OPEN ACCESS
Freely Available Online

OpenAccessPub

JOURNAL OF BIOTECHNOLOGY AND BIOMEDICAL SCIENCE
ISSN NO: 2576-6694

Research Article

DOI : 10.14302/issn.2576-6694.jbbs-20-3466

Effects of Selected Secondary Metabolites in Leaf Extract of *Jatropha Tanjorensis* on Some Gonadal Hormones in Male Wistar Rats.

Akighir John[1,*], Inalegwu Bawa[1], Anyam John[1], Ojochenemi Eje Yakubu[2], Odama Ikani Richard[3]

[1]Department of Biochemistry, College of Science, Federal University of Agriculture, Makurdi P.M.B. 2373 (970001) Nigeria

[2]Department of Biochemistry , Faculty of Pure and Applied Sciences, Federal University Wukari P.M.B. 1020,Wukari, Taraba State, Nigeria

[3]Department of Medical Biochemistry, College of Medicine, University of Nigeria, Enugu Campus, Enugu State, Nigeria

Abstract

Background and Objective

The use of medicinal plants in industrialized societies for extraction and development of many drugs and other chemotherapeutics and traditionally for herbal remedies has increased in recent times. Plant–based medicine is essential in health care services with about 80% global population relying on it because of its cheap source and availability. *Jatropha tanjorensis* is one such plant used by males and females of childbearing age for treatment of reproductive problems such as infertility. Literature on isolation and characterization of the secondary metabolites in this plant may not be common. Against this backdrop, this research work was carried out to isolate, characterize and determine the effects of *J. tanjorensis* on the gonadal hormones of male wistar rats.

Materials and Methods

The secondary metabolites were isolated, characterized, and identified using nuclear magnetic resonance. The experiment was conducted using 25 male wistar rats weighing between 180-200 g randomized into 5 groups, 3 controls and 2 treatment groups of 5 rats each. The treatment groups received 25 mg/kg body weight of phytol and lupeol orally by gastric lavage for 14 days. The animals were anaesthetized and blood samples collected for hormonal assay.

Result

The experimental data was analyzed using one-way analysis of variance (ANOVA) with Statistical Package for Social Sciences (SPSS) version 17.0, while the post hoc test assessed using Duncan Multiple Range Test at $p \geq 0.05$. There was a significant decrease ($p < 0.05$) in the levels of FSH, LH and TST in the treatment groups when compared to the control groups. The motility and sperm count decrease significantly ($p < 0.05$) when treatment groups were compared to the control animals. The secondary metabolites, phytol and lupeol present in the leaf extract of *Jatropha tanjorensis* were responsible for the decrease in some of the gonadal hormones studied.

OPEN ACCESS
Freely Available Online

Corresponding author: Akighir John, Department of Biochemistry, College of Science, Federal University of Agriculture, Makurdi P.M.B. 2373 (970001) Nigeria, Email: johnakighir2016@gmail.com

Keywords: Characterization; gonadal hormones; isolation; *Jatropha tanjorensis;* Secondary metabolites; rat.

Received: Jun 29, 2020 **Accepted:** Jul 17, 2020 **Published:** Jul 25, 2020

Editor: Monica Butnariu, Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, Timis, Romania.

Introduction

An increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of many drugs and chemotherapeutics from these plants as well as from traditionally used herbal remedies. A large and increasing number of patients use medicinal herbs or seek the advice of their physician regarding their use [8].It has been reported that about 70 % of the human population is dependent wholly or partially on plant-based medicine. This plant-based traditional medical system plays an essential role in health care with about 80 % of the world's population relying on it due to its availability and cheap source [20]. In Nigeria and most developing countries of the world, many people still rely heavily on herbal preparations for the treatment of various diseases despite availability of orthodox medicine. Many of these medicinal plants are used by males and females of reproductive age and for treating reproductive problems such as infertility [26]. *Jatropha. tanjorensis* is one such plant used by males and females of childbearing age for treating reproductive problems such as infertility. Literature on isolation and characterization of the secondary metabolites in this plant may not be common. Against this backdrop, this research was conducted to isolate secondary metabolites in leaf extract of *J. tanjorensis* and determine their effects on some gonadal hormones of male wistar rats.

Materials and Methods

Equipment

Stat Fax 3300 chemistry analyzer, Precision pipette, Beakers, Digital balance (model: SF-400), Blender, Incubator, Improved Neuber counting chamber, Glass column, Binocular Microscope (Olumpus),Test tubes (anticoagulant free),General laboratory glass wares, Test tube rack, Syringe and needle, Conical flask, Separation funnel, Glass wool, Spatula, Aluminum foil and Kitchen Knife etc.

Chemicals/Reagents

Enzyme immunoassay test kitsSilica gel, Hexane, Distilled water, Chloroform, Ethyl acetate, Methanol etc, Silica gel, Hexane, Distilled water, Chloroform, Ethyl acetate, Methanol etc.

Methods

Fresh leaves material of *J. tanjorensis* was collected in a home garden, at the University of Agriculture Makurdi and authenticated by the Department of Botany, College of Science, Federal University of Agriculture, Makurdi and Voucher specimen of the plant deposited in the Department's herbarium. The leaves were thoroughly washed with clean water and allowed to drain, air-dried at room temperature. The crispy leaves were pulverized using an electric blender and preserved in a moisture-free airtight laboratory containers for extraction. The homogenized *J. tanjorensis* was subjected to extraction as described by Agarwal *et al.*, 2007 [1]. Thus 100 g of the powdered material was macerated in 1000 ml of 80% (v/v) methanol and allowed to stay for 48 hours with intermittent agitation at a cool temperature of 4ºC. The mixture was first filtered with cheese cloth, then filter paper (Whatman No.1) and the methanol evaporated using a rotary evaporator. The concentrate was allowed for complete dryness using a thermostatically controlled water bath at 42 ºC, this yielded 250 g crude extract.

The methanol extract was chromatographed on silica gel (60-120 mesh size) to separate its component fractions and eluted with different solvent combinations based on increasing polarity beginning from Hexane,

chloroform, ethyl acetate and methanol as described by Hostettmann *et al.,* 1999[7]. The following ratios of solvent combinations were sequentially used.

Hexane: Ethyl acetate 9.5:0.5, 7.0:3.0, 6.0:4.0, 5.0:5.0

Chloroform: Methanol 9.5:0.5, 9.0:1.0, 7.0:3.0, 1.0:1.0

Thin layer chromatography was also carried out as described by Tor-Anyiin *et al.*, 2016 [26].

The thin layer chromatography (TLC) tank was prepared: A Hexane /Methanol mixture was prepared in the ratio of 9:1 (10ml) because it gave the best resolution on the TLC plates. Spots were put neatly and deftly to ensure uniform and tidy application of the fraction materials on the TLC plates. The developed plates were charred in a hot frying pan for two minutes and the pooling of the fractions was done on the basis of the similarities of the spots, color and the retention factor on the TLC plates. The individual bioactive compounds from the fractions were determined using Nuclear Magnetic Resonance (NMR) technique

Nuclear magnetic resonance was carried out as described by Komiya *et al.*, 1999 [10] Always a dilute solution is analyzed. The compound to be studied was generally mixed with solvent (CC14 or etramclhyl silane) and the dilute solution was filled in a tube. The sample under investigation was placed in the magnetic field and subjected to RF field of oscillator the particular combination of the oscillator frequency and strength, the RF energy was absorbed by certain nuclei and an RF signal was picked up by the detector.

Twenty five male wistar rats were procured from the animal house of College of Health Sciences, Benue state University, Makurdi. The animals were acclimatized for one week, housed in cages under room temperature (25±2ºC) , and relative humidity (55±5%) and a 12 hour light/ dark cycle in animal house of laboratory department, College of Veterinary Medicine, University of Agriculture, Makurdi. The animals were allowed free access to chow and tap water *ad libitum* with all the experimental procedures approve by the university research and ethics committee. Oral acute toxicity was carried out as described by Lorke, 1983 [13].

Experimental Design

The design consisted of 25 male wistar rats grouped into five (5) groups of five (5) rats each. The groups were normal control (NC), positive control (PC), standard control (SC) and two treatment groups. The doses used were based on the LD^{50} determined for the fractions and the predetermined LD^{50} for the crude extract obtained from preliminary studies. The normal control group received normal feed and water, Positive control group received 25 mg/kg body weight of Sustanon, the standard control group received 10 mg/kg standard drug Amlodipine. The treatment groups' received 25 mg/kg body weight of phytol and lupeol through oral intubation daily for 14 days.

Collection and Preparation of Sera Samples

The rats were anesthetized with phenobarbital, cardiac puncture performed and blood sample collected. The sera samples were separated, then assayed for Testosterone, follicle stimulating hormone and luteinizing hormone using enzyme-link immune-absorbent assay (ELISA) as described by Marshall, 1975; Knobil, 1980 and Chen *et al.*, 1991 [14,9,5] methods.

Cauda epididymis sperm concentration and motility was assessed according to the method employed by Prasad *et al.*, 2008 [21]

Statistical Analysis

Experimental data were expressed as mean± standard error of mean (SEM) and analyzed using one-way analysis of variance using Statistical Package for Social Sciences (SPSS) version 17. The post hoc test was assessed using Duncan Multiple Test Range (DMRT) at $p \leq 0.05$

Results

Acute Toxicity Study of Jatropha Tanjorensis

The result showed lethal dose determination of methanolic leaf extract of *Jatropha tanjorensis* in wistar rats for 48 hours. There was no mortality and toxicity sign within 48 hours after oral administration of 3500, 5000 and 6500 mg/kg body weight. The LD_{50} of this plant was considered to be > 5000 mg/kg (Tab 1 &2)

Nuclear Magnetic Resonance (NMR)

^{13}C-NMR spectra were taken with JEOL, JNM-400. Structural determination of J25 was done using ^{1}H-NMR ($CDCl_3$), ^{13}C-NMR ($CDC1_3$), 'H-'H-COSY [(Table 3), HMQC [(Table 4) and HMBC

Carbon-13 NMR of J25

The Carbon-13 NMR of J25 showed the following signals: ^{13}C NMR (101 MHz, Chloroform-*D*) δ

140.40, 123.20, 59.53, 40.01, 39.50, 37.56, 37.49, 37.42, 36.80, 32.93, 32.83, 29.84, 28.11, 25.27, 24.94, 24.61, 22.86, 22.77, 19.89, 19.85, 16.31 (Table 3 & 4).

Proton NMR of J25

The fraction J25 had the following ^{1}HNMR signals: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 5.40 (dt, *J* = 7.9, 3.8 Hz, 1H), 4.14 (d, *J* = 6.9 Hz, 2H), 2.16 – 1.91 (m, 9H), 1.69 – 1.55 (m, 7H), 1.52 (dt, *J* = 13.2, 6.8 Hz, 1H), 1.41 (d, *J* = 5.7 Hz, 1H), 1.39 – 1.30 (m, 7H), 1.30 – 1.26 (m, 5H), 1.26 – 1.20 (m, 8H), 1.19 – 1.01 (m, 12H), 0.86 (d, *J* = 6.4 Hz, 11H), 0.83 (d, *J* = 2.8 Hz, 5H) (Table 6 and 7).

The Carbon-13 NMR of J25 showed the following signals: ^{13}C NMR (101 MHz, Chloroform-*D*) δ 140.40, 123.20, 59.53, 40.01, 39.50, 37.56, 37.49, 37.42, 36.80, 32.93, 32.83, 29.84, 28.11, 25.27, 24.94, 24.61, 22.86, 22.77, 19.89, 19.85, 16.31 (Table 3 & 4) (structure1)

Proton NMR of J7

The proton NMR of J7 revealed the following data: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.68 (d, *J* = 2.6 Hz, 1H), 4.56 (d, *J* = 2.5 Hz, 1H), 4.46 (dd, *J* = 10.7, 5.5 Hz, 1H), 2.32 (ddt, *J* = 21.7, 8.8, 5.9 Hz, 3H), 2.04 (d, *J* = 5.3 Hz, 4.4H), 1.67 (s, 9H), 1.40 – 1.36 (m, 3H), 1.02 (d, *J* = 3.1 Hz, 3H), 0.95 (d, *J* = 5.1 Hz, 5H), 0.87 – 0.80 (m, 21H), 0.78 (s, 2H). Tab 5 & 8)

Discussion

Column chromatography of the leaf extract of *Jatropha tanjorensis* was done with solvent system of gradually increasing polarity, beginning from Hexane, chloroform, ethyl acetate and methanol, thereby confirming earlier studies [7].

The thin layer chromatography (TLC) of fractions collected from the column chromatography of *Jatropha tanjorensis* leaf extract showed retention factor of 0.4 for lupeol and 0.90 for phytol, which is within the range of values (0.31 and 0.45) obtained by [24] for lupeol, and 0.80 for phytol [3].The differences in the results obtained by [24, 3] is chiefly due to solvents combination in the mobile phase. Better resolutions are obtained when a combination of polar and non-polar solvents are used.

The structure of J25 was identified based on spectroscopic analysis (^{1}H NMR, ^{13}C NMR, 2D NMR) and by comparison of it spectral data with those reported previously in the literature [10] (Table 4). The proton NMR of J25 revealed the presence of three distinct regions: an olefinic region (5.38-5.42 ppm), an alcohol region (4.14-4.15 ppm) and an aliphatic region (0.78-2.09 ppm). The olefinic signal [5.40 (td, *J* = 6.3, 3.1 Hz, 2H)] showed characteristics (an ABX coupling pattern), while the alcohol signals [4.14 (d, *J* = 7.0 Hz, 2H)].

The number of carbons in J25 was revealed to be twenty by ^{13}CNMR data. Prominent HMQC correlations showed that the proton at δ 5.40 ppm was attached to the carbon at δ 123.19 ppm other correlations showed features consistent with those of a diterpene alcohol.

Prominent HMBC correlations showed correlation between protons at C-1 to carbons C-2 (2*J*)(δ 123.20 ppm) and C-3 (3*J*)(δ 140.40 ppm) while the proton at C-2 was correlated to C-4 (δ 40.01 ppm) and C-20 (δ 16.31 ppm) proving that C-20 was a substituent at C-3. Literature comparison with the work of [10] confirmed the compound was phytol (Structure 1), a diterpene alcohol and was in full accord with other previously reported values [23].

^{1}H-NMR data of J7 revealed two signals olefinic protons at δH 4.56 (d, *J* = 2.5 Hz, H-29a) and 4.68 (d, *J* = 2.6 Hz, H-29b) characteristic of lupine-type triterpenes like lupeol and betulin. A proton signal at δH 4.46 (dd, *J* = 10.7, 5.5 Hz, 1H) showed that the proton at C-3 was not a carbinol proton while signals at 2.04 (s, 3H) consistent with those of an acetyl methyl group showed that there was an acetyl group attached through an oxygen at position C-3. A literature search showed that the HNMR spectra data of J7 was consistent with that of lupeol acetate in the literature [11].

Acute toxicity study is > 5000 mg/kg because neither death nor any other sign of toxicity (mortality, fast respiratory rate, convulsion and dullness) was observed during the period of the experiment. Earlier studies have proven that LD_{50} of the aqueous extract of *J. tanjorensis* was > 5000 mg/kg [2], confirming its ameliorative effects on phenylhydrazine induced anaemia.

Table 1. Acute Toxicity Study of *Jatropha tanjorensis*

	Dose (mg/kg)	Number of animal	% mortality
First phase			
Group 1	3500	3	0
Group 2	5000	3	0
Group 3	6500	3	0

Toxicity sign: no-toxicity sign observed within the 48 hours of observation LD_{50} > 5000 mg/kg

Table 2. The result of thin layer chromatography (TLC) of fractions collected from the column chromatography of *Jatropha tanjorensis* leaf extract as presented

No. Spot	**Fraction range**	**Rf value**	**New fractions**
1	J1- J5	0.9	J 25
4	J6- J8	0.9	J25
4	J9- J11	0.9	J25
2	J12- J14	0.7	J10
3	J15- J20	0.4	J7
1	J21- J26	0.4	J7
1	J27- J29	0.4	J7
2	J30- J39	0.4	J7

Formula for RF=Distance travelled by the spot/Distance travelled by solvent front

KEY: RF=Retention Factor

J1- J5 = *Jatropha* fraction 1-5

J6- J8 = *Jatropha* fraction 6-8

J9- J11 = *Jatropha* fraction 9-11

J12- J14 = *Jatropha* fraction 12-14

J15- J20 = *Jatropha* fraction 15-20

J21- J26 = *Jatropha* fraction 21-26

J27- J29 = *Jatropha* fraction 27-29

J30- J39 = *Jatropha* fraction 30-39

Table 3. Nuclear Magnetic Resonance Analysis Characterization of J25 as Phytol ((2*E*)-3, 7, 11, 15-tetramethylhexadec-2-en-1-ol)

Position	**^{1}H multiplicity (J in Hz)**	**^{13}C**	**^{1}H Literature Komiya *et al.,* 1999**	**^{13}C Literature Komiya *et al.,* 1999**
1	4.14 (d, J = 6.9 Hz, 2H)	59.53	4.09	59.4
2	5.40 (dt, J = 7.9, 3.8 Hz,1H)	123.20	5.34	123.0
3	-	140.40	-	140.4
4	1.98	40.01	-	39.9
5	0.84	25.27	-	25.1
6	1.37	36.80	-	36.7
7	1.52	32.83	-	32.7
8	1.21	37.56	-	-
9	1.25	24.61	-	24.5
10	1.26	37.49	-	37.4
11	1.57	32.93	-	32.8
12	1.25	37.42	-	37.3
13	1.26	24.94	-	24.8
14	1.25	39.50	-	39.4
15	1.25	28.11	-	27.9
16	0.79 (d, 3H)	22.77	-	22.6
17	0.84 (d, 3H)	22.87?	-	22.7
18	0.84 (s, 6H)	19.85	-	19.7
19	0.84	19.89	-	19.7
20	1.66 (s, 3H)	16.31	1.60	16.2

Table 4. Nuclear Magnetic Resonance Analysis of J25

Position	**^{1}H multiplicity (J in Hz)**	**^{13}C**	**HMBC**	**HMQC**
1	4.14 (d, J = 6.9 Hz, 2H)	59.53	C-2, 3	C-1
2	5.40 (dt, J = 7.9, 3.8 Hz, 1H)	123.20	C-4, 20	C-2
3	-	140.40		
4	1.98	40.01	C-4, 20	C-4
5	0.84	25.27	C-4, 7, 8	C-5
6	1.37	36.80		
7	1.52	32.83		
8	1.21	37.56		
9	1.25	24.61		C-9
10	1.26	37.49		
11	1.57	32.93		
12	1.25	37.42		
13	1.26	24.94		
14	1.25	39.50		C-14
15	1.25	28.11		C-15
16	0.79 (d, 3H)	22.77		C-20
17	0.84 (d, 3H)	22.87?		C-17
18	0.84 (s, 6H)	19.85		C-18
19	0.84	19.89		C-19
20	1.66 (s, 3H)	16.31	C-4, 20	C-20

Table 5. Structures of Phytol and Lupeol Isolated from Methanolic Leaf Extract of *Jatropha tanjorensis*

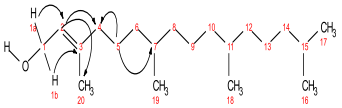
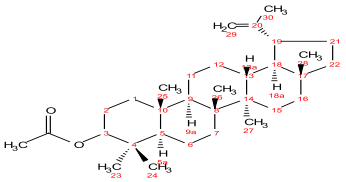
S/NO	Name of the compound	Structure of the compound	Rentention factor (RF)
1	Phytol (2*E*)-3, 7, 11, 15-tetramethylhexadec-2-en-1-ol)		**0.9**
2	Lupeol acetate		**0.4**

Table 6. Effects of Secondary Metabolites in Leaf Extract of *Jatropha tanjorensis* on the Levels of TST, FSH and LH in Sustanon-Induced Wistar Rats

Groups	FSH (ng/mL)	LH (ng/mL)	TST(ng/mL)
Normal Control	5.96 ± 0.81[a]	7.45 ± 0.66[a]	0.45 ± 0.13[a]
Positive Control	7.52 ± 0.02[a]	6.48 ± 0.57[a]	20.88 ± 0.04[b]
Standard Control	3.40 ± 0.99[b]	2.87 ± 0.17[b]	13.30 ± 0.63[c]
Sus + 25 mg/ phytol	3.22 ± 0.13[b]	2.38 ± 0.29[b]	13.67 ± 0.74[c]
Sus + 25 mg/kg lupeol	3.00 ± 0.24[b]	3.08 ± 0.13[b]	12.72 ± 0.79[c]

Data are expressed as mean ± standard error of mean (SEM) with n: 5, values with different superscripts as alphabet down the columns are considered statistically significant ($p < 0.05$).

Table 7. Effects of Phytol and Lupeol on Testicular Weight of Sustanon- Induced Wistar Rats

Groups	Weight (g)
Normal Control	1.20 ± 0.03^{a}
Positive Control	1.67 ± 0.05^{b}
Standard Control	1.24 ± 0.08^{b}
Sus + 25 mg/kg phytol	1.47 ± 0.13^{b}
Sus + 25 mg/kg lupeol	1.19 ± 0.11^{b}

Data are expressed as mean ± standard error of mean (SEM) with n: 5, values with different superscripts as alphabet down the columns are considered statistically significant ($p < 0.05$).

Table 8. The Percentage Concentration and Sperm Motility of Sustanon-Induced Rats Treated with Phytol and Lupeol

Groups	% Motility
Normal Control	61.64 ± 2.15^{a}
Positive Control	66.67 ± 2.36^{a}
Standard Control	40.33 ± 2.62^{b}
Sus + 25 mg/kg phytol	39.32 ± 2.26^{b}
Sus + 25 mg/kg lupeol	41.61 ± 2.24^{b}

Data are expressed as mean ± standard error of mean (SEM) with n: 5, values with different superscripts as alphabet down the columns are considered statistically significantly ($p < 0.05$).

Follicle stimulating hormone (FSH) recorded significant decrease ($p < 0.05$) in treatment groups when compared to the control rats. The findings of Udoh *et al.*, 2009 [27], however, disagree with the submission of Modares and Heidari, 2015 [16]. FSH regulates the development, growth, pubertal maturation and reproductive process of the human body. In both male and female FSH stimulates the maturation of germ cells. In male induces sertolic cell to secrete androgen-binding protein (ABP), regulated by inhibin's negative feedback mechanism on anterior pituitary. Specifically, activation of sertolic cell by FSH sustains spermatogenesis and stimulates inhibin-B secretion [4]. It is suggested that the low sperm count recorded in this study may have been orchestrated through this mechanism.

Luteinizing hormone (LH) recorded a significant decrease ($p < 0.05$) in all the treatment groups when compared to the control group. Udoh *et al.*, [27] also recorded reducing effects in the LH levels at 150 mg/kg body weight in non-induced rats, which decreased significantly ($p < 0.05$) when compared to the control group. Contrary to these findings of Modares and Heidari 2015 [16] observed that *Allium Sativum* at 800 mg/kg body weight increased LH levels on heat stress female mice (3.2 Iμ/ml). These variations in the levels of the hormones may be as the result of varying bioactive compounds in the various plants used by the researchers. Also, sex is another determining factor in organisms' reaction to drugs or substances as in the case of [16]. LH is produced by gonadotropic cells in the anterior pituitary gland. In females, an acute rise of LH ("LH surge") triggers ovulation and development of the corpus luteum [6]. In males, LH in synergy with FSH, stimulate Leydig cell production of testosterone [16].Suggestion is therefore made that, the reduction created by the treatment substances on the levels of these hormones may be the reason for the reduction in the testosterone levels recorded in treated rats.

It was observed that the testosterone levels in treated rats decrease significantly ($p < 0.05$) with lupeol orchestrating the highest reducing effect when compared to the control rats. The also agrees with the findings of Udoh *et al.*, 2009[27] on Caricpryl-99 seed alkaloid extract on the serum of testosterone, which showed that the extract decreased the hormone levels significantly in non-induced rats.

The findings in some gonadal hormones (Testosterone, Follicle stimulating hormones and Luteinizing hormones) decreasing significantly ($p < 0.05$) as recorded in all the treatment groups when compared to the control rats may be the likely cause of spermatogenic arrest and failure of spermatogenesis in the histology of rats' testes. Oluwole *et al.*, 2012[19] in his work on assessment of hepatic and renal functions administered methanolic leaf extract of *Jatropha tanjorensis* also recorded a disruption in hepatic and renal functions in rats. The findings collaborates the ascension that LH through specific receptors controls the production and secretion of testosterone and testosterone is critical for the completion of meiosis and entry into progress through spermatogenesis in rats [15]. This also agrees with the earlier works that, the crude extract of *Allium sativum* (Garlic) caused a decrease in serum testosterone levels with effects being evoked at a very low dose [18].

The testicular weights of the wistar rats induced and treated with phytol and lupeol in leaf extract of *Jatropha tanjorensis* recorded no significant decrease ($p < 0.05$) when the treatment groups was compared to the control. Contrary to these findings of Munir *et al.*, 2017 [17] who observed a significant decrease ($P < 0.05$) in the testicular weight of rats treated with Bisphenol® as compared to the control group. These findings however agree with the findings in this study with reference to amlodipine control group. Amlodipine and bisphenol are both antihypertensive drugs [17]. The findings of Rabia *et al.* (2008) also agree with the findings in this study that amlodipine significantly decreased ($P < 0.05$) the testicular weights of albino rats. However, this contradicts the findings in this study on the isolated secondary metabolites; the reason may simply be time dependent.

The percentage motility of rats treated with phytol and lupeol recorded a significant decrease ($P < 0.05$) when compared to the control group. The reduction in the percentage motility of sperm and its total count may chiefly be as a result of the secondary metabolites present in the methanolic leaf extract of *J. tanjorensis* affecting the leydig cells which are responsible for production of testosterone, and

testosterone is responsible for the completion of spermatogenetic process [12].

Conclusion

In the study, the secondary metabolites; phytol and lupeol were isolated and characterized from the methanolic leaf extract of *Jatropha tanjorensis* and have demonstrated to have significant reducing effects on some gonadal hormones and hence be used as local birth control agents.

Conflict of Interest

The authors declare that there is no conflict of interest

Acknowledgement

We are very grateful to the staff of the Department of Biochemistry, Federal University of Agriculture Makurdi and Professor John Igoli of the Department of Chemistry Federal University of Agriculture Makurdi and Mrs Regina Akighir for their assistance in making sure that research gained the light of the day.

References

1. Agarwal, S.P., Rajesh, K., Khanna, M.D. and Roop, K.K. Shilajit: review:phytotherapy research 2007, 21 (5)1786-1789
2. Ameloko, U.J. Anti-anemic effect of aqueous and methanolic leaf extracts of Jatropha janjorensis on phenylhydrazine induced hemolytic anemic rats Ph.D dissertation Dept. of Biochemistry, ABU Zaria, Kaduna State 2010
3. Basarkar, U.G. Phytol-A chemical constituent of Leucas aspera through HPTLC American International Journal of Research in Formal Research in Formal, Applied and Natural Sciences 2005 Available online at http://www.iasir.net retrieved on 2018/07/29 at 9:30 PM
4. Boron, W.F. and Boulpaep, E.LMedical physiology: a cellular and molecular approach. 2nd revised Edition: Elsevier Saunders. 2012. p. 112
5. Chen,A., Bookstein ,J.J .and Meldrum, D.R..Diagnosis of testosterone secreting adrenal adenoma by selective venous catheterization Fertility and Sterility 1991, 55 (6) 1202-1203.
6. Hammes, S.R. The further redefining of steroid-mediated signaling Proceedings of Natural and Academic Science 2003,100 (5): 2168-2170.
7. Hostettmann, K., Maraton, A.S. and Hostettmann, M. Preparative chromatography Techniques in Natural Product Isolation (2nd Ed) HAFTAD EN-GELSKA 1998
8. Iwalewa, E.O., Adewunmi, C.O., Omisore, N.O.A. O.A., Azike, C.K., Adigun, A.O and Olowayo, O.G. Pro-and antioxidant effects and Cytoprotective potentials of nine edible vegetables in Southwest Nigeria Journal of Medicinal Food, 2005, 8 (4) 539-573.
9. Knobil, E. The neuroendocrine control of the menstrual cycle, Rec Program Hormone 1980, 36: 52-88.
10. Komiya, T., Kyohkon, M., Ohwaki, S., Eto, J., Katsuzaki, H., Imai, K., Kataoka, T. and Yoshioka, K.,Ishi,Y. and Hibasami, H. Phytol induces pro-grammed cell death in human lymphoid leukaemia Molt 4B cells. International Journal of Molecular Medicine 1999, 4(4) 377-457
11. Lakshmi, V., Mishra, V., Palit, G., Gupta, S. and Agarwar, S.K. Lupeol and lupeol esters protect the gastric ulcer in rats. Natural Product: An Indian Journal 2014, 10(4)113-122.
12. Lee, B.S. and William, H.W. The regulation of spermatogenesis by androgens: *Seminars in Cell and Developmental Biology* 2014, 30:2-3
13. Lorke, D.A. A new approach to practical acute toxicity testing. *Archive of toxicology*; 1983, 54(4) 275-287
14. Marshall, J.C. Clinical Endocrinology and Metabolism Investigative procedures clinical *Endocrinology and Metabolism* 1975, 4(3):545-612.
15. MchLachlan, R.I., O'Domell, L., Meachem, S.J., Stanton, P.G., de Kretser, D.M., Pratis, K. and Robertson, D.M. Identification of specific sites of hormonal regulation in spermatogenesis in rats, monkeys, and man. *Recent Program Hormone* 2002, 57:149-228.
16. Modares, M. and Heidari, M. (2015). Effect of Allium sativum extract on pituitary-gonad axis in heat stressed female mice. *Journal of Chemical and*

Pharmaceutical Research cal and Pharmaceutical Research 2015, 7(6):7-9

17. Munir, B., Qadir, A. and Tahir, M. Effects of bisphenol A on testicular functions in albino rats and their abolitions with Tribulus terristeris L. *Brazilian journal of pharmaceutical Sciences* 2017, 53 (3):e00104
18. Olayiwola, G., Iwalewa, E.O., Omobuwajo, O.R., Adeniyi, A.A. and Verspohi, E.J.Anti-diabetic potential of *Jatropha tanjorensis* leaves extract. *Nigerian Journal of Natural Products and Medicine* 2004, 8:55-58.
19. Oluwole, I. O., Oluwaseun T. O. and Bukola V. A. Assessment of renal and hepatic functions in rats administered methanolic leaf extract of *Jatropha tanjorensis Annas of Biological Research* 2012, 3 (2):837-841.
20. Owolabi, J., Omogbai E.K. and Obasuyi O. Antifungal and antibacterial activities of the ethanolic and aqueous extracts of Kigelia africana (Bignoniaceae) stem bark *African Journal of Biotechnology 2007, 6(*14):882-887.
21. Prasad, S., Kalra, N., Singh, M. and Shukla, Y. Protective effects of lupeol and mango extract against androgen induced oxidative stress in Swiss albino mice *Asian Journal of Androl*ogy 2008,10 (2): 313-321
22. Rabia, L., Ghulam, M.L. and Muhammad, A. Effects of Amlodipine on serum testosterone, testicular weight and Gondo-somatic index in adult rats *Journal of Ayub Medical College* 2008, 20(4):8-10.
23. Rajab, M.S., Charles, L. Cantrell, C.L., Franzblau, S.C. and Fischer, N.H .Anti-mycobacterial Activity of (E)-Phytol and Derivatives: a Preliminary Structure- Activity Study. *Planta Medica,* 1998, 64 (1) 2-4.
24. Rohini, R.M. and Amit, K.D. Determination of Lupeol, β-Sitosterol and Quercetin from Ethyl Acetate Extract of Rhizophora Mucronata Barkby HPTLC Technique *Asian Journal of Pharmaceutical and Clinical Research* 2011,4(1):103-105.
25. Sakpa, C.L. and Uche-Nwachi O.E. Histological effects of aqueous leaf extract of chaya (Cnidoscolus aconitifolius) on the testes and epididymis of adult Wistar rats. *Journal of Medicine and Biomedical Research* 2014, 13 (1): 120-122.
26. Tor-Anyiin, T.A., Igoli, J.O. and Anyam, J.N. (2016). Studies on Dacryodes edulis 111. Isolation and characterization of Galic acid from methanolic extract of raw (untreated) seeds of Dacryodes edulis and its Antimicrobial properties *Journal of Chemical Society of Nigeria* 2016, 41(1) 6-9.
27. Udoh, P. B., Udoh, F. V., Umoren, E. B., James, U. W., Okeke, C. P. and Agwu, B. The comparative effects of chronic consumption of kola nut (cola nitida) and caffeine diet on locomotor behavior Effect of Caricapryl-99 Seed Alkaloid Extract on the Serum Levels of Sex Hormones and Pituitary Gonadotrophins in Male Albino Ratsand body weights in mice Nigerian. *Journal of Physiological Sciences* 2009, 24 (1):73-81.