Anethum graveolens as a possible modulator of testicular steroidogenesis

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Abstract. The aim of this study was to evaluate the possible in vitro effects of Anethum graveolens L. (dill) extract on the steroidogenesis in testicular tissue. Specifically, we focused to determine the dominant chemical components of the dill extract followed by a subsequent assessment of its in vitro effects on the production of cholesterol, dehydroepiandrosterone, androstenedione and testosterone by rat testicular fragments. Dill extract was subjected to high performance liquid chromatography (HPLC) which determined cyanosid, carvone, chlorogenic acid, p-hydroxybenzoic acid and salicylic acid among the predominant biomolecules. For the in vitro experiments, testicular fragments from 12 sexually mature Wistar rats were incubated in the absence (control) or presence of 10, 100 or 1000 μg/mL extract for 24 h. Cholesterol levels were quantified via photometry and the hormones were evaluated by Enzyme-Linked Immunosorbent Assay (ELISA). Our data showed that the release of cholesterol and androstenedione (but not dehydroepiandrosterone and testosterone) by the testicular fragments obtained from seminiferous tubules was significantly affected by the dill extract in a dose-dependent manner. Exposure to the extract led to a significant decrease of both cholesterol as well and androstenedione (P<0.01 in case of 10 μg/mL; P<0.001 with respect to 100 and 1000 μg/mL extract). Our results suggest that the bioactive molecules present in Anethum graveolens L. could have dose-dependent impact on the secretion of selected male reproductive hormones, playing a role in the regulation of testicular steroidogenesis. Nevertheless, the exact effect of individual biomolecules on the endocrine testicular function has yet to be investigated.

1. Introduction
The role of medicinal herbs in the management of health issues has been known for centuries, and plant-based therapy has contributed significantly to the creation of modern drugs and nutraceuticals. Moreover, more than 60 % of the world’s population relies on bioactive ingredients from herbs used for medical purposes [1]. The World Health Organization is a strong advocate in favour of the use of medicinal herbs and encourages scientists to emphasize on a rational use of a vast array of plants for the development of new pharmaceutical remedies [2]. A variety of plant extracts are nowadays used in the prevention or treatment of male subfertility, including hypoactive sexual desire disorder, hormonal imbalance, erectile and ejaculation dysfunction as well as sub-standard semen parameters [3].

Multiple plants have been traditionally used to counteract male infertility, based on their enhancing
effects on the testicular steroidogenesis and spermatogenesis. Rose mallow (*Hibiscus*) and Malabar Spinach (*Basella alba*) have been shown to stimulate the testosterone secretion in adult males and are currently recommended as a complementary therapy to counteract sexual fatigue [4]. Extracts obtained from Peruvian ginseng (*Lepidium meyenii*) have been shown to activate different stages of spermatogenesis in healthy rats, as well as to alleviate testicular stress caused by heavy metal intoxications [5]. In the meantime, pomegranate (*Punica granatum*) juice administered to rats has exhibited the ability to increase sperm count and motion characteristics, density of spermatogonia and spermatocytes, as well as the volume of seminal vesicles and male reproductive cells [6]. These examples indicate emphasize on the need to further investigate the potential modulatory effects plant extracts may exhibit on the testicular structure and function.

*Anethum graveolens* L. (dill) has been known as an ethnomedicinal plant since ancient times and it is still a popular herb widely used as a spice and as a staple for the production of essential oils. Dill is an aromatic herb found in the *Apiaceae* family. The most prominent volatile biomolecules found in dill include carvone (dill seeds), α-phellandrene, limonene, dill ether and myristicin (dill herb) [7, 8].

Dill is used to relieve colic pain and flatulence in young children. The essential oil relieves intestinal spasms and gripping, improves appetite and aids digestion. Dill triggers milk production in lactating females, and it also helps to treat urinary complaints, piles and mental disorders [8, 9].

A number of *in vivo* studies have suggested that dill extracts have the ability to stimulate the spermatogenic as well as steroidogenic capacity in rodents [10, 11]. *In vitro* studies on primary cell cultures and tissues are nevertheless a useful strategy to understand the behaviour of potentially beneficial or harmful biomolecules because of their consistent functional characteristic and increased sensitivity [12, 13]. Taking into account the essential roles of sex steroids in the growth, differentiation and function of the male reproductive system, it is important to investigate the impact of plant extracts on male steroidogenesis. We are unconscious of any reports that have investigated the direct *in vitro* effect of the dill extract on the endocrine processes involved in reproductive functions. As such, our aim was to assess the impact of selected concentrations of the dill extract on the secretion of cholesterol, testosterone, dehydroepiandrosterone (DHEA) and androstenedione by rat testicular fragments. At the same time, we determined the predominant bioactive compounds present in the dill extract and associated their occurrence with the potential to modulate testicular steroidogenesis.

2. Materials and methods

2.1. Plant collection and preparation of the extract

Dill was harvested at the Botanical Garden, Slovak University of Agriculture (Nitra, Slovakia) in the summer of 2016. Fresh plant material was dried and crushed, weighed and immersed in 96% ethyl alcohol (Centralchem, Bratislava, Slovakia). The maceration took two weeks at 20-22°C in the absence of light. The resulting extract was placed into a rotary evaporator (Cole-Parmer, Stone, UK) and processed under reduced pressure at 40°C (vacuum pump, KNF Neuberger, Freiburg, Germany) in order to dispose of any remaining ethyl alcohol. The obtained plant material was resuspended in DMSO (dimethyl sulfoxide; Sigma-Aldrich, St. Louis, Missouri, USA). The final concentration of the stock solution was set to 1000 mg/mL [14].

For the HPLC assessment, fresh plant material was freeze dried and pulverized. Twenty-five mL of 80% methanol (suitable for HPLC; Sigma-Aldrich, St. Louis, Missouri, USA) were added to 1 g of the plant material. The resulting mixture was agitated using a shaker (250 RPM; 20-22°C; 8 h), filtered and stored at 5°C.

2.2. HPLC-DAD analysis

Specific HPLC standards, methanol (suitable for HPLC), acetonitrile (suitable for HPLC) and phosphoric acid (ACS grade) were provided by Sigma-Aldrich (St. Louis, Missouri, USA). Double deionized water was obtained using a purification system (Simplicity 185; Millipore SAS, France). Each standard (0.5 mg) was dissolved in 10 mL methanol. The methanolic standards and plant extracts were
filtered through a filter paper (no. 390, Munktell & Filtrak, Niederschlag, Germany) followed by the Q-
Max syringe filter (0.22 mm diameter; Frisenette, Knebel, Denmark).

HPLC assessment of the dill extract was performed with the 1260 Infinity II LC System (Agilent
Technologies, Santa Clara, California, USA). The analysis was executed using a Purosphere reverse
phase C18 column (4 mm x 250 mm x 5 mm; Merck, Kenilworth, New Jersey, USA). The mobile phase
was composed of acetonitrile and 0.1% phosphoric acid in double deionized water. The starting flow
rate was set to 1 mL/min with 10 mL of the injection volume. The column temperature was set to 30°C
while the standards and samples were in the sample manager at 4 °C. Data collection and analysis was
carried out using the Aglient OpenLab ChemStation software for LC 3D Systems [15]. The extract was
analysed in triplicate.

2.3. Collection of testicular fragments and in vitro culture
Male Wistar rats (n=12, 120 days old) obtained from the Institute of Experimental Pharmacology (Dobrá
Voda, Slovakia) were kept in plastic cages at 24±1 °C and 12 h light/12 h dark regime. The animals were
provided with a standard laboratory feed and water ad libitum. Institutional and national regulations for
the care and use of laboratory animals were followed, and all procedures were approved by the Slovak
State Veterinary and Food Institute and Ethics Committee (no. 3398/11-221/3).

The rats were anesthetized by intraperitoneal injection with chloral hydrate, the testes were removed,
deposited into a sterile Petri dish with fresh, sterile-filtered Dulbecco's phosphate buffered saline (PBS;
Sigma-Aldrich, Missouri, USA) and dissected into 4 approximately equal fractions.

Seminal tubules were isolated from the interstitial tissue and mechanically dissociated with the help
of tweezers and a blade knife. In order to confirm the steroidogenic activity within the heterogenous cell
suspension collected from the testicular fragments, the 3β-hydroxysteroid dehydrogenase (3β-HSD)
staining was performed following the protocol introduced by Klinefelter et al. [12] and modified by
Jambor et al. [13]. Sample aliquots were incubated with a mixture consisting of nitrotetrazolium blue
chloride (Sigma-Aldrich, Missouri, USA), dehydroisoandrosterone-3-sulfate (Sigma-Aldrich, Missouri,
USA) and nicotinamide adenine dinucleotide (Sigma-Aldrich, Missouri, USA) at 37°C for 2 h.
Subsequently the cell suspensions were observed under a light microscope (Leica, Wetzlar, Germany)
for the occurrence of blue formazan crystals, indicating the activity of 3β-HSD.

The collected fragments were washed twice in PBS and placed into 1 ml DMEM (Dulbecco's
Modified Eagle Medium, Sigma Aldrich, Missouri, USA) enriched with 10% foetal bovine serum
(Sigma Aldrich, Missouri, USA), 1% penicillin-streptomycin solution (Sigma Aldrich, Missouri, USA)
and without (control) or with the Anethum extract supplemented at various concentrations (10, 100,
1000 µg/mL) for 24 h. The in vitro culture was carried out in 24-well plates at 37 °C and 5% CO₂. Four
independent experiments were carried out on 4 individual days using separate pools of testes collected
from 12 rats.

2.4. Assessment of cholesterol and steroid hormones
Following incubation, the culture medium was collected and centrifuged (300 x g, 4 °C, 10 min). The
supernatant was subjected to the quantification of cholesterol and sex hormones. Cholesterol levels were
assessed using the DiaSys commercial kit (Diagnostic Systems, Holzheim, Germany) and the Rx Monza
photometer (Randox Laboratories, Crumlin, UK). Testosterone, DHEA, and androstenedione were
quantified using commercial ELISA (enzyme-linked immunosorbent assay) kits (DiaLab, Wiener
Neudorf, Austria). Concentrations of steroid hormones were measured at 450 nm using the Glomax
Multi+ plate spectrophotometer (Promega, Wisconsin, USA). The analysis was performed in duplicates.

2.5. Statistical analysis
GraphPad Prism 3.02 (GraphPad Software, San Diego, California, USA) was used for the statistical data
evaluation. One-way analysis of variance (ANOVA) and the Dunnett's test were selected for advanced
statistical calculations. Bartlett's test was used to test the assumption that the obtained data came from
populations with equal variances, while the F test assessed the homogeneity of the variance. The results
are expressed as the mean±standard deviation (SD). The level of significance was set at ***P<0.001; **P< 0.01 and *P< 0.05.

3. Results and discussion
Concentrations of the specific biomolecules identified by HPLC are displayed in Table 1. The predominant biomolecule found in the extract was cyranosid (618.15±9.94 mg/kg). With respect to the phenolic acids; chlorogenic, neochlorogenic, p-hydroxybenzoic, salicylic acid and vanillic acid were identified, amongst which chlorogenic acid was the most abundant (533.36±8.89 mg/kg).

Table 1. Major biomolecules determined [mg/kg] in the dill extract.

| Biomolecule          | Concentration (mg/kg) |
|----------------------|-----------------------|
| Cynarosid            | 618.15±9.94           |
| Carvone              | 45.60±4.09            |
| Limonene             | 17.51±2.66            |
| Myristicin           | 11.35±1.22            |
| Chlorogenic acid     | 533.36±8.89           |
| Neochlorogenic acid  | 107.67±1.98           |
| p-hydroxybenzoic acid| 12.04±2.27            |
| Salicylic acid       | 17.27±1.89            |
| Vanillic acid        | 8.77±2.01             |

(Figure 1). The in vitro impact of the dill extract on the cholesterol levels in cells collected from rat seminiferous tubules. Each bar represents mean (± SD) values for cholesterol expressed as % of the control (0 μg/mL extract) and experimental groups (exposed to 10, 100 and 1000 μg/mL extract). ***P< 0.001; **P< 0.01.

As a progenitor of steroid molecules, cholesterol plays an essential role in the synthesis of sex hormones. As revealed in figure 1 the cholesterol levels were noticeably different between the control (0.22±0.11 μg/mL) and experimental groups (0.14±0.05 μg/mL in case of 10 μg/mL extract; 0.08±0.02 μg/mL with respect to 100 μg/mL extract; 0.07±0.03 μg/mL in relation to 1000 μg/mL extract). We detected a significant decrease of the cholesterol quantity, depending directly on the dose of extract used (P<0.01 with respect to 10 μg/mL; P<0.001 in case of 100 and 1000 μg/mL extract).

Similarly to cholesterol, androstenedione production of testicular fragments exposed to the dill extract (figure 2) was affected significantly (P<0.01 in terms of 10 μg/mL; P<0.001 in case of 100 and 1000 μg/mL extract). The levels of androstenedione decreased depending on the concentration of the extract used in the experiment (18.29±2.26 ng/mL in case of 10 μg/mL extract; 15.25±1.99 μg/mL with respect to 100 μg/mL extract; 5.61±0.67 μg/mL in relation to 1000 μg/mL extract) in comparison with the control (23.08±3.01 ng/mL).
Figure 2. The in vitro impact of the dill extract on the androstenedione production in cells collected from rat seminiferous tubules. Each bar represents mean (±SD) values for cholesterol expressed as % of the control (0 μg/mL extract) and experimental groups (exposed to 10, 100 and 1000 μg/mL extract). ***P<0.001; **P<0.01.

As shown in figure 3, dehydroepiandrosterone concentrations were not significantly different between the control and experimental groups. A small decrease was observed following exposure to 100–1000 μg/mL (9.07±0.84 – 8.55±0.91 μg/mL) of the extract, however without significant differences (P>0.05) in comparison with the control (9.57±0.74 μg/mL). The analysis showed that the Anethum graveolens extract did not affect the concentration of dehydroepiandrosterone in the culture medium.

Figure 3. The in vitro impact of the dill extract on the dehydroepiandrosterone levels in cells collected from rat seminiferous tubules. Each bar represents mean (±SD) values for cholesterol expressed as % of the control (0 μg/mL extract) and experimental groups (exposed to 10, 100 and 1000 μg/mL extract).

Similarly, testosterone concentrations were not significantly affected by the exposure to the Anethum extract (figure 4; P>0.05). A minor decrease of testosterone production was recorded in all experimental groups (14.87±2.09 ng/mL in case of 10 μg/mL extract; 15.18±2.50 ng/mL with respect to 100 μg/mL extract; 14.22±2.91 ng/mL in relation to 1000 μg/mL extract), however without significant differences (P>0.05) in comparison with the control (15.82±1.92 ng/mL). The analysis showed that the dill extract did not have an impact on the quantity of testosterone in the culture medium.
Outcomes from scientific research focused on the use of plants and their extracts traditionally used in ethnotherapy may be appropriately interpreted following a hierarchy of significance. Data obtained from properly designed in vivo studies primarily contribute to the overall understanding of the beneficial or adverse effects plant extracts have on the living organism. Nevertheless, we chose an in vitro approach, which may provide invaluable information and serve as a reasonable predictor of possible clinical outcomes. Such in vitro strategy has the ability to clarify the contradictory and often controversial knowledge gathered so far on the impact of plant extracts on the living organism [16]. In this case, we focused to assess the chemical composition of the Anethum graveolens extract and subsequently to investigate its effects on the steroidogenic activity of rat testicular tissue.

Preliminary data are available on the biochemical description and possible biological effects of dill and its extracts or essential oils. A complex assessment of the biomolecules present in 8 dill cultivars was provided by Al Ahl and Omer [17]. Similarly to our results, limonene (9.60–18.0%) and carvone (81.35–89.98%) were the predominant biomolecules detected in European cultivars. Interestingly, and correspondingly to our data, neither dillapiole nor piperitone were detected in essential oils prepared from European cultivars, whereas abundant amounts of these biomolecules were found in African dill cultivars. As such, we may agree that the exact biocomposition of medicinal plants and their products depends heavily on the cultivar, region, climate conditions as well as the age of the plant during collection [17, 18]. Szopa and Eikert [19] furthermore state that in vitro cultures of Anethum graveolens could become a suitable model to understand the accumulation of a variety of phenolic acids, as well as a possible source of p-hydroxybenzoic acid and o-hydroxybenzoic (salicylic) acid for biotechnological use. The occurrence of both acids was confirmed by our HPLC analysis as well.

To our knowledge, no report exists on the analysis of the production of reproductive hormones by testicular tissue in vitro following exposure to dill extract. A number of in vivo studies have however assessed the effects of aqueous Anethum extract on the male reproductive system in rats. Contrary to our observations, neither Iamsaar et al. [11] nor Monsefi et al. [10] detected no negative or harmful effects of the exposure to the dill extract on the body growth, sperm concentration, motility or morphology, and as such, the extract did not exhibit any negative effect on the epididymal sperm maturation. Based on both studies, we may speculate that the spermatogenic function of testicular tissue may not be affected by the presence of the dill extract or its biomolecules. Furthermore, morphometrical evaluations revealed that the diameter of seminiferous tubules and density of spermatogenic cells were not different between the control and experimental groups, indicating no significant damage to the structural integrity of male reproductive organs potentially caused by the Anethum extract. Surprisingly, both studies also observed normal testosterone levels in the groups receiving treatment in comparison

![Figure 4](image.png)

**Figure 4.** The in vitro impact of the dill extract on the testosterone levels in cells collected from rat seminiferous tubules. Each bar represents mean (±SD) values for cholesterol expressed as % of the control (0 μg/mL extract) and experimental groups (exposed to 10, 100 and 1000 μg/mL extract).
with the control, suggesting that the complex hypothalamic-pituitary-gonadal axis in males was not disrupted by the dill extract. Nevertheless, our in vitro study shows that the Anethum extract dos exhibit significant modulatory effects on the endocrine activity by decreasing the levels of cholesterol as well as androstenedione by rat testicular fragments.

Assuming that the dill extract does not exhibit a substantial effect on the response of male gonads towards the luteinizing hormone (LH) and follicle-stimulating hormone (FSH), the decrease of cholesterol and androstenedione detected in our study may be linked to the actual testosterone biosynthesis within testicular Leydig cells. Testosterone as a steroid hormone, is derived from cholesterol, with two subsequent pathways that may be followed. While the Δ-4 pathway is based on the conversion of androstenedione, the most important intermediate of the Δ-5 pathway is DHEA [20].

In our study, a significant dose-dependent decrease of cholesterol was recorded. Logically, its decrease should trigger a subsequent decline of all observed hormones, as recorded in a number of previous reports [12, 21-23], however that was not the case in our experiments. We may speculate that the decreasing concentration of cholesterol did not affect the Δ-5 pathway, being reflected on non-significant changes in the production of DHEA. This may subsequently become the primary source for further conversion to testosterone as previously suggested by Miller [24]. On the other hand, the decreasing trend of cholesterol was well-mirrored by a significant decrease of the most important intermediate of the Δ-4 pathway, androstenedione. As such, we may hypothesize that the bioactive compounds present in the dill extract have led to a shift in the balance of the testicular steroidogenesis towards the Δ-5 pathway via DHEA-S, leading to lower levels of cholesterol available for androstenedione synthesis. A similar observation was recorded by Zimmermann et al. [25]. Cholesterol is considered to be the building block of all the steroid hormones, and it is well known that low cholesterol levels contribute to poor hormone production [26]. In our case, the decreasing levels of cholesterol might have had an impact on the dynamics of male steroidogenesis, however the end product (testosterone) was not significantly affected. We may assume that although lower, the levels of cholesterol were still high enough to provide substrate for subsequent reactions of steroid hormone biotransformation [25, 27]. At the same time, based on the fact, that two pathways exist to produce testosterone, we may hypothesize that the Δ-5 pathway was less affected by the biomolecules present in Anethum than the Δ-4 pathway, and thus compensated for the imbalance caused by the administration of dill extract. Similar changes in the balance of testosterone biosynthesis were reported by Jambor et al. [13]. Nevertheless, to verify our hypothesis, it would be necessary to quantify the intermediates of both pathways, i.e. progesterone and androstenediol. Furthermore, although a non-significant, but a decreasing trend was observed in case of both DHEA and testosterone, which may become more pronounced with the increasing time of in vitro culture. As such, it may be important to prolong the culture of testicular fragments in the presence of the dill extract in order to re-evaluate its effect on the steroidogenesis from a long-term point of view.

The interplay of bioactive molecules present in the dill extract is another point to be addressed in further studies. While it has been shown that cynamosid, carvone, limonene and myristisicin present in the dill extract may exhibit beneficial effects on the male reproductive system in vivo [28-30], it is important to assess their synergy or antagonism in a complex mixture such as a plant extract. Moreover, the exact in vitro effect of individual biomolecules on the endocrine testicular function has yet to be investigated. Finally, future in vitro studies are highly anticipated in order to assess the impact of medicinal plants and their properties in specialized cells and tissues in order to predict their behavior in a complex organism.

4. Conclusion
Our preliminary results offer new evidence on the biological impact of the Anethum graveolens extract on male reproductive tissues. The application of medicinal plants with a wide array of beneficial properties into practical andrology is unquestionably necessary. Our data evidently cannot predict an indisputable in vivo or in vitro conclusion as a direct impact of dill extract on fertility in males has to be addressed further. To transform our observations into practice, reports on the possible toxicity,
pharmacokinetic behaviour and bioavailability of dill and its products in relation to the functional activity of the male reproductive system are more than necessary.

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References
[1] Kumar S, Paul S, Walia Y K, Kumar A and Singhal P 2015 Therapeutic potential of medicinal plants: A review J. Biol. Chem. Chron. 1 46-54
[2] WHO 2002 WHO Traditional Medicine Strategy 2002–2005 J D Quick, J Sawyer, P Thorpe, D Whitney and X Zhang eds. (Geneva: World Health Organization)
[3] Kamal R, Gupta R S and Lohiya N K 2003 Plants for male fertility regulation Phytother. Res. 17 579-90
[4] Moundipa F P, Kamtchouing P, Koueta N, Tantchou J, Foyang N P and Mbiapo F T 1999 Effects of aqueous extracts of Hibiscus macranthus and Basella alba in mature rat testis function J. Ethnopharmacol. 65 133-9
[5] Rubio J, Riqueros M I, Gasco M, Yucra S, Miranda S and Gonzales G F 2006 Lepidium meyenii (Maca) reversed the lead acetate induced-damage on reproductive function in male rats Food Chem. Toxicol. 44 1114-22
[6] Lin W H, Tsai M T, Chen Y S, Hou R C, Hung H F, Li C H, Wang H K, Lai M N and Jeng K C 2007 Improvement of sperm production in subfertile boars by Cordyceps militaris supplement Am. J. Chin. Med. 35 631-41
[7] Blank I and Grosch W 1991 Evaluation of potent odorants in dill seed and dill herb (Anethum graveolens L.) by aroma extract dilution analysis J. Food Sci. 56 63-7
[8] Jana S and Shekhawat G S 2010 Anethum graveolens: An Indian traditional medicinal herb and spice Pharmacogn. Rev. 4 179-84
[9] Duke J A 2001 Handbook of Medicinal Herbs (London: CRC Press)
[10] Monsefi M, Zahmati M, Masoudi M and Javidnia K 2011 Effects of Anethum graveolens L. on fertility in male rats Eur. J. Contracept. Reprod. Health Care 16 488-97
[11] Iamsaard S, Prabsattroo T, Sukhorum W, Muchimapura S, Srissaard P, Uabundit N, Thukhammee W and Wattanathorn J 2013 Anethum graveolens Linn. (dill) extract enhances the mounting frequency and level of testicular tyrosine protein phosphorylation in rats J. Zhejiang Univ. Sci. B. 14 247-52
[12] Klinefelter R G, Hall F P and Ewing L L 1987 Effect of luteinizing hormone deprivation in situ on steroidogenesis of rat Leydig cells purified by a multistep procedure Biol. Reprod. 36 769e783
[13] Jambor T, Greifova H, Kovacik A, Kovacikova E, Tvrda E, Forgacs Z, Massanyi P and Lukac N 2018 Parallel effect of 4-octylphenol and cyclic adenosine monophosphate (cAMP) alters steroidogenesis, cell viability and ROS production in mice Leydig cells Chemosphere 199 747-54
[14] Tvrda E, Michalko J, Matušiková I and Lukáč N 2016 In vitro effects of the Chlamydomonas reinhardtii extract on bovine spermatozoa JMBFS 6 972-5
[15] Lukšic L, Árvay J, Vollmannová A, Tóth T, Skrabanja V, Treck J, Germ M and Kreft I 2016 Hydrothermal treatment of Tartary buckwheat grain hinders the transformation of rutin to quercetin J. Cer. Sci. 72 123-4
[16] Brandt K and Seal C 2006 Advantages and pitfalls of different types of studies for investigations of the impact of food on health Paper presented at Joint Organic Congress (Odense, Denmark: University of Newcastle upon Tyne, School of Agriculture)
[17] Al Ahl H S and Omer E 2016 Essential oil content and chemical composition of eight dill
Anethum graveolens L) cultivars cultivated under Egyptian conditions Int. J. Pham. Pharm. Sci. 8 227-31

[18] Aminzadeh M, Amiri F, Ashor Abadi E, Mahdevi K and Fadai S 2010 Factors affecting on essential chemical composition of Thymus kotschyanus in Iran World Appl. Sci. J. 8 847-56

[19] Szopa A and Eikert H 2015 Anethum graveolens L. in vitro cultures – a potential source of bioactive metabolies, phenolic acids and furanocoumarins Acta Biol. Cracoviens Botanica 57 29-37

[20] Winters S J and Clark B J 2003 Testosterone synthesis, transport, and metabolism Androgens in Health and Disease Contemporary Endocrinology C J Bagatell and W J Bremner eds. (Totowa, NJ: Humana Press) pp 3-22

[21] Hou J W, Collins D C and Schleicher R L 1990 Sources of cholesterol for testosterone biosynthesis in murine Leydig cells Endocrinology 127 2047-55

[22] Sriraman V, Sairam M R and Rao A J 2003 Evaluation of relative roles of LH and FSH in regulation of differentiation of Leydig cells using an ethane 1,2-dimethylsulfonate-treated adult rat model J. Endocrinol. 176 151-61

[23] Gupta R S, Kachhawa J B S, Khushalani V, Tanwar K and Joshi Y C 2006 Effect of Cressa cretica methanol extract on testicular function of albino rats Pharm. Biol. 44 382-8

[24] Miller W L 2002 Androgen biosynthesis from cholesterol to DHEA Mol. Cell Endocrinol. 198 7-14

[25] Zimmerman Y, Eijkemans M J, Coelingh Bennink H J, Blankenstein M A and Fauser B C 2014 The effect of combined oral contraception on testosterone levels in healthy women: A systematic review and meta-analysis Hum. Reprod. Update 20 76-105

[26] Kelly D M and Jones T H 2013 Testosterone: a metabolic hormone in health and disease J. Endocrinol. 176 R25-45

[27] Zimmerman Y, Eijkemans M J, Coelingh Bennink H J, Blankenstein M A and Fauser B C 2014 The effect of combined oral contraception on testosterone levels in healthy women: A systematic review and meta-analysis Hum. Reprod. Update 20 76-105

[28] Tajuddin, Ahmad S, Latif A, Qasmi I A and Amin K M 2005 An experimental study of sexual function improving effect of Myristica fragrans Houtt. (nutmeg) BMC Complement. Altern. Med. 5 16

[29] Wilson M J, Lindgren B R and Sinha A A 2008 The effect of dietary supplementation with limonene or myo-inositol on the induction of neoplasia and matrix metalloproteinase and plasminogen activator activities in accessory sex organs of male Lobund-Wistar rats Exp. Mol. Pathol. 85 83-9

[30] Wang X, Tao R, Yang J, Miao L, Wang Y, Munyangaju J E, Wichai N, Wang H, Zhu Y, Liu E, Chang Y and Gao X 2017 Compounds from Cynomorium songaricum with estrogenic and androgenic activities suppress the oestrogen/androgen-induced BPH process Evid. Based Complement. Alternat. Med. 2017 6438013