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PHYTOCHEMICAL CHARACTERIZATION AND FEMALE FERTILITY-ENHANCING POTENTIAL OF *AJUGA IVA* (L.) EXTRACT: INSIGHTS FROM *IN VIVO* AND TOXICOLOGICAL STUDIES

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ABSTRACT. This study investigated the phytochemical composition, evaluated the acute toxicity, and potential fertility-enhancing effects of a hydroethanolic extract obtained from *Ajuga iva* (L.) aerial parts. The extraction yielded 23.8%, and analysis revealed a high concentration of phenolics (182.21± 0.51mg Eq GAE/g DW extract) and flavonoids (46.75± 0.85mg Eq QE/g DW extract). Using HPLC-ESI-MS/MS, we identified twenty-one metabolites, with phytoecdysteroids and flavonoids being the most abundant. No signs of mortality or toxicity were recorded over 14 days following a single orally administered dose of 2000 mg/kg. Notably, the extract significantly increased the weights of the ovaries and genital tract ($p < 0.01$), resulting in a more than 2.5-fold rise compared to controls. Histological study confirmed enhanced follicular development, especially at the 20 mg/kg dose, characterized by a greater number of tertiary and Graafian follicles. Molecular docking analysis further revealed strong binding affinities of apigenin, naringenin-7-O-rutinoside, naringenin, campestanol trans-ferulate, and ajugasterone C with key reproductive hormone receptors, suggesting their possible role in stimulating folliculogenesis. Altogether, these findings highlight the promise of *Ajuga iva* (L.) as a natural fertility enhancer and support the need for further research.

KEY WORDS: *Ajuga iva (L)*, Acute toxicity, Fertility, Histological study, Hydroethanolic extract, Molecular docking

INTRODUCTION

Female infertility poses a significant challenge to many couples, affecting not only individual psychological and physical well-being but also the social stability of partnerships, particularly among young couples [1]. Its prevalence is increasing globally, according to a World Health

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Organization (WHO) report, with an estimated 1 to 6 people (17,5%) in the adult population [2]. Infertility is clinically defined as the failure to achieve pregnancy after 12 months of regular, unprotected sexual intercourse [2]. Despite advances in modern reproductive technologies, many individuals continue to seek both conventional and traditional treatments. Since antiquity, medicinal plants have served as a cornerstone in the management of various health conditions, including infertility [3, 4]. *Ajuga iva* (L.) (*A. iva* (L.)), with its long-standing use in North African and Mediterranean traditional medicine for female reproductive health, represents a promising candidate for further pharmacological investigation within this context [5, 6].

Ajuga iva (L. Schreber) is a plant utilized in traditional Algerian remedies under the name "*Chendgoura*", and it is part of the Lamiaceae family and the botanical genus *Ajuga*, with more than 300 different species. This plant is broadly found throughout the arid regions of Africa, Asia, Europe, and Australia [7]. The reported biological activity includes antidiabetic, antimicrobial [8], and antioxidant [9]. It can also decrease the oxidative stress in hypercholesterolemia [10]. Overall, the composition of *A. iva* (L.) includes several flavonoids, terpenes, tannins, and phytoecdysteroids. It also has phenolic acids, anthocyanins, and other compounds [9, 11].

In traditional medicine, *A. iva* (L.) has been demonstrated to be an effective treatment for female infertility [5, 6, 11]. While *A. iva* (L.) has been explored for many properties [9-11], its direct impact on female fertility, particularly, no studies have investigated the female fertility-enhancing potential via a hydroethanolic extract from *Ajuga iva* (L.) through integrated in vivo and toxicological studies, highlighting the specific research gap our work addresses. Unlike general reviews on herbal male infertility treatments [12] or studies focusing on different plants for female fertility [5], our work uniquely provides a comprehensive assessment of *Ajuga iva* (L.)'s female fertility potential using both in vivo and molecular docking approaches.

The present study aimed to evaluate the acute toxicity and potential effects on fertility, specifically ovarian and genital tract weights and ovarian histology, in immature female mice treated with the hydroethanolic extract of *A. iva* (L.). Additionally, an in-silico study was conducted to clarify the mechanism of action, and the phytochemical composition was evaluated through LC-MS/MS analysis.

EXPERIMENTAL

Chemicals and reagents

All chemicals, solvents, and reagents used for extraction were of analytical or HPLC grade. Ethanol (96%) was supplied by BIOCHEM, France. Formaldehyde solution (35 %) was obtained from Honeywell Reidel-de Haen, and xylene (99.86%) from Fisher Scientific, UK. Folin-Ciocalteu phenol reagent (2N), Sodium carbonate (Na_2CO_3, 99,95%), aluminum trichloride ($AlCl_3$, 99,99%), sodium acetate (CH_3COONa, > 99%), Tween 80 (viscous liquid), Parafine (56-58 °C), Acetic acid (99.99%), and Methanol (> 99.7%) were all obtained from Sigma-Aldrich, USA.

Solution preparation

A hydroalcoholic solution (75% v/v) was obtained by diluting ethanol (96%) with distilled water. The Folin–Ciocalteu reagent was used after dilution to 10% (v/v) from the commercial 2 N stock. Sodium carbonate solution was prepared at 20% (w/v) using analytical grade Na_2CO_3 (99.95%). An aluminum chloride solution was made at 10% (w/v) by dissolving $AlCl_3$ (99.99%) in distilled water. Sodium acetate was prepared as a 1 M solution (> 99% purity), and 0.1 mL was withdrawn from it. Acetic acid (99.99%) was diluted to 0.1% (v/v) before use. Tween 80 was used as a vehicle at 0.05% (v/v). Formaldehyde solution (10%) was prepared by diluting a concentrated stock (35%) with distilled water.

Instruments and apparatus

An electric grinder (SAYONA SZJ-1307 equipped with a 300-watt motor was used to grind the plant material. A rotary evaporator (Heidolph Hei-Vap, France) with heating bath adjustable between 20 °C and 220 °C (±1 °C), and a rotation speed range of 10-280 rpm, coupled to a vacuum pump (8 mbar, Flow rate 1,2 m^3 h^{-1}) was used for evaporation. A UV-Vis spectrophotometer (Shimadzu UV-1900, Japan) with a wavelength range of 190-1100 nm (±0.5 nm) was utilized for the determination of total phenolic content (TPC) and total flavonoid content (TFC). LC/UV/ESI-MS Agilent 1100 HPLC system, equipped with a photodiode array (PDA) detector. The separation was carried out on a KNAUER column (250 mm × 4.6 mm; 5 µm particles). UV analysis was performed between 230 and 350 nm. Mass spectrometry was performed in positive ionization mode with the following parameters: source temperature at 120 °C, capillary voltage at 3.2 kV, probe temperature at 350 °C, and cone voltage at 40 V. The mass spectrum was recorded in an m/z range from 150 to 750.

Plant material

This study was carried out using the plant *Ajuga iva* (L.) (*A. iva* (L.)), a medicinal species recognized for its pharmacological properties. Specimens were collected in the Chréa region, located approximately 50 km southwest of Algiers, specifically in the Chréa National Park (Blida Province). The GPS coordinates of the collection site are: 36°21'36.23"N; 2°49'04.2"E. Botanical identification was performed in the herbarium of the Botany Department of the National School of Agronomy (ENSA), El Harrach (Algeria), where a control specimen was deposited under the reference number CTA 133/03/11.

Only the aerial parts of *A. iva* (L.) were used in the analyses (leaves, stems, and inflorescences). The carefully selected plant material was cleaned with distilled water to eliminate surface residues and dried in a climate-controlled room maintained at (30 ± 3 °C) with adequate ventilation, drying for 14 days. The moisture content was determined according to the AOAC method [13], yielding a value of 10%. The samples were ground using an electric grinder (SAYONA SZJ-1307) and then passed through a 1 mm mesh sieve to ensure a homogeneous particle size distribution. The resulting powder was preserved in tightly sealed amber glass containers at room temperature, protected from light and moisture, until use.

Phenolic compound content

Preparation of the ethanolic extract of A. iva (L.): In the present study, the extraction was carried out using a homogenizer (HG-15A-set-A package), blending 10 g of *A. iva* (L.) powder with 150 mL of 75% (v/v) ethanol. The mixture was homogenized for 50 minutes at 40 °C. Afterwards, the extract was filtered under vacuum through a 185 mm filter, and the filtrate was evaporated to dryness. The dried extract was weighed and stored in an amber glass flask at 4 °C until further analysis and biological testing.

Total phenolic content

The determination of total phenolic was conducted using the Folin–Ciocalteu colorimetric method [14], based on the phenolic groups' ability to reduce the phospho-molybdic-phosphotungstic complex, forming a blue complex measurable at 760 nm [15]. In practice, 100 µL of the ethanolic extract was combined with 500 µL of 10% Folin–Ciocalteu reagent, followed by the addition of 1 mL of 20% sodium carbonate (Na_2CO_3). The reaction mixture was maintained in the dark at room temperature for 30 minutes. Absorbance was determined with a UV-visible spectrophotometer (Shimadzu UV-1900 Pharmaspec). Quantification was performed against a

gallic acid standard curve, and results were expressed as mg of gallic acid equivalents per gram of dry extract (mg Eq GAE/g DW extract).

Total flavonoid content

Total flavonoid content was determined by the aluminum trichloride ($AlCl_3$) colorimetric method, according to the procedure described by Chang *et al.* [16]. In this method, 2 mL of the extract or standard solution was mixed with 0.1 mL of 10% (w/v) $AlCl_3$, 0.1 mL of 1 M sodium acetate (CH_3COONa), and 2.8 mL of distilled water. After 30 minutes of incubation at room temperature, the absorbance was read at 415 nm with the Shimadzu spectrophotometer. Quantification was performed by interpolation from a quercetin standard curve, and the results were expressed as mg quercetin equivalents per gram of dry extract (mg Eq QE/g DW extract).

LC/UV/ESI-MS analysis

The chemical profile of phenolic compounds in the ethanolic extract was conducted according to the method reported by Ma *et al.* [17]. The analyses were performed on an Agilent 1100 HPLC system. A volume of 20 µL of sample was injected, with a flow rate of 1 mL/min, using a binary gradient composed of solvent A (0.1% acetic acid) and solvent B (HPLC-grade methanol). The gradient program was as follows: 0-55 min (95% A / 5% B), 55-56 min (5% A / 95% B), 56-58 min (95% A / 5% B), 58-60 min (95% A / 5% B). The various phenolic compounds were detected between 230 and 350 nm.

Data analysis was performed using CHEMSTATION software. The identification of the compound was performed by comparison with available authentic standards. Phenolic compounds were further characterized through co-chromatography with authentic standards. PDA and mass spectral data were employed to verify the identity of compounds that were previously described in the literature [9, 18, 19].

Acute toxicity of A. iva (L.) extract

The acute oral toxicity evaluation was performed in accordance with the Organization for Economic Co-operation and Development (OECD) Test Guideline 423 [20]. For this study, female BALB/c mice, 6 to 8 weeks old and weighing between 17 and 21 g, were randomly divided into four experimental groups. The animals were housed in polypropylene cages under controlled environmental conditions: temperature of 25 ± 2 °C, relative humidity of 55 ± 10%, and a 12 h/12 h light/dark cycle.

The animals were acclimated for five days before the experiment. Four hours before administration, the mice were fasted (with free access to water). The dry extract of *A. iva* (L.) was dissolved in Tween 80 vehicle (0.05% v/v) concentration and administered orally as a single dose at 0 (only vehicle), 50, 300, and 2000 mg kg^{-1} body weight (see Table S1). The animals were carefully observed for the first 30 minutes after injection, then at regular intervals for the first 24 hours, and daily for a period of 14 days to detect any signs of toxicity, behavioral changes, or death.

This entire procedure was conducted in accordance with the ethical standards established by the Algerian Association of Laboratory Animal Sciences (AASEA) and approved by the Institutional Ethics Committee of the Houari Boumediene University of Science and Technology (USTHB), under approval number 45/DGLPAG/DVA.SDA.14. dated 2022.

Fertility effect of A. iva (L.) extract in mice

Ovarian and genital weights: To explore the potential effects of *A. iva* (L.) extract on sexual development and fertility, a study was conducted on recently weaned female BALB/c mice, aged 20 to 21 days and weighing between 13 and 19 g. This age stage was chosen because it precedes the onset of puberty, marked in particular by vaginal opening, which generally occurs around day 25 [21]. At this age, the hypothalamic-pituitary-gonadal axis begins to be activated, notably by the release of GnRH [22].

Four groups of mice received oral gavage treatments once daily over five days with *A. iva* (L.) extract at increasing doses of 0 (Normal control only vehicle), 5, 10, and 20 mg kg^{-1} body weight. An additional control group of sexually mature adult female mice, weighing 29 and 36 g, corresponding to the reproductive stage with a regular estrous cycle and left untreated, was included to compare the mean ovarian weight of treated animals with that of untreated mature controls.

On day 6, the animals were euthanized by cervical dislocation. The ovaries and reproductive organs (uterus and vagina) were removed, cleaned, and then weighed using a precision balance. These data made it possible to evaluate the effect of the extract on the development of reproductive structures in comparison with the control groups.

Histological study

To characterize the possible histomorphological changes induced by the extract, a histological analysis was performed on the ovaries of the treated animals. Ovarian tissues were immersed in 10% buffered formalin for 24 hours for fixation, then dehydrated in ethanol baths of increasing concentration, clarified in xylene (99,86 %), and then embedded in molten paraffin. Histological sections with a thickness between 2 and 5 µm were made using a rotary microtome. These sections were then stained according to the standard method with hematoxylin and eosin (H&E), and then examined under a light microscope to detect possible alterations in the organization of the ovarian tissue, such as the formation of follicles, the state of the corpus luteum, or the presence of cysts.

Docking studies

To predict potential interactions between bioactive metabolites, present in *A. iva* (L.) and key molecular targets involved in the regulation of human reproduction, a molecular docking study was undertaken. This in silico simulation was performed using PyRx 8.0 software, a widely used tool for virtual ligand screening and assessment of binding affinity between small molecules and biological macromolecules.

Structures of the target proteins were extracted in three-dimensional format from the Protein Data Bank (PDB). Hormone receptors selected in this study include: estrogen receptor β (ERβ, PDB ID: 1X7B) [23], estrogen receptor α (ERα, PDB ID: 1ERE) [24], human gonadotropin receptor GnRH (GnRH1R, PDB ID: 7BR3)[25], progesterone receptor (PR, PDB ID: 1E3K) [26], luteinizing hormone receptor (LHR, PDB ID: 7FIG)[27], and follicle-stimulating hormone receptor (FSHR, PDB ID: 1XWD) [28]. Ligands of interest were selected from the bioactive constituents identified in *A. iva* (L.). Their three-dimensional chemical structures were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in SDF format. Before the docking step, the receptor and ligand structures were prepared according to standard protocols, including the removal of crystalline water molecules, the addition of partial charges, and energy minimization to optimize the conformation.

The actual molecular docking was performed using the PyRx 8.0 interface and generated essential data such as binding energies, affinity scores, and root-mean-square deviations (RMSD)

less than or equal to 2 Å. These parameters were used to predict the stability of the complexes formed between the ligands and the target receptors.

Finally, the resulting complexes were visualized and analyzed using BIOVIA Discovery Studio Visualizer software (version 2021). This step allowed us to identify the types of molecular interactions involved in the bond, following the methodological approaches previously described in the scientific literature [29, 30].

Statistical analysis

The results are presented as mean ± standard deviation (SD). Comparisons between control and treated groups, including ovarian and genital weights, were performed using the student t-test. A 95% confidence interval was applied for the interpretation of results, and statistical significance was considered at $p < 0.01$. All statistical analyses were carried out using SPSS software, version 26.0 (IBM Corp., 2015).

RESULTS AND DISCUSSION

Extract yield, total phenolic, and total flavonoid content

The present study highlights the significant extraction yield and rich phytochemical composition of the hydroethanolic extract of *A. iva* (L.) aerial parts. The high levels of phenolics (182.21± 0.51mg Eq GAE/g DW extract) and flavonoids (46.75± 0.85mg Eq QE/g DW extract) support the traditional medicinal use of this species (Table S2).

The high phenolic content observed indicates the strong antioxidant potential of our extract, as phenolics are well known for their strong free radical scavenging activities [31]. Our results show that it substantially exceeds the values reported by Ammar *et al.* [9] , who found the phenolic content of 143 mg Eq GAE/g DW extract in the ethanolic extract of *A. iva* (L.) collected from Tunisia. In contrast, the Algerian *A. iva* (L.) studied here demonstrated significantly higher phenolic content than previously reported for other *Ajuga* species [18]

Similarly, the total flavonoid content obtained in this study aligns with findings in related studies, such as Ammar *et al.* [9], who reported flavonoid contents of 40-50 mg Eq QE/g DW extract for Tunisian populations of *A. iva* (L.), which are in agreement with our results. These findings are in line with other phytochemical screenings of Algerian species [32], which noted abundant flavonoids and phenolics, suggesting that these constituents are characteristic and potentially contribute to biological activities beyond antioxidants [33], which could significantly contribute to fertility-enhancing effects by modulating inflammation and oxidative stress. Such mechanisms are supported by reports of *A.iva* (L.) improving reverse cholesterol transport in diabetic rats and implications for inflammatory pathways in fertility [10].

The variations observed in phenolic compounds content can be influenced by genetic diversity, the phenological stage, environmental conditions, as well as the sample preparation and extraction methods. These factors are major determinants of polyphenolic compounds content. Elements such as the drying method, storage conditions, particle size of the powder, the nature, and especially the polarity of the solvent used, along with extraction conditions and the choice of reference standard for calibration (quercetin, catechin, or rutin), can all significantly affect the final measured content [34].

Overall, the pharmacological potential of the *A. iva* (L.) plant looks promising. The combination of high extraction yield and abundant bioactive components makes *A. iva* (L.) a strong candidate for further pharmacological studies and natural product-based therapies.

LC/UV/ESI-MS analysis

To identify the chemical components of *A. iva* (L.) extract, LC/UV/ESI-MS analysis was conducted. The chromatographic profile obtained is illustrated in the Figure. 1, while the analytical data of the different peaks, including retention times, absorption wavelengths (λmax), molecular ions $[M-H]^-$, and major fragments, are summarized in Table 1.

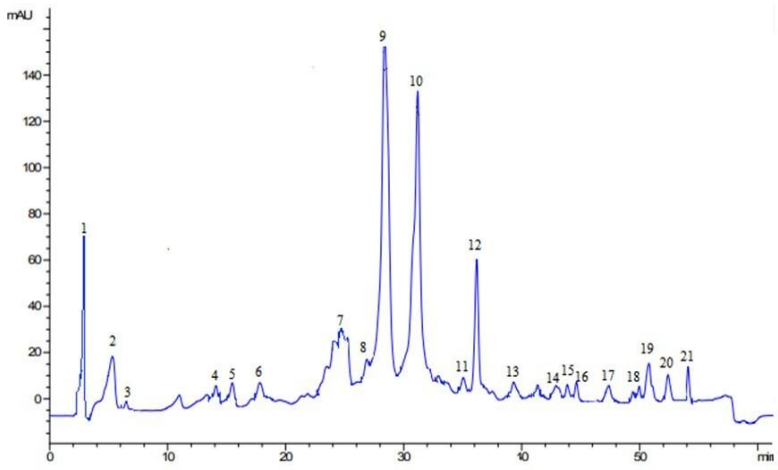


Figure 1. Chromatogram of the hydroethanolic extract from the aerial parts of *A. iva* (L.) plant.

Table 1. Retention time (RT), maximum UV absorption (λmax), mass spectral data, and tentative identification of the phenolic compounds in the hydroethanolic extract of *A. iva* (L.) from the aerial (parts n=3 samples, 3 replicates per sample).

N°	RT (min)	UV (Max)	$[M-H]^-$ m/z	Main fragment	Tentative identification	Classes	%
1	2.85	270	169		Gallic acid	Phenolic acid	2.56
2	5.32	290	137	93	P- Hydroxybenzoic acid	Phenolic acid	7.48
3	6.50	280	341	269	Apigenin-8-C-glucoside	Flavonoid	0.70
4	14.09	350	447	285	Luteolin-7-O-gluside	Flavonoid	0.52
5	15.48	331	269		Apigenin	Flavonoid	0.87
6	17.81	237	497		Polypodine B	Phytoecdysteroid	1.01
7	25.18	271-331	593	269	Apigenin-dihexoside	Flavonoid	2.49
8	26.88	249	463		B-Ecdysone	Phytoecdysteroid	1.05
9	28.38	249	479		20-hydroxyecdysone	Phytoecdysteroid	30.14
10	31.18	249	493		Makisterone A	Phytoecdysteroid	23.54
11	35.05	249	493		D24-28Makisterone	Phytoecdysteroid	0.73
12	36.19	249	519		Cyasterone	Phytoecdysteroid	5.64
13	39.32	249	477		22-Oxocyasterone	Phytoecdysteroid	0.92
14	42.90	271-329	579	271	Naringenin-7-O-rutinoside	Flavonoid	1.02

15	43.86	271-329	579		Naringenin	Flavonoid	0.66
16	44.64	271-329	579	271	Naringenin-7-O-neohesperidoside	Flavonoid	0.72
17	47.38	249	517		24-Dehydropre cyasterone	Phytoecdysteroid	0.95
18	49.96	197	363		Harpagide	Iridoid	0.63
19	50.78	208	577		Campestanol *trans* ferulate	Sterol	2.46
20	52.39	249	481		Ajugasterone C	Phytoecdysteroid	1.23
21	54.09	249	481		7,8-dihydroajugasterone C	Phytoecdysteroid	1.06

This approach allowed the tentative identification of 21 metabolites, including a significant structural diversity: phytoecdysteroids (n = 10), flavonoids (n = 7), phenolic acids, sterols, and iridoids. Among the major compounds are several ecdysteroids characteristic of the genus Ajuga, including peaks 6, 8, 9, 10, 11, 12, 13, 17, 20, and 21. 20-Hydroxyecdysone (peak 9), for example, represents 30.14% of the relative abundance and was identified by an acetic acid adduct ion at m/z 539, releasing a main fragment at m/z 479. Makisterone A (peak 10), the second most abundant compound (23.54%), was detected with a precursor ion at m/z 553 and a fragment at m/z 493, consistent with an acetic acid adduct. Other steroids, such as polypodin B (peak 6), β-ecdysone (peak 8), cyasterone (peak 12), and ajugasterone C (peak 20), also exhibit characteristic profiles. Flavonoids, another dominant class, were identified by their distinctive UV spectra and specific fragments. Apigenin-8-C-glucoside (peak 3), luteolin-7-O-glucoside (peak 4), and free apigenin (peak 5) were confirmed by $[M-H]^-$ ions at m/z 341, 447, and 269, respectively. The diglycosylated derivative of apigenin (peak 7) exhibits a molecular mass $[M-H]^-$ at m/z 593, suggesting the loss of two hexose units (324 amu). Glycosylated naringenins (peaks 14, 15, 16) were identified by a parent ion $[M-H]^-$ at m/z 579, with a dominant fragment at m/z 271, characteristic of the naringenin aglycone.

Phenolic acids detected include gallic acid (peak 1; m/z 169) and p-hydroxybenzoic acid (peak 2; m/z 137 → 93). Finally, the iridoid harpagide (peak 18; m/z 363; λmax = 197 nm) and the sterol campestanol trans-ferulate (peak 19; m/z 577; λmax = 208 nm) complete the complex composition of this extract. This varied chemical composition highlights the plural pharmacological potential of *A. iva* (L.), particularly in the areas of hormonal regulation, reproduction, antioxidation, and cytoprotection.

These results are consistent with the overall abundance of phytoecdysteroids, particularly 20-hydroxyecdysone, previously reported in *Ajuga* species [18]. Early phytochemical investigations of *A. iva* (L.) enabled the isolation of ecdysterone and related compounds [35]. Later research identified more ecdysteroids and flavonoids, demonstrating the sustained scientific interest in its chemical diversity and supporting the interpretation of its biological effects [36], [37].

Our analysis identified two subclasses of flavonoids in *A. iva* (L.): naringenin (derivatives of flavanones) and apigenin8-C-glucoside, apigenin, apigenin-dihexoside, as well as Luteolin-7-O-gluside (derivatives of flavones). Among these, naringenin-7-O-rutinoside and apigenin-dihexoside were the predominant compounds. In agreement with Boudjelal *et al.* [38], apigenin and naringenin were confirmed as the predominant flavonoids in aqueous infusions of *A. iva* (L.). Phytoecdysteroids present in *A. iva* (L.), exhibit structural similarity to arthropod molting hormones and affect reproductive physiology in mammals through hormone-like mechanisms, explaining observed biological effects [39]. In addition, the flavonoids present in *A. iva* (L.) apigenin and naringenin, when combined, showed greater estrogen receptor activation than when tested individually [40]. These findings support traditional North African and Mediterranean uses of *A. iva* (L.) for female reproductive health and suggest its promising therapeutic potential for infertility [5], [6].

Acute toxicity of A. iva (L.) extract

An acute toxicity study was conducted to determine the safety profile of the hydroethanolic extract of *A. iva* (L.) when administered orally. Mice were administered a single dose of up to 2000 mg kg^{-1} and observed for 14 days (Table S1). No mortality or signs of suffering were noted in any of the treated groups. These observations indicate that the no-observed-adverse-effect-level (NOAEL) was determined to be 2000 mg kg^{-1}, while the median lethal dose (LD_{50}) is considered to be higher than this value [41]. Post-mortem macroscopic examination of vital organs (heart, liver, kidneys, lungs, spleen) revealed no alterations, thus confirming the acute safety of the extract under the tested conditions.

Fertility effects of A. iva (L.) extract in mice

The potential effect of the extract on reproductive system development was explored in prepubertal female mice. They received daily oral administration for five days at increasing doses of 5, 10, and 20 mg kg^{-1}. The experimental results showed a statistically highly significant increase ($p < 0.01$) in both genital tract weight and ovarian weight between immature mice treated with *A. iva* (L.) extract and untreated mice (Normal control), with an increase of more than 2.5-fold. (Table 2).

Specifically, the 20 mg kg^{-1} dose induced a higher mean ovarian weight than that of untreated adult mice, suggesting accelerated sexual maturation.

Anatomically, adult mice exhibit significantly greater reproductive system and ovarian weights compared to recently weaned prepubescent mice. Our experimental results confirm this ontogenetic difference, demonstrating statistically significant increases in both genital tract weight and ovarian weight ($p < 0.01$). Although the mean ovarian weight of treated immature mice was comparable to that of untreated adult female mice, no statistically significant difference ($p > 0.01$) was observed in genital tract weights between treated immature and untreated mature mice. Statistical analysis using the student's t-test confirmed substantial differences between the treated groups and the immature controls (Table 2).

It is also important to note that immature mice treated at 20 mg kg^{-1} had genital weights that exceeded those of adults, indicating hormonal mimetic or stimulatory effects. These observations are supported by the data presented in Tables S3-S7.

Table 2. Fertility effect of *A. iva* (L.) extract (5 mg kg^{-1}, 10 mg kg^{-1}, 20 mg kg^{-1}) in mice: Student's t-test results.

Dose	Genital tract weight (mg)	Ovarian weight (mg)
Mature mice	198.93±38.17[a]	23.58±2.0[a]
Normal control	30.18±5.35	10.48±1.44
5 mg kg^{-1}	42.05 ± 5.63[a]	18.25 ± 1.65[a]
10 mg kg^{-1}	44.05 ± 5.64[a]	25.47 ± 3.48[ab]
20 mg kg^{-1}	48.38 ± 5.83[a]	28.55 ± 3.53[ab]

[a] ($p < \alpha$=0,01), when compared with the control normal (only vehicle), and [b] ($p < \alpha$=0,01) mature mice.

The increases in ovarian weight of more than 2.5-fold may be ascribed to the occurrence of phytoestrogenic substances in the plant extract. The characterization confirms the presence of phytoecdysteroids and flavonoids, which are recognized as phytoestrogens [42]. Plant ecdysteroids are natural polyhydroxylated compounds derived from the biosynthesis of cholesterol or other plant sterols [43]. Flavonoids, on the other hand, belong to the main classes of phytoestrogens (along with isoflavones and stilbenes) [42]. In this study, naringenin (flavanone) and apigenin (flavone) were identified as molecules with high estrogenic potential. Their combination enhances their affinity for estrogen receptors, particularly Erα [40]. The results

suggest that *A. iva* (L.) extract contains compounds capable of interacting with hormone receptors, potentially promoting ovarian steroidogenesis.

The ethnomedicinal relevance of the plant lies in its use for treating infertility, as reported by Salhi *et al.* [6]. This activity is likely related to the stimulation of ovarian cell multiplication, mimicking the action of FSH and promoting gamete maturation and fertilization [44, 45]. These effects are attributable to the combined presence of flavonoids and phytoestrogens [45-47].

Histological study

Histopathological analysis of the ovaries showed morphological changes consistent with follicular stimulation in mice treated with *A. iva* (L.) extract. Compared with the control group, mice receiving the extract had an increased number of follicles at different stages of maturation (Figure 2). At the highest dose (20 mg kg^{-1}), an abundance of tertiary follicles and homogeneous Graafian follicles at the cavitary stage was observed, indicating advanced ovarian development. Lower doses (5 and 10 mg kg^{-1}) also showed stimulatory effects, although less so, with a predominance of secondary and primary follicles and a few primordial follicles.

These results suggest that *A. iva* (L.) extract possesses folliculogenesis-inducing properties, probably via endogenous hormonal modulation or an ecdysteroid-mimetic effect. This opens up interesting perspectives in the field of phytotherapy for fertility support.

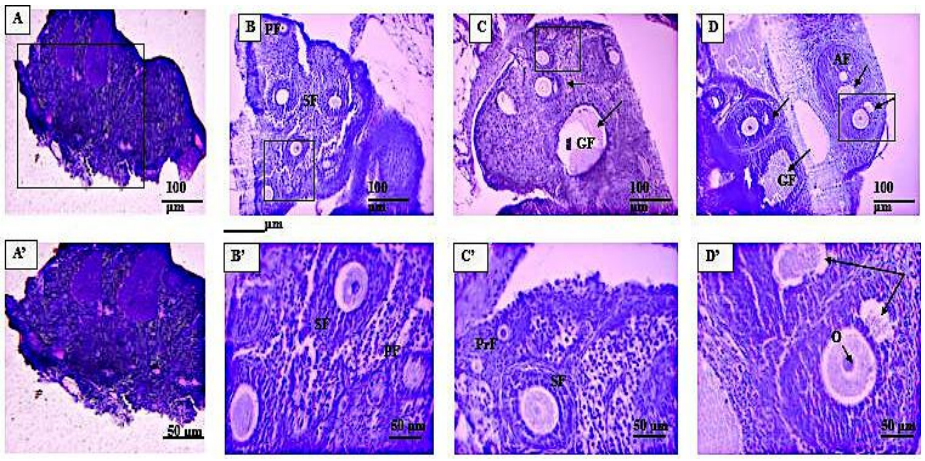


Figure 2. Histopathological observation of the ovaries. (A) The control group, (B) The group treated with 5 mg $kg^{-1}$of *A. iva* (L.) extract, (C) The group treated with 10 mg kg^{-1} of *A. iva* (L.) extract, and (D) The group treated with 20 mg $kg^{-1}$of *A. iva* (L.) extract. PrF: Primordial follicle; PF: Primary follicle; SF: Secondary follicle; GF: Graafian follicle; O: Oocyte. Bars in 1 = 100 µm, bars in 2 = 50 µm.

The extract's capacity to stimulate or enhance FSH activity regulates the growth and differentiation of ovarian follicles, which could be attributed to its bioactive phytoconstituents. These findings are aligned with the reported physiological effects [48]. *A. iva* (L.) Compounds including flavonoids and phytoecdysteroids may bind with reproductive system receptors, especially estrogen receptors ERα. This receptor-mediated way can explain the observed increase in follicular recruitment and growth. This will be further investigated through molecular docking studies.

The present findings highlight the therapeutic potential of *A. iva* (L.) in enhancing female reproductive health. Its folliculogenesis-enhancing effect, especially at a 20 mg kg^{-1} dose, supports the ethnopharmacological use of the plant in fertility enhancement.

Molecular docking

To elucidate the molecular mechanisms underlying the activity of the bioactive compounds identified in the leaf extract, an in-depth molecular docking study was performed using PyRx software. This in silico approach allowed examining the potential interactions of 21 compounds identified by LC-MS with different protein targets implicated in the regulation of the female reproductive system. Compared to the reference ligands co-crystallized with these proteins, the majority of the tested compounds showed remarkable binding affinities, illustrating their bioactive potential. These results are summarized in Table S8 and illustrated in Figure 3. For the estrogen receptor alpha (ERα), three compounds particularly stood out for their high binding affinities: naringenin-7-O-rutinoside, campestanol trans-ferulate, and Ajugasterone C, with binding free energies (ΔG) of −7.8 and −7.5 kcal mol^{-1}. These interactions are mainly stabilized by hydrogen bonds formed between the carboxyl groups of the ligands and the amino acid residues Asn 439, His 513, and Lys 362, known for their crucial role in ligand binding. At the FSH receptor level, naringenin-7-O-rutinoside was the most efficient, with an estimated binding affinity of $\Delta G = -7.6$ kcal mol^{-1}. Three hydrogen bonds were observed between this flavonoid compound and the essential residues Ser 55, Ser 34, and Cys 32, suggesting a specific interaction with the receptor active site. Regarding the luteinizing hormone (LH) receptor, several compounds showed exceptional affinity, including apigenin, naringenin-7-O-rutinoside, naringenin, campestanol trans-ferulate, and ajugasterone C, with ΔG ranging from −8.5 to −8.0 kcal mol^{-1}. These bonds are reinforced by hydrogen interactions involving phenolic hydroxyl groups and functional residues such as Tyr 612, Ser 450, Pro 347, Cys 280, Asp 355, Gly 358, Thr 446, and Asp 140. In parallel, the compound campestanol trans-ferulate also showed significant affinity towards the GnRH receptor, characterized by a binding energy of −8.4 kcal mol^{-1}, and predominantly mediated by the Pro 173, Tyr 211, Ala 209, Phe 210, and Phe 213 residues, which play a central role in ligand recognition. Progesterone receptors (PR) also exhibited marked interactions with several flavonoids and phytoecdysteroid derivatives. Compounds such as naringenin-7-O-rutinoside, campestanol trans-ferulate, and 20-hydroxyecdysone displayed $\Delta G \leq -8.0$ kcal mol^{-1}. Hydrogen bonds were detected between these ligands and residues Gly 923, Asp 882, Tyr 753, Thr 829, Thr 753, His 881, and His 888, confirming their potential as natural hormone modulators.

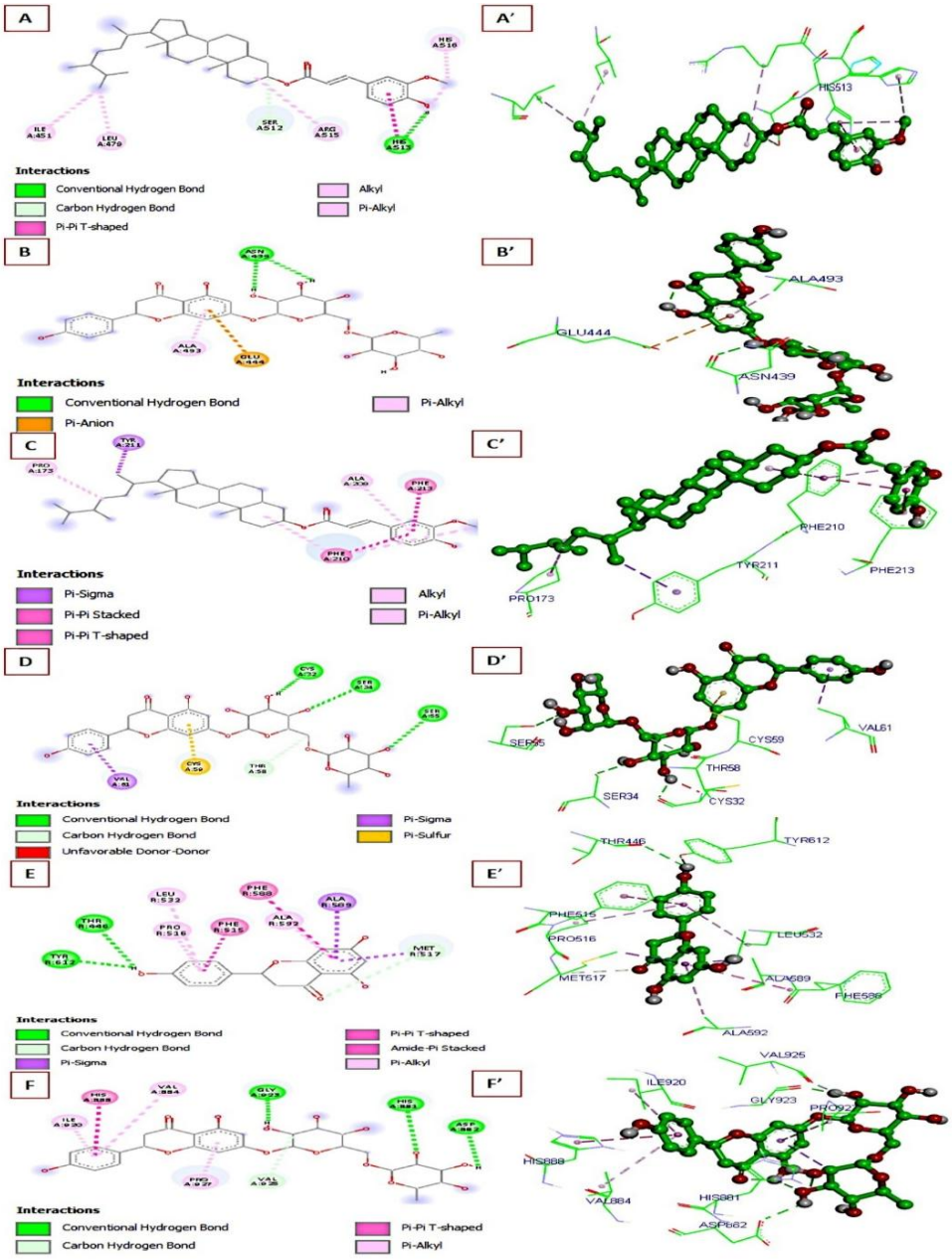


Figure 3. 2D and 3D representations of (A, A') Campestanol trans ferulate, and (B, B') Naringenin-7-O-rutinoside, with Estrogen α Receptor (ER α), (C, C') Campestanol trans ferulate, with Human Gonadotropin Receptor (GnRH), (D, D') Naringen-in-7-O-rutinoside with Human Follicle Stimulating Hormone Receptor (FSH), (E, E') Naringenin with Luteinizing Hormone receptor (LHR), (F, F') Naringenin-7-O-rutinoside with Progesterone Receptor (PR).

According to molecular modeling, drug-likeness, and ADMET analysis, in silico data show that apigenin and naringenin exhibit strong potential for modulating the progesterone receptor (PR), suggesting an ability to improve endometrial receptivity. Furthermore, campestanol trans-ferulate, formed by esterification of campestanol with ferulic acid, also demonstrated high affinity for the ERα and ERβ receptors [49], [50]. Our study revealed that apigenin, naringenin-7-O-rutinoside, naringenin, campestanol trans-ferulate, and ajugasterone C exhibit particularly significant binding energies (−8.5 to −7.8 kcal mol^{-1}) towards the Erα, LH, GnRH, and P receptor. Furthermore, naringenin showed a high affinity toward LHR (−8.5 kcal mol^{-1}).

These compounds are good candidates for new oral therapeutic drug designs with a mechanism of stimulating the growth of ovarian follicles and enhancing endometrial receptivity. These findings suggest that the estrogen-like effect of *A. iva* (L.) compounds is likely the result of a synergistic interaction that triggers transcriptional processes and/or signaling events, ultimately regulating gene expression involved in the development and function of the reproductive system. This study highlights the therapeutic potential of these compounds in addressing female fertility issues via their interaction with reproductive system receptors. These compounds may have a significant impact on the reproductive health of women by controlling hormone activity, increasing the development of ovarian follicles, and stimulating ovulation. Moreover, their anti-inflammatory properties may also help treat fertility issues caused by inflammation.

CONCLUSIONS

The current study reveals the remarkable potential of the hydroethanolic extract from *Ajuga iva* (L.) aerial parts to enhance the reproductive health of females. The extract shows a high extraction yield and greater content of phenolics and flavonoids in the hydroethanolic extract of *A. iva* (L.) aerial parts, confirming the species' traditional therapeutic usage. The main compounds in the extract were determined to be phytoecdysteroids and flavonoids by LC/UV/ESI-MS analysis. By increasing ovarian weights in immature female mice, oral administration of this extract may improve female fertility. This is most likely due to follicle development, which is demonstrated by changes in size and structure. Campestanol trans-ferulate, Ajugasterone C, apigenin, naringenin-7-O-rutinoside, and naringenin all appear to have promising therapeutic potential for reproductive system receptors, according to an in-silico study. With the potential for therapeutic applications in female reproductive health, these findings start to raise hopes for improved management and treatment of reproductive issues. Regarding these results, more investigation is required to identify the fundamental mechanisms. To further elucidate the extract's method of action and validate its therapeutic potential, future studies should focus on long-term safety and fertility trials in addition to mechanistic analyses such as hormone measurement and gene expression profiling in ovarian tissues.

CRediT authorship contribution statement

Zineb Salem: Conceptualization, Investigation, Formal analysis, Methodology, Writing – original draft, Visualization. Faiza Zermane: Supervision, Resources, Writing – review & editing. Benamar Cheknane: Investigation, Formal analysis. Henni Chader: Investigation, Formal analysis. Salim Djellouli: Investigation, Formal analysis. Anita Kumari: Investigation, Formal analysis. Nabil Touzout: Investigation, Validation, Formal analysis. Hossam S. El-Beltagi: Supervision, Writing – review, editing & Funding acquisition. Othman Al-Dossary: Investigation, Validation, Formal analysis. Bader Alsubaie: Writing – review, editing & Funding acquisition. Adel A. Rezk: Supervision, Resources, Writing – review, editing & Funding acquisition. Ahmed Mahmoud Ismail: Resources, Writing – review & editing. Wael Elmenofy: Writing – review,

editing & Funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Data availability

The authors can provide the supporting information upon request.

REFERENCE

1. Boivin, J.; Bunting, L.; Collins, J.A.; Nygren, K.G. International estimates of infertility prevalence and treatment-seeking: Potential need and demand for infertility medical care. *Hum. Reprod*. **2007**, 22, 1506-1512.
2. World Health Organization report. *1 in 6 people globally affected by infertility: WHO*. April 2023. Available at: https://www.who.int/news/item/04-04-2023-1-in-6-people-globally-affected-by-infertility.
3. El-Amier, Y.A.; El-Halawany, E.F.; H.M. Soliman,E.F.; El Hayyany. L.Y. Primary GC-MS chemical analysis of alcoholic extract of *Emex Spinosa* (L.) Campd. and screening of their antioxidant, antibacterial, and cytotoxic characteristics. *Bull. Chem. Soc. Ethiop*. **2023**, 37, 101-114.
4. Patel, F.R.; Modi, N.R. Secondary metabolite production from medicinal plants for the treatment of female infertility: a review. *Res. J. Life Sci. Bioinform. Pharm. Chem. Sci*. **2018**, 4, 544-551.

5. Benkaddour, M.; Djahra, A.B.; Benkherara, S.; Benine, C.; Benmoussa, O. Ethnobotanical Importance and Pharmacological Properties of Plants Used in Southestern Algeria (El Oued) for the Treatment of Women's Infertility Problems. *J. Herbs. Spices. Med. Plants*. **2025**, 31, 427-443.
6. Salhi, S.; Fadli, M.; Zidane, L.; Douira, A. Études floristiques et ethnobotaniques des plantes médicinales de la ville de Kénitra (Maroc). *Mediterr. Bot*. **2010**, 31, 133-146.
7. Ni, B.; Dong, X.; Fu, J.; Yin, X.; Lin, L.; Xia, Z.; Zhao, Y.; Xue, D.; Yang, C.; Ni, J. Phytochemical and biological properties of *Ajuga decumbens* (Labiatae): a review. *Trop. J. Pharm. Res*. **2015**, 14, 1525-1536.
8. Saidi, S.; Remok, F.; Handaq, N.; Drioiche, A.; Gourich, A.A.; Menyiy, N.E.; Amalich, S.; Elouardi, M.; Touijer, H.; Bouhrim, M. Phytochemical profile, antioxidant, antimicrobial, and antidiabetic activities of *Ajuga iva* (L.). *Life*. **2023**, 13, 1165.
9. Ammar, H.; Touihri, I.; Kholif, A.E.; M'Rabet, Y.; Jaouadi, R.; Chahine, M.; Marti, M.E.d.H.; Vargas-Bello-Pérez, E.; Hosni, K. Chemical composition, antioxidant, and antimicrobial activities of leaves of *Ajuga iva*. *Molecules* **2022**, 27, 7102.
10. Hamden, K.; Carreau, S.; Jamoussi, K.; Ayadi, F.; Garmazi, F.; Mezgenni, N.; Elfeki, A. Inhibitory effects of 1α, 25-dihydroxyvitamin D3 and *Ajuga iva* extract on oxidative stress, toxicity, and hypo-fertility in diabetic rat testes. *J. physiol. Biochem.* **2008**, 64, 231-239.
11. Bouyahya, A.; El Omari, N.; Elmenyiy, N.; Guaouguaou, F.E.; Balahbib, A.; El-Shazly, M.; Chamkhi, I. Ethnomedicinal use, phytochemistry, pharmacology, and toxicology of *Ajuga iva* (L.,) Schreb. *J. Ethnopharmacol*. **2020**, 258, 112875.
12. Shakeri, M.; Saberi, M.; Boojar, M.M.A. Herbal treatments and their impact on male fertility: A comprehensive review. *Qeios*. **2025**, 7, 2632-3834.
13. Horwitz, W.; Latimer, G.W. *Official method of analysis of the Association of AOAC International*, 18th ed., Association of Official Analytical Chemistry International, Maryland, USA; **2005**, 3, 1-30.
14. Lister, E.; Wilson, P. Measurement of total phenolics and ABTS assay for antioxidant activity (personal communication). *Crop Res. Inst. Lincoln. New Zealand*. **2001**.
15. Talbi, H., Boumaza, A., El-Mostafa, K., Talbi, J., & Hilali, A. Évaluation de l'activité antioxydante et la composition physico-chimique des extraits méthanolique et aqueux de la Nigella sativa L. *J. Mater. Environ. Sci*. **2015**, 6, 1111-1117.
16. Chang, C.C.; Yang, M.H.; Wen, H.M.; Chern, J.C. Estimation of total flavonoid content in propolis by two complementary colometric methods. *J. Food Drug Anal*. **2002**, 10, 178-182.
17. Ma, C.; Dunshea, F.R.; Suleria, H.A.R. LC-ESI-QTOF/MS characterization of phenolic compounds in palm fruits (jelly and fishtailpalm) and their potential antioxidant activities. *Antioxidants* **2019**, 8, 483.
18. Bakrim, A.; Ngunjiri, J.; Crouzet, S.; Guibout, L.; Balducci, C.; Girault, J.P.; Lafont, R. Ecdysteroid profiles of two Ajuga species, *A. iva* and *A. remota. Nat. Prod. Commun*. **2014**, 9, 1934578X1400900804.
19. Stevens, J.F.; Reed, R.L.; Morré, J.T. Characterization of phytoecdysteroid glycosides in meadowfoam (*Limnanthes alba*) seed meal by positive and negative ion LC-MS/MS. *J. Agric. Food.Chem.* **2008**, 56, 3945-3952.
20. Schlede, E. Oral acute toxic class method: OECD Test Guideline 423. *Rapporti. istisan*. **2002**, 41, 32-36.
21. Ajayi, A.F.; Akhigbe, R.E. Staging of the estrous cycle and induction of estrus in experimental rodents: an update. *Fertil. Res. pract*. **2020**, 6, 5.
22. Moon, S.; Yun, B.; Lee, M.; Seok, E.; Ha, J.; Yang, H. Gonadotropins regulate the mRNA expression of gonadotropin-releasing hormone and its receptors in the mouse ovary and uterus. *Development and Reproduction* **2024**, 28, 1-22.

23. Manas, E.S.; Unwalla, R.J.; Xu, Z.B.; Malamas, M.S.; Miller, C.P.; Harris, H.A.; Hsiao, C.; Akopian, T.; Hum, W.T.; Malakian, K. Structure-based design of estrogen receptor-β selective ligands. *J. Am. Chem. Soc*. **2004**, 126, 15106-15119.
24. Brzozowski, A.M.; Pike, A.C.; Dauter, Z.; Hubbard, R.E.; Bonn, T.; Engström, O.; Öhman, L.; Greene, G.L.; Gustafsson, J.A.; Carlquist, M. Molecular basis of agonism and antagonism in the oestrogen *receptor. Nature* **1997**, 389, 753-758.
25. Yan, W.; Cheng, L.; Wang, W.; Wu, C.; Yang, X.; Du, X.; Ma, L.; Qi, S.; Wei, Y.; Lu, Z. Structure of the human gonadotropin-releasing hormone receptor GnRH1R reveals an unusual ligand binding mode. *Nat. Commun*. **2020**, 11, 5287.
26. Matias, P.M.; Donner, P.; Coelho, R.; Thomaz, M.; Peixoto, C.; Macedo, S.; Otto, N.; Joschko, S.; Scholz, P.; Wegg, A. Structural evidence for ligand specificity in the binding domain of the human androgen receptor: implications for pathogenic gene mutations. *J. Biol. Chem*. **2000**, 275, 26164-26171.
27. Heitman, L.H.; Oosterom, J.; Bonger, K.M.; Timmers, C.M.; Wiegerinck, P.H.; IJzerman, A.P. [3H] Org 43553, the first low-molecular-weight agonistic and allosteric radioligand for the human luteinizing hormone receptor. *Mol. Pharmacol*. **2008**, 73, 518-524.
28. Fan, Q.R.; Hendrickson, W.A. Structure of human follicle-stimulating hormone in complex with its receptor. *Nature* **2005**, 433, 269-277.
29. BIOVIA, D.S. Discovery Studio Visualizer, v21.1.0.20298. *Dassault Systèmes*. **2021**. Available at: https://discover.3ds.com/discovery-studio-visualizer-download
30. Trott, O.; Olson, A.J. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem*. **2010**, 31, 455-461.
31. Yisak, H.; Redi-Abshiro, M.; Chandravanshi, B.S.; Yaya, E.E. Total phenolics and antioxidant capacity of the white and brown teff [Eragrostic tef (Zuccagni) Trotter] varieties cultivated in different parts of Ethiopia. *Bull. Chem. Soc. Ethiop*. **2022**, 36, 749-766.
32. Lekmine, S.; Benslama, O.; Bensalah, B.; Touzout, N.; Moussa, H.; Tahraoui, H.; Ola, M.S.; Hafsa, H.; Zhang, J.; Amrane, A. Bioactive phenolics of *Hyoscyamus muticus* L. Subsp. Falezlez: A molecular and biochemical approach to antioxidant and urease inhibitory activities. *Int. J. Mol. Sci.* **2025**, 26, 370.
33. Lekmine, S.; Benslama, O.; Ola, M.S.; Touzout, N.; Moussa, H.; Tahraoui, H.; Hafsa, H.; Zhang, J.; Amrane, A. Preliminary data on Silybum marianum metabolites: Comprehensive characterization, antioxidant, antidiabetic, antimicrobial activities, LC-MS/MS profiling, and predicted ADMET analysis. *Metabolites* 2025, 15, 13.
34. Alara, O.R.; Abdurahman, N.H.; Ukaegbu, C.I. Extraction of phenolic compounds: A review. *CRFS* **2021**, 4, 200-214.
35. Ikan, R.; Ravid, U. The isolation and identification of ecdysterone from *Ajuga iva*. *Planta. Med*. **1971**, 20, 33-35.
36. Coll, J. New minor ecdysteroids from *Ajuga iva* (Labiatae) and complete ^{1}H-NMR assignment of cyasterone. *Afinidad. Quím. Teór. Apl*. **2007**, 64, 242-250.
37. Boukada, F.; Meddah, B. Flavonoids from aerial part of Algerian *Ajuga iva* (l.) schreb.: The HPLC-UV analysis and antioxidant capacity. *Kragujevac. J. Sci.* **2021**, 43, 23-34.
38. Boudjelal, A.; Siracusa, L.; Henchiri, C.; Sarri, M.; Abderrahim, B.; Baali, F.; Ruberto, G. Antidiabetic effects of aqueous infusions of artemisia herba-alba and *Ajuga iva* in alloxan-induced diabetic rats. *Planta. Med*. **2015**, 81, 696-704.
39. Aly, R.; Ravid, U.; Abu-Nassar, J.; Botnick, I.; Lebedev, G.; Gal, S.; Ziadna, H.; Achdari, G.; Smirov, E.; Meir, A.; Ghanim, M. Biological activity of natural phytoecdysteroids from Ajuga iva against the sweetpotato whitefly *Bemisia tabaci* and the persea mite *Oligonychus perseae*. *Pest. Manag. Sci*. **2011**, 67, 1493-1498.

40. Yang, L.; Allred, K.F.; Dykes, L.; Allred, C.D.; Awika, J.M. Enhanced action of apigenin and naringenin combination on estrogen receptor activation in non-malignant colonocytes: Implications on sorghum-derived phytoestrogens. *Food. Funct*. **2015**, 6, 749-755.
41. Alexeeff, G.V.; Broadwin, R.; Liaw, J.; Dawson, S.V. Characterization of the LOAEL-to-NOAEL uncertainty factor for mild adverse effects from acute inhalation exposures. *Regul. Toxicol. Pharmacol*. **2002**, 36, 96-105.
42. Rochester, J.R.; Millam, J.R. Phytoestrogens and avian reproduction: Exploring the evolution and function of phytoestrogens and possible role of plant compounds in the breeding ecology of wild birds. *Comp. Biochem. Physiol. A Mol. Integr. Physiol*. **2009**, 154, 279-288.
43. Lafont, R.; Harmatha, J.; Marion-Poll, F.; Dinan, L.; Wilson, I. The Ecdysone handbook, online. *Cybersales sa: Prague, Czech Republic*, 2002. Available at: http://ecdybase.org.
44. Mbemya, G.T.; Vieira, L.A.; Canafistula, F.G.; Pessoa, O.D.L.; Rodrigues, A.P.R. Reports on in vivo and in vitro contribution of medicinal plants to improve the female reproductive function. *Reprod. Climatér* **2017**, 32, 109-119.
45. Shoorei, H.; Khaki, A.; Ainehchi, N.; Taheri, M.M.H.; Tahmasebi, M.; Seyedghiasi, G.; Ghoreishi, Z.; Shokoohi, M.; Khaki, A.A.; Raza, S.H.A. Effects of *Matricaria chamomilla* extract on growth and maturation of isolated mouse ovarian follicles in a three-dimensional culture system. *Chin. Med. J*. **2018**, 131, 218-225.
46. Goka, C.; Telefo, P.; Mbemya, G.; Awouafack, M.; Lienou, L.; Yemele, D.; Njina, S.; Donfack, N.; Tagne, R.; Fekam, F. Potentialisation of pregnant mare serum gonadotropin inducing effect on ovarian follicles growth by the aqueous extract of *Aloe buettneri*, *Dicliptera verticillata*, *Hibiscus macranthus* and *Justicia insularis* leaves in immature rats. *Pharmacol.* **2016**, 7, 328-336.
47. Naji, T.; Hossenzadeh Sahafi, H.; Saffari, M. The effects of phytoestrogens Matricaria recutita on growth, maturation of oocytes in the three-spot gourami (*Trichogaster trichopterus*). *ISFJ* **2014**, 23 85-94.
48. Kumar, T.R.; Wang, Y.; Lu, N.; Matzuk, M.M. Follicle-stimulating hormone is required for ovarian follicle maturation but not male fertility. *Nat. Genet.* **1997**, 15, 201-204.
49. Li, D.; Sun, S.; Chen, J. Research progress on classification, source, application of phytosterol esters, and their thermal oxidation stability. *Grain Oil Sci. Technol*. **2024**, 7, 1-11.
50. Slighoua, M.; Amrati, F.E.Z.; Chebaibi, M.; Mahdi, I.; Al Kamaly, O.; El Ouahdani, K.; Drioiche, A.; Saleh, A.; Bousta, D. Quercetin and ferulic acid elicit estrogenic activities in vivo and in silico. *Molecules* **2023**, 28, 5112.