguinarine Affects Swine Granulose Cell Activity." *Reprod. Toxicol.* 21: 335-40.
30. Browning, R., Schrick, F. N., Thompson, F. W., & Wakefield, T. (1998). "Reproductive Hormonal Responses to Ergotamine and Ergonovine in Cows during the Luteal Phase of the Estrous Cycle." *J. Anim. Sci.* 76: 1448-54.
31. Simoni, M., & Nieschlag, E. (1995). "FSH in Therapy: Physiological Basis, New Preparations and Clinical Use." *Reprod Med. Rev.* 4: 163-77.
32. Iguchi, I. & Sato, T. (2000). Endocrine disruption and developmental abnormalities of female reproduction. *Am. Zool.* 40, 402-411.
33. Gallo, R. V. (1981). "Pulsatile LH Release during the Ovulatory Surge on Proestrous in the Rat." *Biol. Reprod.* 24: 100-4.
34. Bennatau-Pelissiero, C. (2018). Natural estrogenic substances, origins and effects. In: Merillon, J. M. & Ramwat, K.(eds) *Bioactive molecules in food. Reference Series in Phytochemistry.* Springer Cham. [https://doi.org/10.1007/978-3-319-54528-8\\_10-1](https://doi.org/10.1007/978-3-319-54528-8_10-1)
35. Pal, D., Mazumdar, U. K. & Gupta, M. (2012). Fractionation of stigmasterol derivative and study of effects of *Celsia coromandelina* aerial parts petroleum ether extract on appearance of puberty and ovarian steroidogenesis in immature mice. *Pharm. Boil.* 50 (6), 747-753.
36. Havranex, F., Stroufova, A., Kozlova, J., Herzmann, J. & Hejda, J. (1973). On the mechanism of contraceptive action of estrogens administered after ovulation. *Ceska Gynekol.* 38, 617-619.
37. Dande, P. & Patil, S. (2012). Evaluation of saponin from *Tigonella foeman Craecum* seed for its anti-fertility activity. *Asian J. Pharm. Clin. Res.* 5 (3), 154-157.
38. Hsia, S. M., Yeh, C. L., Kuo, Y. H., Wang, P. S., & Chiang, W. (2007). "Effects of Adlay (CoixLachryma-Jobi L. Var. Ma-yuenStapf.) Hull Extracts on the Secretion of Progesterone and Estradiol *in Vivo and in Vitro.*" *Expal. Biol. and Med.* 232: 1181-94.
39. Hadley, M. E. (2000). "Hormones and Female Reproduction Physiology." In Hadley ME, Ed. *Endocrinology* (5th edEd.). Englewood Cliffs, NJ: Prentice Hall Inc., 445-72.
40. Nordic Federation of Societies of Obstetrics and Gynaecology (NFSOG). *Acta Obstetricia et Gynaecologica Scandinavica.* 94(2015) 8-16.
41. Schneider M. A., Davies M. C. & Honour J. W. (1993). The timing of placental competence in pregnancy after oocyte donation. *Fertil Steril.* 1993;59:1059-59:1059-63.
42. Obeten, K. E., Isaac, V. N. & Ije, E. L. (2013). The Electrolytic Effect of *Sida acuta* Leaf Extract on the Kidney Electrolyte of Adult Wistar Rats, *Journal of Biology, Agriculture and Healthcare* HYPERLINK "<http://www.iiste.org>" ISSN 2224-3208 (Paper) ISSN 2225-093X (Online) Vol.3, No.10, 2013
42. [Note: Write the journal names in full](#)

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