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RESEARCH ARTICLE

Antiproliferative and ameliorative effects of *Tetracera potatoria* and its constituent

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Abstract
The effect of *Tetracera potatoria* leaves and its isolated constituent on polycystic ovarian syndrome (PCOS) and associated gynaecological cancers was investigated. Crude extract showed the lowest level of luteinizing hormone and the highest level of estradiol and follicle stimulating hormone, comparable to the normal control group. The hexane ($IC_{50} = 34.8 \pm 0.3$ µg/mL), and dichloromethane ($IC_{50} = 41.3 \pm 0.8$ µg/mL) fractions inhibited the proliferation of Chinese Hamster ovarian (CHO) cells. The dichloromethane fraction was subjected to purification using column chromatography, which led to the isolation of apigenin. The structure of the isolated compound was confirmed by the reported spectroscopic data. Apigenin inhibited the proliferation of both CHO and HeLa cells with an IC_{50} values of 22.2 ± 0.5 and 6.2 ± 0.6 µg/mL, respectively. The apigenin was isolated and reported for the first time in *T. potatoria*. The leaves extract of *T. potatoria* showed curative effect on irregular estrus cycle and hormonal imbalance. The isolated constituent showed anticancer potential, hence it could reduce the risk of gynaecological cancers among PCOS patients.

Keywords *Tetracera potatoria* · Antiproliferative · Flavonoids · Polycystic ovary syndrome · Luteinizing hormone · Follicle stimulating hormone

Introduction

Polycystic ovary syndrome (PCOS) is the leading endocrine disorder affecting women worldwide. It is connected with ovulatory dysfunction, polycystic ovarian morphology and hyperandrogenism (Legro et al. 2013). In most studies, the incidence of PCOS among women is assessed to be between 5 and 20%. The criteria used and ethnicity of the studied population have been identified as factors responsible for the variation in the prevalence of PCOS (Azziz et al. 2004; Ehrmann 2005). In South-eastern Nigeria, prevalence rate of 18.1% was reported when presence of numerous ovarian cysts was used as diagnostic criteria (Ugwu et al. 2013). Women affected with PCOS are at 2.7-fold increased risk of developing gynaecological cancers such as ovarian and cervical cancers. However, there is no established relationship between PCOS and breast cancer (Dumesic and Lobo 2013; Ding et al. 2017). Abnormal *p53* tumour suppressor gene was recently discovered in the endometrium of PCOS patients (Shafiee et al. 2015; Gadducci et al. 2016).

Tetracera potatoria is a medicinal plant found in wooded areas of Senegal, southern part of Nigeria, Central and Eastern Africa (Dalzel 1937). The leaves or a portion of the stem are boiled in its own sap and used as a powerful diuretic, vermifugal and purgative, as well as for the treatment of gastrointestinal and other stomach complaints (Burkill 1985; Betti 2004). The sap is also used for the treatment of cough and toothache (Oliver-Bever 1960). In south-western Nigeria, the aqueous extract from the root is an active remedy for intestinal disorders (Adesanwo et al. 2003). It is used in traditional treatment of inflammation, skin infection and ulcer (Adesanwo et al. 2013). Several classes of phytochemicals such as tannins, alkaloids, cardiac glycosides, flavonoids

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and saponins have been reported to be present in *Tetracera potatoria* (Adesanwo et al. 2013), however, betulinic acid, N hydroxy imidate-tetracerane, β-stigmasterol, stigmast-5-en-3β-yl acetate, tetraceranoate, lupeol and botulin are the few compounds isolated from various parts of *T. potatoria* so far (Adesanwo et al. 2013; Fomogne-Fodjo et al. 2017). In an ethnobotanical survey conducted in the course of this study, *T. potatoria* leaf juice was mentioned for management of menstrual disorder, stomach upset and infertility among premenopausal women in South-western Nigeria (Ogunlakin and Sonibare 2020). This study therefore reported the effect of *Tetracera potatoria* leaves and its constituent on polycystic ovarian syndrome and associated gynaecological cancer.

Materials and methods

Plant material

Fresh leaves of *Tetracera potatoria* were collected in Oluponna (7° 35′ 34.7″ N 4° 11′ 27.5″ E), Ayedire Local Governmet Area, Osun state of Nigeria on 19th of February, 2017. The plant was identified and authenticated by Mr. G. Ibhanesebhor, a taxonomist-in-charge of the Herbarium of Obafemi Awolowo University, Ile-Ife, Nigeria where voucher specimen (IFE Herbarium-17794) was also deposited.

Extraction and solvent partitioning

The air-dried plant material was pulverised. Pulverised plant material (1300 g) was extracted with methanol for 72 h at room temperature. The extract was filtered and the filtrate was concentrated *in vacuo* yielding dark-green sticky extract. The extract was stored in refrigerator. Solvent–solvent partitioning was done in accordance with standard procedure (Van Wagenen et al. 1993). The *n*-hexane, dichloromethane (DCM) and ethyl acetate fractions obtained were concentrated *in vacuo* to give residues, which were kept in air tight containers for subsequent bioassay. The percentage yields of *n*-hexane, DCM and ethyl acetate fractions were calculated.

In vitro antioxidant assay and quantification of phenolics and flavonoids

The antioxidant potential of the methanol extract and the fractions of *T. potatoria* was evaluated using 1, 1-diphenyl-2-picryl-hydrazyl-hydrate (Mensor et al. 2001; Bursal and Gülçin 2011). Total phenolic content (TPC) of the methanol extract and fractions were measured using Folin-Ciocalteu spectrophotometric method (Miliauskas et al. 2004). The aluminium chloride colorimetry method was used for quantification of flavonoids according to the standard procedure (Woisky and Salatino 1998).

Spectroscopic analysis

Nuclear magnetic resonance (NMR) spectra were recorded on *Bruker AV (Avance)* spectrometer (^{1}H NMR at 400 MHz; ^{13}C NMR at 600 MHz) using the nondeuterated solvent peaks as internal standard. Low resolution electron impact mass spectra were recorded on a finnigan *MAT 312* and *MAT 312* spectrometer. TLC was carried out using silica gel 60 GF_{254} pre-coated aluminium sheets by Sigma Aldrich, Germany. Melting point of the compound was determined on Buchi® M-560 melting point apparatus.

Isolation and characterisation of apigenin from DCM fraction

Light-green coloured DCM fraction (2.62 g) was subjected to column chromatography and eluted with hexane, DCM, ethyl acetate and methanol in different ratios to give 80 fractions of 200 mL each. The fractions were analysed by TLC using pre-coated TLC plates (Silica gel G_{60} F_{254} sheets 20 × 20 cm, 0.5 mm thickness) using appropriate solvent systems as mobile phases. Twelve different pooled fractions, D_1 (fractions 1–4), D_2 (fractions 5–8), D_3 (fractions 9–13), D_4 (fractions 14–20), D_5 (fractions 21–23), D_6 (fractions 24–25), D_7 (fractions 26–28), D_8 (fractions 29–39), D_9 (fraction 40), D_{10} (fractions 41–50), D_{11} (fractions 51–66), D_{12} (fractions 67–80) were obtained. Fraction D_9 (greenish yellow, 200 mg) vial prep-TLC technique (developed with DCM-ethyl acetate—80:20 and few drops of acetic acid) yielded compound **1** (6.28 mg, yellow coloured powder).

Antiproliferative studies

Antiproliferative effect of crude and solvent fractions of *Tetracera potatoria* on HeLa and CHO cell lines (ATCC, Manassas, USA) was evaluated by standard MTT colorimetric assay (Mosmann 1983). One hundred microliter (100 µL) of viable cells (5×10^4 cells/mL of HeLa and 6×10^4 cells/mL of CHO cells) cultured in DMEM (Dubecco's modified Eagle's medium; Sigma chemical Co., St. Louis, MO, USA) supplemented with 10% FBS were seeded into 96-wells microliter plate and incubated overnight at 37 °C in 5% CO_2. The extracts, solvent fractions and Doxorubicin at three different concentrations (1, 10 and 100 µg/mL) prepared in triplicate were added to the plate and incubated for 48 h. Fifty microliter (50 µL) of 0.5 mg/mL MTT was added to each well after 4 h incubation of the mixture. The same volume of DMSO was then added to all test and control wells and the absorbance was measured at 540 nm using spectrophotometer (Spectra

Max plus, Molecular Devices, CA, USA). The antiproliferative effect of all the test samples was reported as IC_{50} value (µg/mL). This assay was repeated for the isolated compound **1**.

In vivo studies

Experimental animals

Laboratory-bred virgin female Wistar rats, weighing between 150 and 200 g with regular estrous cycles were used. All animals were housed in cages under 12 h to 12 h light–dark cycle at a temperature of 20–25 °C. The cages were lined with soft wood shavings, used as beddings to absorb waste products from the animals, and changed regularly. They had free access to constant food pellets (Ladokun feeds Nig. Ltd.) and water ad libitum during the course of this study. Animals were acclimatized for minimum of 1 week before the start of experimental procedures. The experiment was conducted in accordance with the directions of Guide for the Care and Use of Laboratory Animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. In addition, the experiment was approved by University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC/19/0051).

Induction of PCOS and administration of test samples

The in vivo study was based on the crude extract of *T. potatoria*. Twenty non-pregnant, female albino rats (150–200 g) with normal estrous cycle were randomly grouped into four made of five albino rats per group. Groups I-III were treated with l mg/kg letrozole orally for a period of 2 l consecutive days using 0.5% w/v carboxymethyl cellulose (CMC) as vehicle for the induction of PCOS. Group I received 100 mg of *T. potatoria* methanol extract per kilogramme body weight, while Group II received l mg of clomiphene citrate (Colid, Pfizer pharamceuticals, USA) per kilogramme body weight. Groups III (untreated disease control) and IV (normal control) received 2 mL of 5% w/v CMC in distilled water (Kafali et al. 2004). The test samples were administered daily for 15 days via oral route. The dose level of 100 mg/kg *b.w.* was used in this study based on human therapeutic dose mentioned in the preliminary ethnobotanical survey conducted for managing irregular menstrual disorder and associated gynaecological disorders. The dose for rat was calculated considering human to albino rat conversion factor (conversion factor = 0.018) according to body surface area (Nair and Jacob 2016).

Determination of oestrous cycle pattern and hormonal analysis

The phases of the oestrous cycle were detected by examining vaginal cytology (Marcondes et al. 2002). Vaginal lavage was obtained with a Pasteur pipette filled with 0.1 mL of normal saline (0.9% NaCl), gently inserted into the rat's vagina. The withdrawn vaginal fluid was dropped on a glass slide and immediately evaluated microscopically. The oestrus cycle was monitored and the proportion of leukocytes, epithelial and cornified cells was expressed as phase index (%). The levels of Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH) and estradiol in the serum of the rats were measured using the Enzyme Linked Immuno Sorbent Assay (ELISA). Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) were assessed in the serum samples using the microwell kits manufactured by Fortress Diagnostics limited, United Kingdom, while estradiol level was measured by microwell kits manufactured (Dialab, Austria). The samples and the test reagents were equilibrated at room temperature prior the test. Then 0.05 mL of calibrators and rat's samples were pipetted inside the wells followed by addition 0.1 mL of dilute enzyme conjugate to each well excluding the blank well. These mixtures were incubated for 60 min at room temperature. The mixtures in the microwells were thrown out and the wells were cleaned with 0.2 mL of distilled water. This was done twice in order to remove water in the well. Solution of the substrate (0.1 mL) was pipetted into each microwell in the same order and interval as for the enzyme conjugate, blank well was included and incubated for 20 min at room temperature in the dark. Stop solution (0.1 mL) was added into each microwell using the same order and timing as for the reaction of the substrate solution. Absorbance of each microwell was read at 450 nm against blank using a microplate reader. The developed colour was stabled for at least 30 min and the optical densities were read during this time.

Ovarian histology

The animals were euthanized 24 h after last treatment, between 0900 and 1100 h to minimise diurnal variation, with 2% sodium pentobarbital (30 mg/kg) for laparotomy to collect abdominal aorta blood and ovaries of the overnight fasted rats. The tissue architecture of the dissected ovaries was examined according to standard method (Avwioro 2010). Haematoxylin and Eosin stain technique was used. The tissues were observed and dissected into small pieces of not more than 4 mm thick into pre-labelled cassettes. These small pieces of ovary tissues were immersed in 10% formal saline for 24 h for fixing. Tissue processing was done automatically using automatic tissue processor (Leica TP 1020). The tissues were dehydrated by passing them through

various dehydrating reagents such as 10% formal saline and alcohol (70%, 80%, 90% and 95%). Formal saline is the mixture of 100 mL of 40% of formaldehyde, 9 g of NaCl and 900 mL of distilled H_2O. The tissues were immersed in the molten paraffin wax, dispensed into a metal mould, and was transferred to a cold plate to solidify. The tissue block formed was separated from the mould and were trimmed to expose the tissue surface using a rotary microtome at 6 µm. The surfaces were placed on ice and sectioned at 4 µm (ribbon section). The sections floated on water bath (Raymond lamb) set at 55 °C were picked using clean labelled slides, dehydrated on a hotplate (Raymond lamb) set at 60 °C for 1 h and viewed under the light microscope using × 100 and × 400 objective.

Statistical analysis

The results of all assays were expressed as mean ± standard error of mean. The experiments were carried out in triplicate. The data were analysed with GraphPad (Version 5, GraphPad Prism Software Inc., San Diego, CA,). One-way ANOVA followed by Dunnett's Multiple Comparison Test were employed to test for the statistical differences between the groups at $p < 0.05$.

Results

Plant extraction

The percentage yields of the crude methanol extract, *n*-hexane, DCM and ethyl acetate fractions were 13.49% (175.37 g), 6.40% (11.23 g), 4.49% (7.87 g) and 13.71% (24.11 g), respectively.

In vitro antioxidant assay and quantification of phenolics and flavonoids

The dichloromethane (DCM) and ethyl acetate fractions of *T. potatoria* displayed free radical scavenging activity with IC_{50} values of 89.15 ± 0.50 and 9.52 ± 0.35 µg/mL, respectively, among which ethyl acetate fraction was found to be more potent, compared with the standards, ascorbic acid and rutin (IC_{50} values of 2.76 ± 0.01 and 20.6 ± 9.26 µg/mL, respectively) (Table 1). The result of total phenolic content (TPC) showed that ethyl acetate fraction of *T. potatoria* had the highest TPC (7150.18 ± 110.00 µg GAE/g). The crude extract had the highest Total flavonoid content (190.28 ± 12.30 mg QE/g).

Spectroscopic analysis

Isolation and characterisation of apigenin from DCM fraction

Compound **1,** isolated as a yellow powder (MP = 345.2–347.1 °C) from the DCM fraction of *T. potatoria* had the following spectroscopic data; ^{1}H NMR spectrum (400 MHz, $CDCl_3 + CD_3OD$): δ (ppm) 7.69 (2H, d, $J = 8.8$ Hz, H-2′), 6.85 (2H, d, $J = 8.8$ Hz, H-3′), 6.43 (1H, s, H-3), 6.34 (1H, d, $J = 2$ Hz, H-8), 6.17 (1H, d, $J = 2.4$ Hz, H-6). ^{13}C NMR (600 MHz, $CDCl_3$): δ (ppm) 182.4 (C=O, C-4), 164.5 (C, C-7), 163.8 (C, C-2), 161.4 (C, C-5), 160.6 (C, C-4′), 157.8 (C, C-9), 128.1 (CH, C-2′), 128.1 (CH, C-6′), 122.1 (C, C-1′), 115.8 (CH, C-3′), 115.8 (CH, C-5′), 104.4 (C, C-10), 103.1 (CH, C-3), 99.0 (CH, C-6), 94.2 (CH, C-8). EI-MS *m/z* (% rel. abun.): 271 (M^++1, 19), 270 (M^+, 100), 269 [M–(H)], 253 [M–(H)–(OH)]. The NMR spectroscopic data of the compound (Fig. 1) is presented in Table 2.

Antiproliferative studies

The hexane and DCM fractions of *T. potatoria* inhibited the proliferation of CHO cells with IC_{50} values of 34.8 ± 0.3 and 41.3 ± 0.8 µg/mL, respectively, while the crude and ethyl acetate fraction had insignificant effect on CHO cells proliferation. Crude extract and solvent fractions of *T. potatoria* exerted no inhibitory effect on HeLa cells proliferation (Table 3). The compound, apigenin, inhibited proliferation of HeLa (IC_{50} value of 6.2 ± 0.6 µg/mL) and CHO cell lines

Table 1 The DPPH (IC_{50}), TPC and TFC values of the extract and solvent fractions of *T. potatoria*

Extracts	Solvents	DPPH (IC_{50}) (µg/mL)	TPC (µg GAE/g)	TFC (mg QE/g)
T. potatoria.	Crude	220.89 ± 6.99***/***	3404.67 ± 6.13	190.28 ± 12.30
	Hexane	–	1963.17 ± 110.93	12.85 ± 0.18
	DCM	89.15 ± 0.50***/***	2518.33 ± 96.17	3.68 ± 0.16
	Ethyl acetate	$9.52 \pm 0.35^{NS}/^{NS}$	7150.18 ± 110.00	79. 64 ± 0.15
Ascorbic acid		2.76 ± 0.01		
Rutin		20.6 ± 9.26		

Data represented as mean ± (SEM) (n = 3). One-way ANOVA followed by Dunnett's Multiple Comparison Test at $p = 0.05$. IC_{50} DPPH of each extract was compared with standards (Ascorbic acid and rutin) with level of significant difference represented by *** or asterisk separated by (/) indicate order of significance from Ascorbic acid and rutin respectively, *NS* no significant difference from the standards

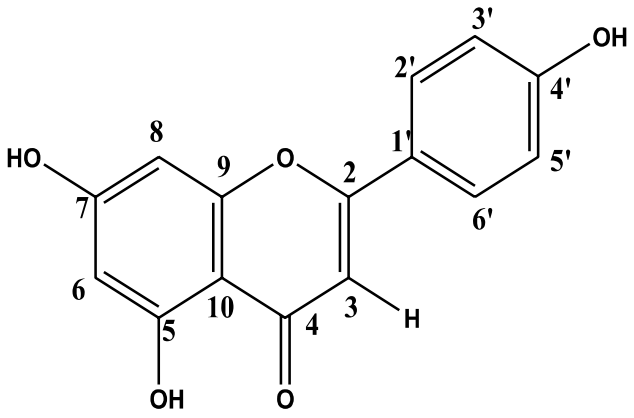

Fig. 1 Structure of 5, 7-dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one (apigenin) (1)

(IC_{50} value of 22.2 ± 0.5 µg/mL). The standard, Doxorubicin, had IC_{50} values of 0.8 ± 0.0 l µg/mL and 3.1 ± 0.2 µg/mL on CHO and HeLa, respectively.

In vivo studies

Effect of plant extract on oestrous cycle pattern and hormonal analysis

Oral administration of letrozole for 2 l days influenced reproductive cycle irregularity in female albino rats (Fig. 2). For the period of letrozole administration the estrous and proestrous phases were absent in the letrozole treated rats. As treatment commenced all treated rats exhibited acyclic conditions, however, *T. potatoria* extract improved the estrous cycle (Fig. 2) by increasing the appearance of estrous phase (index = 24.25%) and decreasing the duration of diestrous phase (index = 24.25%) when compared with disease control group (estrous index = 7.1%; diestrous index = 24.26%). *Tetracera potatoria* influenced reduction in the LH level and increased the level of FSH as shown in Table 4. The LH circulatory level in all groups ranged from 0.19 ± 0.05 mIU/mL to 0.23 ± 0.03 mIU/mL. Group I, having rats treated with *T. potatoria* methanol extract, had the least value (0.19 ± 0.05 mIU/mL). The level of FSH in *T. potatoria* treated group (0.81 ± 0.04 mIU/mL) was comparable with that of the control group (0.93 ± 0.19 mIU/mL). There was a significant ($p < 0.00$ l) reduction in the level of estradiol (5.70 ± 0.77 pg/

Table 3 The IC_{50} (µg/mL) of crude extract, fractions and isolated compound of *Tetracera potatoria* on Chinese Hamster Ovarian (CHO 1) cell line and HeLa cell line

Sample (s)	IC_{50} (µg/mL)	
	CHO 1 cell line	HeLa cell line.
Tetracera potatoria crude	> 100	> 100
Tetracera potatoria hexane fraction	34.8 ± 0.3	>100
Tetracera potatoria DCM fraction	41.3 ± 0.8	> 100
Tetracera potatoria ethyl acetate fraction	> 100	> 100
Compound **1**	22.2 ± 0.5	6.2 ± 0.6
Standard (Doxorubicin)	0.8 ± 0.01	3.1 ± 0.2

Table 2 1H and 13C-NMR data of compound **1** compared with reported literature

Position	Observed		Reported (Nabavi et al. 2015)	
	δ_H (d, $CDCl_3$ + MeOH, 400 MHz)	δ_C ($CDCl_3$, 600 MHz)	δ_H (DMSO, 500 MHz)	δ_C (DMSO, 125 MHz)
l	–	–	–	–
2	–	163	–	163
3	6.43 (s, lH)	103	6.78 (s, lH)	102
4	–	182	–	181
5	–	161	–	161
6	6.17 (d, *J* = 2 Hz, IH)	99	6.19 (d, *J* = 2 Hz, IH)	98
7	–	164	–	164
8	6.34 (d, *J* = 2 Hz, lH)	94	6.48 (d, *J* = 2 Hz, IH)	93
9	–	157	–	–
10	–	104		103
l′	–	122		121
2′	7.69 (d, *J* = 8.8 Hz, 2H)	128	7.93 (d, *J* = 8.8 Hz, 2H)	128
3′	6.85 (d, *J* = 8.8 Hz, 2H)	115	6.93 (d, *J* = 8.8 Hz, 2H)	115
4′	–	160	–	161
5′	6.85 (d, *J* = 8.8 Hz, 2H)	115	6.93 (d, *J* = 8.8 Hz, 2H)	115
6′	7.69 (d, *J* = 8.8 Hz, 2H)	128	7.93 (d, *J* = 8.8 Hz, 2H)	128

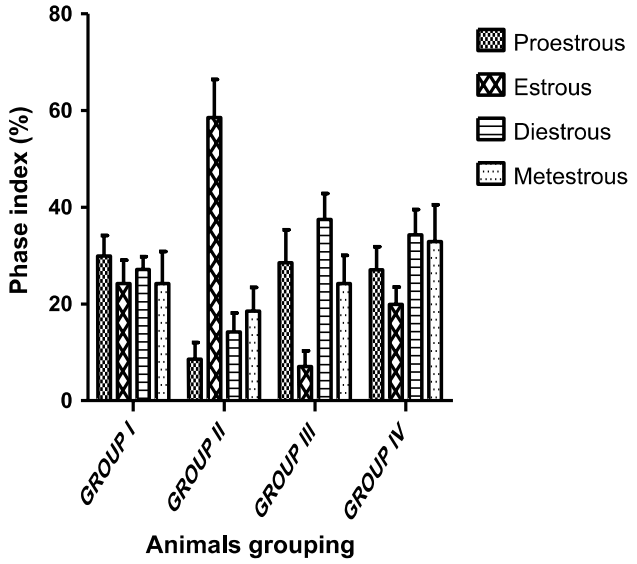

Fig. 2 Index of estrous cycle phases of albino rats after 15 days of treatment with 5% w/v CMC (in distilled water), clomiphene citrate and selected plant extracts. The data represent the mean ± SEM animals, n = 5. Data represented as mean ± (SEM) (n = 5). Group I—100 mg/kg body weight of *Tetracera potatoria*, Group II—Clomiphene citrate (1 mg/kg bw, p.o.), Group III—disease control group, Group IV—normal control group

Table 4 Effect of various treatments on LH, FSH and estradiol

Parameters	Group I	Group II	Group III	Group IV
LH (mIU/mL)	0.19 ± 0.05*	0.23 ± 0.01*	0.23 ± 0.01*	0.22 ± 0.01
FSH (mIU/mL)	0.81 ± 0.04*	0.75 ± 0.05*	0.73 ± 0.03*	0.93 ± 0.19
Estradiol (pg/mL)	9.36 ± 2.06*	7.63 ± 0.89*	5.70 ± 0.77*	9.84 ± 1.44

Data represented as mean ± (SEM) (n = 5). Evaluated by ANOVA followed by Bonferroni tests. *Indicate $p < 0.001$ versus normal control

Group I—100 mg/kg body weight of *Tetracera potatoria*, Group II—Clomiphene citrate (1 mg/kg bw, p.o.), Group III—disease control group, Group IV—normal control group

mL) in PCOS rats. However, treatment with *T. potatoria* caused an upsurge in the level of estradiol (9.36 ± 2.06 pg/mL) in the rats compared to the normal control group which had 9.84 ± 1.44 pg/mL.

Ovarian histology

Administration of letrozole for 21 days changed the morphology of the ovary in all treated animals (Fig. 3). The morphology of the ovary of animal treated with 100 mg/kg *b.w.* of *T. potatoria* (Group I) revealed presence of normal antral follicle with clear antrum, while oocytes are surrounded by granulosa cells. The stroma revealed hyperplastic luteinisation as shown in Fig. 3.

Discussion

Oxidative stress has been identified as one of the major factors contributing to PCOS and gynaecological cancers pathogenesis among reproductive women (Murri et al. 2013; Krstic et al. 2015). Among women with PCOS, oxidative stress favours the pathogenesis of gynaecological cancers (Ding et al. 2017). Reactive oxygen species (ROS) cause mutation of protooncogenes and tumour suppressor genes in the DNA (Ziech et al. 2011), resulting in uncontrollable cell proliferation. For instance, abnormal *p53* tumour suppressor gene was recently observed in the endometrium of PCOS patients (Shafiee et al. 2015; Gadducci et al. 2016). Therefore, to provide justification for the antioxidant potential of the plant, which might play a significant role in reducing the risk of PCOS, the DPPH radical scavenging activity was measured. The result revealed varying strength of antioxidant potential in the crude extract and fractions of the plant. This outcome matched with the reported findings on antioxidant effect of several medicinal plants (Hussain et al. 2016). Phenolics are significant constituents of medicinal plants, which exert numerous therapeutic activities including free radical scavenging ability due to the presence of OH functional groups. Various studies have shown the comparative relationship between phenolics and anti-oxidant potential (Hussain et al. 2016). The hydroxyl functional groups in the skeleton of phenolic compound's structure might be responsible for high scavenging property of extracts and solvent fractions of *T. potatoria* investigated in this study. The total phenolic content was highest in ethyl acetate fraction. The antioxidant power of *T. potatoria* extract and solvents fractions increased as TPC increased. The quantity of phenolics and antioxidant effect of solvent fractions of *Tetracera potatoria* displayed comparable trends ($TPC_{Hexane} < TPC_{DCM} < TPC_{Ethyl\ acetate}$). Total flavonoid content values of ethyl acetate, DCM and hexane fractions of *Tetracera potatoria* had a well-defined trend ($TPC_{DCM} < TPC_{Hexane} < TPC_{Ethyl\ acetate}$). The results obtained support other research outcomes in which a parallel association between phenolics and antioxidant activity have been established (Zhang et al. 2014).

The data of the isolated compound corresponds with the reported data for 4′,5,7-trihydroxyflavone, also known as apigenin (Nabavi et al. 2015). The compound apigenin is reported for the first time in *T. potatoria*.

Cancer is one of the leading causes of death globally. In women with PCOS, chronic stimulation of estrogen sometimes leads to uncontrollable enlargement of endometrium and endometrial cancer (Gottschau et al. 2015). It has been

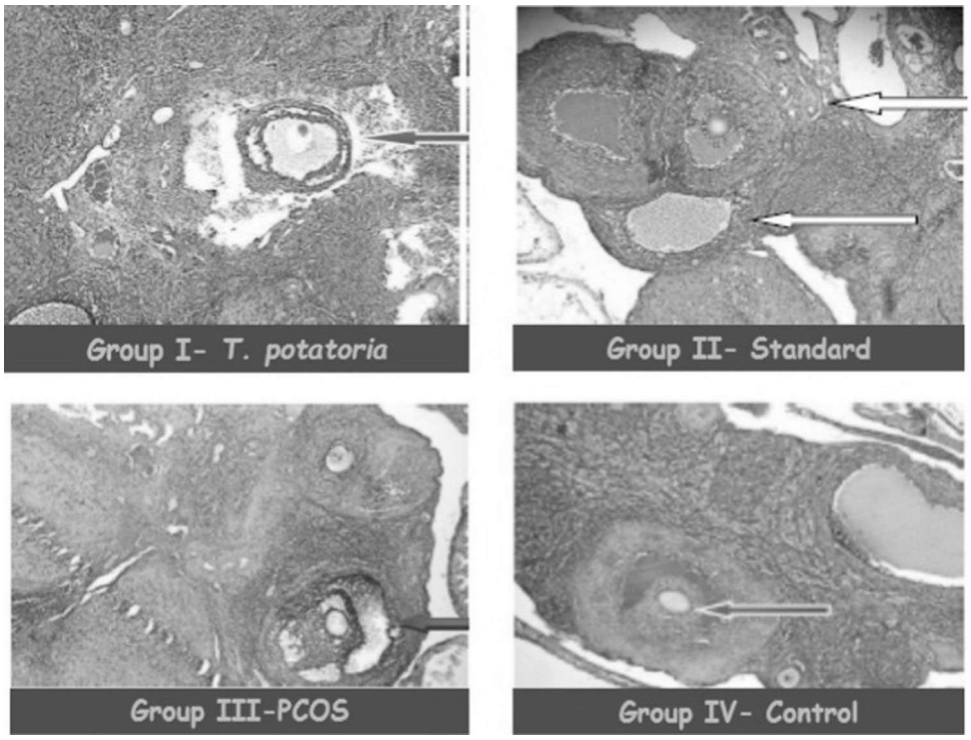

Fig. 3 Section of ovaries of normal rats, controls and rats treated with medicinal plant extract (x 100). Group I received 100 mg of *T. potatoria* extract per kilogramme body weight, Group II received l mg of clomiphene citrate (Colid, Pfizer pharamceuticals, USA) per kilogramme body weight. Groups III is untreated disease control. Group IV (normal control) received 2 mL of 5% w/v CMC in distilled water. Follicle = white arrow; thecal layer = blue arrow

reported that PCOS patients appear to have threefold probability of developing cancer of the endometrium but unaffected by breast cancer. However, PCOS patients have a 2.5-fold greater risk of ovarian cancer than normal control (non-PCOS control). Since abnormal *p53* tumour suppressor gene has been observed in the PCOS patients' endometrium (Shafiee et al. 2015), Chinese Hamster Ovarian (CHO l) cell, tumorigenic cell with characteristic abnormal *p*53 function (Hu et al. 1999) was selected for this study. Also, the human cervical cancer (HeLa) cell was selected for this study based on high global prevalence of gynaecological cancers among reproductive women, especially among women with PCOS (Sankaranarayanan and Ferlay 2006).

The hexane and DCM fractions of *T. potatoria* inhibited the proliferation of CHO-1 cells with 34.8 ± 0.3 and 41.3 ± 0.8 µg/mL IC_{50} values, respectively. The crude extract and solvent fractions had no inhibitory effect on HeLa cells proliferation. The standard (Doxorubicin) had IC_{50} values of 0.8 ± 0.0 l and 3.1 ± 0.2 µg/mL, on CHO l cell line and HeLa cell line, respectively.

Phenolic compounds have been widely investigated for their anti-cancer activities and low toxicity. One particular tumour suppressor gene codes for the protein p53 can induce cell cycle arrest in a DNA damage cell. Then, it can induce transcription of genes involved in DNA repair or, if the damage cannot be repaired, the p53 protein eventually initiates cell suicide, thereby preventing the DNA damaged cell becoming a mutated cell. Interestingly, some flavonoids have shown ability to induce cell cycle arrest at G1/S phase (Yoshida et al. 1992), at G2/M (Lian et al. 1998) or at both G1/S and G2/M phases (Traganos et al. 1992). Apigenin (**1**) was reported to suppress various human cancers in vitro and in vivo by multiple biological effects, such as triggering cell apoptosis and autophagy, inducing cell cycle arrest, suppressing cell migration and invasion, and stimulating an immune response (Cardenas et al. 2016). Recently, apigenin was reported to show anti-cancer activities by stimulating an immune response (Cardenas et al. 2016). The inhibitory effect of apigenin on the proliferation of HeLa and CHO cells was reported in earlier studies (Lepley et al. 1996; Souza et al. 2017). Furthermore, apigenin downregulated CK2α expression and inhibited the self-renewal capacity of sphere-forming cells in HeLa cells (Liu et al. 2015). These support our postulation that any bioactive compounds used for management and/or treatment of PCOS should be able to inhibit proliferation of CHO cells, a non-cancerous cell which possesses one of the main characteristics found recently in PCOS patients' endometrium i.e. abnormal *p53* function (Hu et al. 1999; Shafiee et al. 2015).

Polycystic ovary syndrome remains the commonest hormonal syndrome disturbing womenfolk globally. Variation in LH potentiates severe consequence on the estrous stage of rats. The regulation of estrus cycle is hindered by uncontrollable synthesis of LH hormone evident in PCOS condition (Zangeneh et al. 2012). Androgen surpluses and elevated level of LH are the primary biological irregularity in PCOS patients, while hyperandrogenemia usually manifest at pubertal age (Kakadia et al. 2018). Animal models used for

PCOS studies include neonatal androgenization, administration of estradiol valerate, human chorionic gonadotropin (HCG) administration to hypothyroid rats and maintenance of animals in constant light. None of these models are able to generate PCOS conditions with convincing data mimicking the PCOS conditions in human (Kakadia et al. 2018). Letrozole, which inhibits the action of aromatase inhibitor, yields a PCOS model with features which in several ways portray human-like PCOS condition. It blocks change of androstenedione and testosterone conversion to estrone and estradiol, respectively and mimics PCOS-like condition by effecting circulating hyperandrogenism, hormonal imbalance and intra ovarian androgen excess resulting in manifestation of polycystic ovary. Abnormal follicular development and follicular atresia are detected as a result of constant upsurge in the level of androgen in the ovary. Letrozole also causes hyperglycaemic condition, which may trigger insulin resistance, hyperlipidaemia and associated metabolic syndrome (Choi et al. 2015). Although medicinal plants are effective in restoring menstrual cycle and endocrine disorder among women with PCOS (Zhao 2011), effects of plants such as *T. potatoria* on hormonal imbalance, polycystic ovary conditions and associated risk of gynaecological cancers in PCOS patients have not been extensively investigated.

Oral administration of Letrozole for 21 days influences reproductive cycle irregularity in albino rats as observed in the present study. There was no chance of estrous and proestrous phases observed in rats after treatment with letrozole. The increase in the level of endogenous testosterone has been identified as the main culprit of PCOS. The fluctuations observed in the rat menstrual phase could be related to variations in the concentration of endogenous sex hormones as well as gonadotrophins. These sex hormones regulate the characteristics of the ovaries, hormonal imbalance and follicular maturation, which might initiate irregular oestrous cycle, causing malfunctioning of ovaries (Sun et al. 2013). *Tetracera potatoria* improved the estrous cycle by increasing the appearance of estrous phase and decreasing the period of diestrous phase as compared to untreated PCOS group.

The elevated level of luteinizing hormone (LH) present in most PCOS affected women is connected to the mechanisms associated with high level of circulatory androgen, exposure of the ovarian theca and granulosa cells to LH as well as amplified levels of cAMP. Variation in LH potentiates severe consequence on the estrous stage of rats. The regulation of oestrus cycle is hindered by uncontrollable synthesis of LH hormone evident in PCOS condition. Morphological changes in the ovaries of PCOS rats induced by letrozole are shown by the existence of numerous cysts with hyperplasia in the theca cells as well as thickened capsule of the ovaries. Subcapsular cysts enclosed with a layer of granulosa cells might also be detected. These histopathological features are due to availability of therapeutic levels of FSH, increased LH, and loss of interaction between granulosa and theca cells (Kafali et al. 2004). In our study, *Tetracera potatoria* influenced reduction in the level of LH and increased FSH. Also, a low circulatory estradiol level was found in letrozole-induced PCOS rats. However, treatment with *T. potatoria* extract at 100 mg/kg b.w. increased the estradiol level.

The level of estradiol in *Tetracera potatoria* was 9.36 ± 2.06 pg/mL compared to and PCOS rats, which had 5.70 ± 0.77 pg/mL. Treatment with *T. potatoria* leaf had curative effect on irregular estrual cycle and hormonal imbalance associated with PCOS. Restoration of estrus irregularity and follicular generation to normal following administration of *T. potatoria* could be the physiological effect exerted by phytochemical constituents in the extracts, which uphold the steroidal prestige, allowing fertility to be recuperated.

Conclusion

The antipoliferative effect of the hexane and DCM fractions of *Tetracera potatoria* and its isolated compound **1** (apigenin) on Chinese Hamster Ovarian (CHO) cells is noteworthy. This isolated compound also inhibited human cervical cancer HeLa cells. The plant and its isolated constituent could provide basis for the development of newer antiproliferative agents

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Authors contribution ADO and MAS contributed to the study conception and design. Material preparation, data collection and analysis were performed by all authors. MAS supervised the complete project. AJ, FS and SFS hosted part of the study. The first draft of the manuscript was written by ADO and all authors wrote the manuscript. All authors read and approved the final manuscript. All data were generated in-house and no paper mill was used.

Compliance with ethical standards

Ethical statement Ethical clearance for animal experimental work was obtained from Animal Care and Research Ethics Committee of the University of Ibadan, Nigeria prior to the commencement of experiments (UI-ACUREC/19/0051).

Conflict of interest Akingbolabo Daniel Ogunlakin has no conflict of interest. Mubo Adeola Sonibare has no conflict of interest. Almas Jabeen has no conflict of interest. Farzana Shaheen has no conflict of interest. Syeda Farah Shah has no conflict of interest.

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