

## Fertility control impact of the aerial parts *Ferula tingitana* L. via alteration of hypothalamic-pituitary-gonadal axis responses of female *Wistar* rats

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### ABSTRACT

*Ferula tingitana* L. has been reported for abortive and/or menstruation inducing properties. However, its contraceptive effect has never been deliberately evaluated. Furthermore, no inclusive chemical profiling of its extract was recorded. Many *Ferula* species were known for their effects on the oestrogenic rhythm. During our drug discovery from natural sources *F. tingitana* L. growing in Libya was selected for evaluation of its contraceptive effect. To evaluate the hormonal effects and bioactive molecules of *F. tingitana* ethanol extract of aerial parts (EtOH) using *in vivo* experimental model. Adult female albino rats were divided equally into 4 groups ( $n=6$ ). One group received distilled water for 14 days, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> groups received orally the tested extract at a daily dose of 100, 200 and 300 mgkg<sup>-1</sup> b.wt. for 14 days, respectively. The administration lasted for 14 days (2 weeks) at 9 A.M. Rats body and uterus weight were measured. They fasted overnight and then anaesthetized through a diethylether exposure and blood samples were collected through the ocular puncture. Blood was centrifuged to obtain clear sera for hormonal assay. The serum was subjected by ELISA method for assessment of follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E<sub>2</sub>) and progesterone (P<sub>4</sub>) levels. Biochemical estimations of total cholesterol (TC), triglyceride (TG) and glucose (Glu) level were measured. GC/MS of the lipoidal profile along with HPLC analysis of the phenolic contents were carried out. EtOH and its successive soluble fractions were subjected for chromatographic analysis. The results displayed significant decrease in levels of FSH, LH, E<sub>2</sub> and P<sub>4</sub> of adult female *Wistar* rats. Significant decline in biochemical serum level of TC, TG and Glu were observed. Sesquiterpene daucol, linolenic acid, caffeic acid and hesperidin were the main identified phytoconstituents. CC of EtOH afforded 5 compounds were identified as  $\beta$ -sitosterol **1**, colladonin **2**, scopoletin **3**, caffeic acid **4**, 1-(3,4-dihydroxycinnamoyl) cyclopentane-2',3'-diol **5**. It was concluded that sesquiterpene coumarins as the significant phytoconstituents of EtOH may revealing adverse effect on the menstruation, ovulation of follicles and consequently may impair fertility. EtOH evidenced a hypoglycemic and hypocholesterolemic effects.

**Keywords:** Contraceptive, *Ferula tingitana* L., hypoglycemic, sesquiterpene coumarins.

**Abbreviations:** b.wt = body weight, EtOH = Ethanol extract, FSH = follicle stimulating hormone, LH = luteinizing hormone, E<sub>2</sub> = estradiol, P<sub>4</sub> = progesterone, TC = total cholesterol, TG = triglycerides, Glu = glucose, GC/MS = gas chromatography/mass spectroscopy, HPLC = High performance liquid chromatography, CC = Column chromatography. ELISA = Enzyme Linked Immunoassay, SFA = Saturated fatty acids, USFA = Unsaturated fatty acids, USM = Unsaponifiable matter, SM = Saponifiable matter, FAME = fatty acid methyl esters, PE = petroleum ether extract

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## 1. INTRODUCTION

Complications related to overpopulation comprise the enlarged demand for resources such as clean water and food, starvation and malnutrition, consumption of natural resources faster than the rate of regeneration, and deterioration in living conditions<sup>(1)</sup>. Slowing population growth through lower fertility produces a demographic dividend, whereby the proportion of persons of working age increases with respect to that of children and the elderly.<sup>(1,2)</sup> Lowering fertility facilitates the achievement of key development goals. Countries with lower fertility and therefore slower population growth spend substantially more in the health and education of each child than those with higher fertility.<sup>(3)</sup>

The genus *Ferula* (Apiaceae) comprises about 150 species of flowering plants. Many of the biological features of this genus includes cytotoxic, antibacterial, antiviral, P-glycoprotein (P-gp) inhibitor, antiinflammatory, antileishmanial, antioxidant and others have been attributed to the sesquiterpenes-coumarins present.<sup>(4)</sup> In addition to saponins, essential oils and triterpenes were major constituents.<sup>(4, 5)</sup> Contraceptive carotene sesquiterpenes of *F. jaeschkeana* Vatke. were isolated and evaluated on the histological and biochemical elements of the uterus of ovariectomized rats.<sup>(6)</sup> The hexane extract of *F. jaeschkeana* was studied by Prakash et al<sup>(7)</sup> for its effects on the oestrogenic rhythm following a single administration to immature rats.

*Ferula tingitana* L. is a tall perennial herb and grows in scrubland and rocky areas. It is mainly expanded across Mediterranean coast in Spain, Morocco, Lebanon, Israel, Palestine, Cyprus and Turkey. It is reported for it is known for its abortive and/or menstruation induced properties.<sup>(8)</sup> Miski et al.<sup>(9)</sup> isolated sesquiterpene ester, tingitanol, from *F. tingitana* L., as well as, the petroleum ether extract of the roots of *F. tingitana* yielded daucane esters.<sup>(10)</sup> The volatile oil collected from plant growing in Libya evidenced cytotoxic, antifungal, and mild antibacterial effects.<sup>(11)</sup> Reviewing the current literature revealed that

no experimental study was adapted to prove the folkloric use of the plant under investigation as contraceptive drug. So, the aim of this work is to explore bio-active compounds from the aerial parts of *F. tingitana* L. since the scientific validation of traditionally used plant in treatment is highly demanded. Also, to contribute new knowledge to the currently existing known chemical/or biological data about *F. tingitana* L. is growing in Libya.

## 2. Material and methods

### 2.1. Plant material

Aerial parts of *Ferula tingitana* L. were collected in March 2014 from the West Mountain, Mislata, Libya <https://goo.gl/maps/uzhPwzvWGGwEGAiFA>. Plant identity was kindly authenticated by Dr. Reem Samir Hamdi, professor of plant taxonomy, Botany Department, Faculty of Science Cairo University, Egypt. Drying and grinding of plant material were done in Medicinal, Aromatic and poisonous plant Experimental station, Cairo University. Voucher samples (1-5-2014.) are kept at the herbarium of the Pharmacognosy Department, Faculty of Pharmacy Cairo University <https://goo.gl/maps/Y9Ug8RR7WWb7CCHe6>. EtOH of the aerial parts of *F. tingitana* was dissolved in 1% Tween 80 to prepare solution of concentration of 150 and 300 mg/ml and used for pharmacological studies.

### 2.2. Extraction and fractionation

The air dried powdered aerial parts of *F. tingitana* L.(1.5kg) was extracted with 2.5L ethanol (90%) by cold maceration till exhaustion. The collected EtOH were evaporated to yield 195g of dark green residue. EtOH extract (100g) was suspended in 400 ml of distilled water and partitioned successively. The solvent free extractives were weighed and amounted to 30, 15,7 and 25 g for petroleum ether, methylene chloride, ethyl acetate and *n*-butanol, respectively. In addition, there was 23g of insoluble matter.

### 2.3. Drugs and biochemical kits

Aluminium chloride, Sodium carbonate, Gallic acid and Rutin were obtained from Sigma Aldrich (St. Louis,

MO, USA). All the chemicals used, including the solvents, were of analytical grade. Biodiagnostic kits: A Glucose kit (Bio-Merieux Co, France) was used. Assay kits for progesterone, estradiol, follicle stimulating hormone and luteinizing hormone were supplied by Sigma-Aldrich Chemicals, Pomona/Kempton Park 1619, and Johannesburg, South Africa. Assay kits for glucose, cholesterol and triglycerides were procured from Randox Laboratories Ltd, Co-Atrim, United Kingdom. All other reagents used were of analytical grade and were prepared using glass-distilled water.

#### 2.4. Phytochemical studies

Gas chromatograph/Mass spectrometric (GC/MS) apparatus for unsaponifiable matter analysis. Gas liquid chromatograph, GC Ultra system (Thermo Fisher scientific Co., USA), kept at the central laboratory. Faculty of Agriculture, Cairo University (Giza, Egypt) for analysis of fatty acid. HPLC system (GBC-LC) high performance chromatograph equipped with LC 1150 quaternary gradient pump and LC1210 k program mabledau wavelenegth UV detector (GBC scientific equipment, Melbourne Australia) in the food technology research institute, Faculty of Agriculture, Cairo University (Giza, Egypt) for analysis of phenolics. UV-Visible Spectrophotometer, Shimadzu UV-1650 PC was used for recording UV spectra and measuring the absorbance in UV range. InfraRed spectrophotometer, Shimadzu IR-435, PU-9712 was used for recording IR spectra using KBr discs. EI-MS was recorded on a Varian Mat 711 or SSQ 7000 (Finnigan mat), eV 70 Faculty of Science, Cairo University. Bruker NMR spectrometer<sup>1</sup>H-NMR (400 MHz); <sup>13</sup>C-NMR (100 MHz) Japan were used for structural elucidation of isolated compounds. The NMR spectra were recorded in a suitable solvent (CDCl<sub>3</sub>, DMSO-d<sub>6</sub> or CD<sub>3</sub>OD) using TMS as internal standard and chemical shifts were given in δ ppm value (NMR Laboratory, Microanalytical Unit, Faculty of Pharmacy, Cairo University).

#### 2.5. Investigation of the lipoidal contents

The unsaponifiable and saponifiable lipoids were prepared according to Vogel, <sup>(12)</sup> from the petroleum ether

extract (PE) 1.0 g. The solvent-free residue (0.30g), representing the USM, was saved for further GC/MS analysis. The aqueous alkaline solution, left after separation of the USM, was acidified with dilute hydrochloric acid (5N) to liberate the free fatty acids (FA), yielding a 0.60g residue representing the free FA. <sup>(12)</sup> The FA mixture as well as the standard fatty acids was, saved for GLC analysis.

The USM was subjected to gas chromatography/mass spectrometry (GC/MS) analysis which performed using a Thermo Trace GC 2000 (Thermo Quest, TX, USA)/MS Finnigan mat SSQ7000 system. The instrument was equipped with a DB-5 column (30m × 0.25 mm i.d., 0.25 μm film thickness); J&W Scientific, USA. Operating conditions: Injection volume, 1μl of CH<sub>2</sub>Cl<sub>2</sub> solution of tested samples; oven temperature programming: initial temperature, 40°C (isothermal for 3 min), then increased (4°C/min) to 160°C, followed by further increased to final temperature 280°C (10°C/min); injection temperature: 220°C carrier gas: helium at a flow rate of 1ml/min; mass spectrometer, electronic ionization(EI)mode; ion source, 70eV; mass range:40- 500 amu. Identification of the constituents was achieved by library search on a Wiley 275 L GC-MS data base, observed mass fragmentation patterns to those of the available references as well as of published data. A series of authentic *n*-alkanes was subjected to GC under the same experimental conditions. The individual components were determined by computerized peak area measurement. Compounds of the USM and percentage composition are compiled (Table 1). The FAME sample was analyzed using GLC Trace GC Ultra system equipped with FID detector. Analysis was performed using a Thermo TR-FAME column (70% Cyanopropyl Polysilphenylene Siloxane) (30mx 0.25mmx 0.25μm film thickness); injector temperature 200°C, using N<sub>2</sub> as carrier gas and adopting a temperature programming as initial temperature, 140°C, increased to 200°C by the rate of 5°C/min, then kept isothermal for 3min. Flow rate 30ml/min. with N<sub>2</sub> as carrier gas. Aliquots, 2 μL each, of 2% chloroformic solutions of the analyzed FAME and reference fatty acid methyl esters were analyzed under the same

conditions. Identification was based on comparing the retention time of their peaks with those of the available reference standards. The amount of each component was calculated by peak area measurement using a computing integrator.

#### **Spectrophotometric determination of phenolic and flavonoid contents**

The total phenolic and flavonoid contents were determined in the aerial parts of *F. tingitana* according to published spectrophotometric procedures.<sup>(13, 14)</sup> The total phenolic content was expressed as Gallic acid equivalents (mg GAE/100mg extract) and deduced from the pre-established calibration curve. Triplicate experiments were carried out for each sample. Colorimetric method was adopted, based on measuring the intensity of the color developed when flavonoids are complexed with aluminum chloride method.<sup>(13, 15)</sup> The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg rutin equivalent per 100 mg extract. Experiments were carried out in triplicates, and average absorbance values recorded.

#### **2.6. HPLC analysis of polyphenols contents**

HPLC Agilent (series 1100) equipped with autosampling injector, solvent degasser, ultraviolet (UV) detector set at (280 nm for phenolics determination and 330 nm for flavonoids determination) and quaternary pump. The column temperature was maintained at 35°C temperature for each. The column used for separation was zorbax ODS 5µm (4.5×250mm), Gradient separation was carried out using methanol and acetonitrile (2:1) as a mobile phase at flow rate of 1 ml/min. Authentic phenolics and flavonoids were dissolved in mobile phase and injected into HPLC. The retention time and peak area were used to calculate the phenolic and flavonoids concentrations by the data analysis of Hewlett packard software<sup>(16, 17)</sup>. The simultaneous separation and quantization of flavonoids, catechins and phenolic acids were performed on an analytical HPLC system consisting of GBC-LC high performance chromatograph equipped with a UV detector set at two different wavelengths 280

and 330 nm. Analysis was achieved on a Hypersil BDS C18 column (250 mm× 4.6 mm, 5µm particle size).

#### **2.7. Chromatographic analysis of different extractives of *F. tingitana* L.**

The petroleum ether fraction (10g) was fractionated on a silica gel column VLC (15x20cm). Gradient elution was carried out. Fraction (B): 3g eluted with 30-40% CH<sub>2</sub>Cl<sub>2</sub> in *n*-Hexane) was rechromatographed, to yield pure compound **1** (13 mg, R<sub>f</sub>= 0.37, in S<sub>2</sub>) as white powder. Fraction (C): 2.7g eluted with 50-90% EtOAc in CH<sub>2</sub>Cl<sub>3</sub>) were similarly rechromatographed on a silica gel (60g) column (60×2cm) using *n*-hexane: EtOAc (7:3 v/v) as eluent, fractions (10 mL, each) were collected. For further purification, this residue was applied to a silica gel (20g) column (50×1.5cm) and eluted with CH<sub>2</sub>Cl<sub>2</sub>:MeOH (9:1 v/v), yielding white needle crystals of compound **2** (20 mg, R<sub>f</sub>= 0.67, in S<sub>3</sub>).

Seven grams of methylene-chloride soluble fraction was fractionated on silica columns VLC (15x20cm). Gradient elution was carried out. Fraction II (3g, eluted with methylene chloride:methanol(8:2 v/v), were rechromatographed; sub-fractions II<sub>a</sub> rechromatographed on silica gel column solvent system using CH<sub>2</sub>Cl<sub>2</sub>-EtOAc (1:1) to yield pure compound **3**(13 mg, R<sub>f</sub>= 0.65 in S<sub>3</sub>) as yellow amorphous powder. Sub-fractions II<sub>b</sub> were eluted by CH<sub>2</sub>Cl<sub>2</sub>-MeOH (9:1), showed three spots after spraying with *p*-anisaldehyde, were rechromatographed on silica gel using by gradient elution with EtOAc-CH<sub>3</sub>OH (9:1) solvent system to give two major spot with R<sub>f</sub>-value 0.7 in S<sub>4</sub>, the fractions were pooled and evaporated to yield two compounds **4** (22 mg).

Ethyl acetate fraction (4g) was chromatographed on diaion column (3.5x50 cm), packed with diaion (50 g). Gradient elution was carried out using H<sub>2</sub>O, MeOH and Acetone (increasing percentage 10 – 25%). Similar fractions were pooled together to yield three main fractions coded from D1 to D3. According to the results of thin layer chromatographic investigations the most promising fractions (D2 and D3) were collected and subjected to

further chromatographic purifications using silica gel columns to afford one compound **5** (25 mg,  $R_f = 0.65$  in  $S_3$ ) as yellow amorphous powder.

### Animals

Twenty-four adult female albino rats (*Wistar* strain, 150-200 g) were used for assessment of the hormonal and protective activity on glucose, total cholesterol and triglyceride levels in rats. Animals were obtained from the National Organization for Drug Control and Research (NODCAR), Cairo, Egypt. Animals were housed in an air-conditioned atmosphere, at a controlled temperature of  $24 \pm 1^\circ\text{C}$  with alternating 12 h light and dark cycles and kept on a standard pellet diet and water *ad libitum*. The animals were acclimatized for one week before experimentation. They were screened and observed to exhibit regular estrous cycle. The study protocol complies with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996) and was approved by the Ethics Committee for Animal Experimentation, Faculty of Pharmacy Cairo University, Egypt.

### 2.8. Experimental protocols

The selected animals were randomly divided equally into 4 treatment groups containing 6 six rat each ( $n=6$ ). Group I: Animals were given distilled water for 14 days served as control group. Group II, III and IV: Animals received orally the tested extract at a daily dose of 100, 200 and 300  $\text{mg kg}^{-1}$  body weight /day for 14 days respectively as nearly 1/20, 1/10 and 1/6 of the  $\text{LD}_{50}$ . The administration lasted for 14 days (2 weeks) at 9 AM. EtOH was prepared at fixed dose of 2000 mg/kg, p.o for 14 days according to Organization for economic cooperation and development OECD guideline NO 423. At the end of the experimental period, polyestrous female rats were used for biochemical estimations. They were fasted overnight and then anaesthetized through a diethylether exposure. Blood samples were collected through the ocular puncture into plain sample bottles and left for 15 minutes. Then the samples were centrifuged for 2000rpm for 10 mins to obtain clear sera for hormonal

assay. The serum was then tipped into a separate vial and later subjected by ELISA method for assessment of FSH, LH,  $E_2$  and  $P_4$  levels.

Final body weights of the animal were recorded a day after the last doses administration. Uterus was excised, cleared of supporting tissue and weighted. Blood was collected into plain sample bottles and assayed for FSH, LH,  $E_2$  and  $P_4$  using ELISA according to the principle highlighted by Sakamoto et al.,<sup>(18)</sup> for  $E_2$  and  $P_4$  while that of Uotila et al.<sup>(19)</sup> was used for LH and FSH (Table 7& Figure 2). Serum samples were analyzed for estimating the Glu level by colorimetric methods according to King and Garner,<sup>(20)</sup> levels of serum TC measured according to Henery et al.,<sup>(21)</sup> As well as TG were estimated colorimetrically using high quality kits according to manufacturer's protocol<sup>(22)</sup> (Table 8 and Figure 4).

### 2.9. Statistical analysis

The data are expressed as mean  $\pm$  standard error. Comparisons between animals' groups served as control and three groups treated with different doses of the extract were performed using the one way ANOVA. Significance was accepted at  $P < 0.05$ . The statistical study was conducted by analysis of variance (ANOVA) followed by Tukey's test using the Statistics software IBM-SPSS, (Armonk, NY, U.S.A.) version 20. The significance level was set at  $p < 0.05$ .

## 3. Results

### 3.1. GC/MS analysis of the unsaponifiable matter (USM)

GC/MS analysis of the USM is illustrated in Table 1 which revealed that fenchyl acetate 18.69% was the major hydrocarbon detected. Whereas stigmasterol 1.53% and phytol 35.25% were the major sterol and diterpenes detected, respectively. Phytol was chiefly reported in genus *Ferula*.<sup>(23)</sup> Sesquiterpenes components were up to 24.22% of which daucol 16.79% was the major unique. Terpenoid constituents may justify the anti-inflammatory effect previously reported.<sup>(24)</sup>

### 3.2. GLC analysis of the fatty acid methyl esters (FAME)

GLC analysis of FAME (Table 2) revealed that the USFA constituted 51.45%.  $\alpha$ -Linolenic acid (ALA) 36.75% was the major USFA. Arachidonic acid 11.19% is a fatty acid most found in peanut oil that is responsible for muscle tissue inflammation. (25) Saturated fatty acids (SFA) constituted 22.26%, among which palmitic acid 8.56% was the major one. USFA constituted more than double the percentage of the SFA.

### 3.3. Determination of total phenolic and total flavonoid contents

The average absorbance of ethanol extract were 0.437 and 0.265 corresponding to 19.63 and 61.86 mg gallic acid and rutin/g dry powdered aerial parts respectively. Polyphenolic/ or flavonoids are existing in significant amounts in *F. tingitana* L and further study is needed to explore their constituents. Total phenolic and flavonoid contents of Apiaceae species such as *F.gummosa* Boiss previously reported. (26)

### 3.4. HPLC analysis of polyphenolic compounds

HPLC analysis of the aerial parts of *F. tingitana* L. enabled the identification and quantification of 23 phenolic compounds among which: 13 phenolic acids, 10 flavonoids. The main detected phenolic acids were caffeic acid and gallic acid with concentrations 1192.08 and 344.49 ppm respectively. On the other hand, the major identified flavonoids were hesperidin and quercetin with respective concentrations 1156.3 and 87.75 ppm (Tables 3, 4).

### 3.5. Identification of the bioactive compounds

Five compounds were isolated from the aerial parts of *F. tingitana* L. and characterized through MS, IR, <sup>1</sup>H, <sup>13</sup>CNMR and 2DNMR data as well as by comparison to previously reported ones. Compound **1** is identified as  $\beta$ -sitosterol. (27) Based on the spectral data (Table 5) compound **2** could be identified as sesquiterpene coumarin, colladonin. (28, 10) The <sup>1</sup>HNMR spectrum of compound **3** displayed signals characteristics of a 6,7-dioxygenated coumarin (Table 5) therefore, identified as

scopoletin. On the basis of the previous found and published data (29) compound **4** was identified as caffeic acid (Table 5). The presence of caffeic acid in *F. tingitana* may hint some free radicale quenching effect and\ or cancer chemo preventive property. Comparison of the represented spectroscopic data of **5** (Table 5)with those reported in the literature (30) revealed that **5** is identified as 1-(3,4-dihydroxycinnamoyl)cyclopentane-2,3-diol.

### 3.6. In vivo assessed hormonal activity

#### 3.6.1. Effect on body and reproductive organ weight(uterus)

Results depicted in Table 6 and Figure 3 revealed that administration of the tested extract 200, 300 mg kg<sup>-1</sup> was significantly decreased the body weight gain and reproductive organ (uterus) in dose depended on manner.

#### 3.6.2. Effect on the levels of different serum hormones

Administration of EtOH of *F. tingitana* L. for 3 weeks was significantly decreased the serum levels of FSH, LH, E<sub>2</sub> and P<sub>4</sub> in dose depended on manner (Table7& Figure4).

#### 3.6.3. Effect on the triglycerides, total cholesterol, and glucose level

As compared with the control group, only dose 300mgkg<sup>-1</sup>of ethanol extract of *F. tingitana* L. was able to decrease triglycerides and glucose level (Table 8 and Figure 3). Earlier investigators revealed that *F.tingitana* L. has a hypolipidemic effect marked by decline in the levels of triglyceride on the rats treated with ethanol extract of *F. tingitana* L.

## 4. Discussion

Growing human population through the world particularly in developing and underdeveloped countries has damaging effects on life supporting system on earth. Usually, plants have been used to treat different kinds of ailments. *Ferula gummosa* exhibits change in the body weight of diabetic rats. (31) The reduction in the levels of follicle stimulating hormone may hamper folliculogenesis and delay maturation of the follicle in the pre-ovulatory phase.(32) Prior investigators, Yusufoglu et al.,(33) have

confirmed the hypoglycemic effect of various *Ferula* species. Oral contraceptive gents have been used to reduce fertility rate, but their unusual side effects limit their use. <sup>(34)</sup> Plants has been used to treat different kinds of ailments including. The contraception ability of plants has been reported in several animal models. <sup>(35)</sup> Historically, plants have been a source of drugs, but no scientific experiments prove the importance of herbal medicine as antifertility agents. <sup>(36)</sup> The World Health Organization suggested that effective, locally available plants can be used as alternatives for drugs. <sup>(37)</sup> *Ferula assa-foetida* L. was proved a potential antifertility effect. <sup>(38)</sup> It is likely that the EtOH of *F. tingitana* might have exerted its effect on the anterior pituitary or the hypothalamus since the secretion of stimulating hormone is regulated by the gonadotropic releasing hormone secreted by the hypothalamus. The observed reduction in level of serum LH indicates the inhibitory effect of the extract on the release of LH which may trigger disruption of ovulation. <sup>(39)</sup> This may result in impairment of estrous cycle, hamper conception and normal reproduction in the females. Decrease in P<sub>4</sub> hormone level through 300mgkg<sup>-1</sup> of *F. tingitana* prevent thickening of myometrial lining also ovary can be not imbedded and so increase sensitivity to oxytocin. Decrease in E<sub>2</sub> level may hinder ovulation, preparation of the reproductive tract for zygote implantation and the subsequent maintenance of pregnancy state. <sup>(40)</sup> *Ferula Hermon* was previously reported to induce decrease in the serum hormonal level of LH and FSH. <sup>(41)</sup> *Ferula narthex* Biois showed anti-fertility effect. <sup>(42)</sup> Anti-infertility effect previously reported to several *Ferula* species may be attributed to their sesquiterpenes coumarins contents. <sup>(43)</sup> Sesquiterpenoid compounds were detected as major constituents presented by daucal in *F. tingitana* L. Results observed in this study are comparable to the previously reported. <sup>(10)</sup> Daucane-type sesquiterpenes was isolated

from the methanol extracts of the air-dried roots and stems of *F. kuhistanica*. <sup>(44)</sup> Likewise, aerial part of *F. tingitana* is rich in ALA as seed oil from *Rosa mosqueta* (*Rosa rubiginosa*), sachainchi (*Plukene tiavolubis*), canola, sunflower, and chia (*Salvia hispanica*) which may constitute an alternative that merits research. <sup>(45)</sup> Polyphenolic are existing in significant amounts in *F. tingitana* L. Total phenolic and flavonoid contents of Apiaceae species such as *F. gummosa* Boiss previously reported. <sup>(26)</sup> Reviewing the available literature,  $\beta$ -sitosterol and colladonin were previously isolated from *F. tingitana* L. To the best for our knowledge, this is the first report for the isolation of scopoletin, caffeic acid, and 1-(3,4-dihydroxycinnamoyl) cyclopentane-2,3-diol from the aerial part of *F. tingitana* L.

## 5. Conclusion

Ethanol extract of the aerial part of *F. tingitana* assure its use as a contraceptive agent through diminution of the hypothalamic-pituitary-gonadal axis hormones. An alteration on the hypothalamic-pituitary-gonadal axis responses presented on the female reproductive hormones of adult female *Wistar* rats were noticed as adverse effect on the menstruation, ovulation of follicles and consequently may impair fertility mediated through the synergistic effect of the secondary metabolites mainly, sesquiterpene coumarins. Though, the more comprehensive fundamental mechanism deserved additional inquiry.

## Declaration of competing interest

The authors declare that there is no conflict of interest.

## Authors' contribution

Design of the experiment and writing of the article: A.M.ElSayed, W.A.Algahwaji, K.S.El Deeb. Performing the chemical study: A.M.El Sayed, A.M.El Sayed, W.A. Algahwaji. Performing the pharmacological study: Z.Youseif

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## تأثير التحكم في الخصوبة للأجزاء الهوائية لنبات الفريولا تنجيتانا عن طريق تغيير استجابات محور الوطاء - الغدة النخامية - التناسلية لإناث فئران ويستار

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### ملخص

تم الإبلاغ عن *Ferula tingitana* L لخصائصه المجهضة و/ أو المسببة للحيض. ومع ذلك ، فإن تأثيره في منع الحمل لم يتم تقييمه بشكل متعمد. علاوة على ذلك ، لم يتم تسجيل التتميط الكيميائي الشامل لمستخلصه. عُرفت العديد من أنواع الكيرولا بتأثيرها على إيقاع الإستروجين. أثناء اكتشافنا للعقار من المصادر الطبيعية ، تم اختيار *F. tingitana* L. النامية في ليبيا لتقييم تأثيرها في منع الحمل. لتقييم التأثيرات الهرمونية والجزيئات النشطة بيولوجياً لمستخلص الإيثانول F. تم تقسيم إناث الفئران البيضاء البالغة بالتساوي إلى 4 مجموعات (ن = 6). تلقت مجموعة واحدة الماء المقطر لمدة 14 يوماً، وتلقت المجموعات الثانية والثالثة والرابعة المستخلص المختبر عن طريق الفم بجرعة يومية من 100 و200 و300 مجم / كجم - 1 ووزن. لمدة 14 يوماً على التوالي. استمرت الإدارة لمدة 14 يوماً (أسبوعين) في الساعة 9 صباحاً. تم قياس وزن جسم الفئران والرحم. تم صيامهم طوال الليل ثم تخديرهم من خلال التعرض للحمض الغذائي وتم جمع عينات الدم من خلال ثقب العين. تم طرد الدم للحصول على مصل واضح للمقايسة الهرمونية. تم إخضاع المصل بواسطة طريقة ELISA لتقييم مستويات الهرمون المنبه للجريب (FSH) والهرمون اللوتيني (LH) والإسترايول (E2) والبروجسترون (P4). تم قياس التقديرات البيوكيميائية لمستوى الكوليسترول الكلي (TC) ، والدهون الثلاثية (TG) ومستوى الجلوكوز (Glu). تم إجراء GC / MS للملف الشحمي جنباً إلى جنب مع تحليل HPLC للمحتويات الفينولية. تم إخضاع EtOH وأجزائه القابلة للذوبان المتتالية للتحليل الكروماتوجرافي. أظهرت النتائج انخفاضاً معنوياً في مستويات FSH و LH و E2 و P4 لإناث فئران ويستار البالغة. لوحظ انخفاض كبير في مستوى المصل الكيميائي الحيوي من TC و TG و Glu. كان داوكل سيسكيتيربين وحمض اللينولينيك وحمض الكافيين والهسبريدين من المكونات النباتية الرئيسية التي تم تحديدها. تم تحديد CC من EtOH المنوح لـ 5 مركبات على أنها 1  $\beta$ -sitosterol ، 2 colladonin ، 3 scopoletin ، 4 caffeic acid ، 1-4 ، 2'-cyclopentane-1,3-diol ، 3. استنتج أن sesquiterpene coumarins نظراً لأن المكونات النباتية الهامة لـ EtOH قد تكشف عن تأثير سلبي على الدورة الشهرية، وإباضة الجريبات وبالتالي قد تضعف الخصوبة. أثبت EtOH تأثيرات سكر الدم ونقص الكوليسترول.

الكلمات الدالة: موانع الحمل، *Ferula tingitana* L، سكر الدم، سيسكيتيربين الكومارين.

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