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PHYTOCHEMICAL ANALYSIS AND MOLECULAR MODELING APPROACH OF LEPIDIUM SATIVUM SEEDS ON FUNCTIONAL OVARIAN CYSTS

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ABSTRACT
Lepidium sativum Linn. Belonging to the Brassicaceae (Cruciferae) family. It is an annual herb, containing many pharmacologically active phytochemicals which contribute to the treatment of many diseases and symptoms. the main aim of the study is to re-evaluate the phytochemical components of the seed and to study the effect of its constituents on estrogen receptors. Molecular modeling was used to evaluate the pharmacological effects of the seed constituents on the functional ovarian cyst and to confirm the traditional folkloric use of *L- sativum* seeds as a plant to reduce the size or remove the functional ovarian cyst. Thin-layer chromatography was used to identify and separate the seed constituents using a suitable solvent system. In addition, Spectroscopic methods, UV-visible, fluorescence, and FTIR spectroscopic techniques were used to further evaluate the seed constituents. AutoDock and AutoDock Vina in PyRx virtual screening software were used to study the binding docking energies between many components of *Lepidium sativum* seeds with α and β estrogen receptors. The results of this study showed that *Lepidium sativum* seeds contain many phytochemical compounds, such as phytosterols, phenols, flavonoids, and alkaloids, that bind to human estrogen receptors (α1x7E and β1x7J) and reveal a significant pharmacological effect on functional ovarian cysts.

KEYWORDS: *Lepidium sativum*, Functional ovarian cysts, Molecular docking, Human estrogen receptors.

INTRODUCTION
According to the World Health Organization, today more than 80% of the world's population still relies on traditional drugs, mainly plants or herbs, for primary health care, because it is natural and cause fewer complications.[1]

The mustard family, Brassicaceae, is worldwide distributed. The family is rich in sulfurous compounds, some of which are used medicinally.[2] The family contains 372 genera and 4,060 species, distributed worldwide except Antarctica. The largest genus is *Lepidium* (234 species).[3] From this family, 59 genera and 100 species are present in Libya. *Lepidium sativum* is an annual, fast-growing, erect herb, growing in height up to 15-45 cm, it has white to pinkish small flowers found in racemes. *L.sativum* seeds are brownish red in color, oblong, slightly curved and angular on one side, oval in shape with a rugous surface.[4,5]

Lepidium sativum Common names include: English: cress, common cress, garden cress (GC), land cress, pepper cress; Spanish: lepidio.[2] India: halon, chansur, chandasura.[6] Several regions of Arab countries: "hab el rashaad, Rashad, or thufa".[7] German: gartenkresse; Swedish: smörgåskrasse; Italian: mastruço, agretto.[8]

Ovarian cysts are a very common problem in reproductive-age women. Most ovarian cysts are benign, not cancerous (80-85%), especially functional (physiological) ovarian cysts, which are most common and occur without symptoms.[9]

The major secondary compounds from the *L-sativum* plant are gluconapin, glucosinolates, gluconasturtin, and glucotropaeolin.[10] The plant also contains a significant quantity of calcium, folic acid, iron, and vitamins A and C.[11] Riboflavin, mono-unsaturated fatty acids, arabinose, and cardenolides.[12] The main constituents in seeds are alkaloids, the garden cress (GC) seeds contain seven imidazole alkaloids, lepidine B, C, D, E and F (dimeric) and two new semilepidinoside A and B (monomeric), Glucosinolates, flavonoids as quercetin and kaempferol, sterols, cardiotonic glycosides, triterpene, and tannins are important phytochemical components in the garden cress seed.[10] Phenolic compounds like

protocatechuic acid, gallic acid, caffeic acid, coumaric acid, quinic acid, and ferulic acid are identified in *L-sativum* seeds.[6]

When *Lepidium sativum* seed powder was given to female rabbits in the diet, blood samples were collected to evaluate the level of Luteinizing hormone (LH) and conception rate. The results showed increases in plasma LH hormone level and conception rate, which were referred to as the phytoestrogen component in the seed by permanent or temporary changes of the feedback loop in the hypothalamus through mimicking the endogenous estrogen effects.[13]

L-sativum seeds were largely used for the treatment of many diseases and symptoms, but to our knowledge, no previous studies have been carried out to evaluate the effect of *L- sativum* seeds on functional ovarian cysts. To explain the traditional folkloric use of *L- sativum* seeds in minimizing functional ovarian cysts and relieving their symptoms**.** This study dealt with the analysis of phytochemical components of *L-sativum* seeds by qualitative and quantitative screening, characterized the bioactive constituents present in the seeds by using spectroscopic techniques, and lastly, a molecular docking approach was used to detect any potential estrogen mimics or anti-estrogens in phytochemicals found in *L-sativum* seeds.

MATERIALS AND METHODS

Plant materials*:* *Lepidium sativum* seeds were purchased from Ibn-Sina for herbs and perfumery (located in Tripoli- Libya), the plant seeds were identified, authenticated, and deposited in the Faculty of Sciences Herbarium under voucher number (6823271), by Dr. Mohammad Abuhadra, a plant taxonomist, Department of Botany, Faculty of Sciences, University of Tripoli, Libya in the year (10-2019).

Extraction: The dried seeds were ground by an electrical grinder to obtain a fine powder. the extraction was done by Serial exhaustive extraction (hexane, ethyl acetate, and methanol). Powdered materials were macerated in solvents in a stoppered container for a defined period. The plant powder was continuously shaken, and after that, the extracts were filtered using Whatman 1 filter paper, the filtrates were concentrated using a rotary evaporator, and the concentrates were used for phytochemical and spectroscopic analysis.

Phytochemical screening: The preliminary phytochemical screening of hexane, ethyl acetate, and methanol extracts of *L-sativum* seeds was conducted using standard qualitative identification methods. Quantitative analysis was done for the determination of alkaloids (by using alkaline precipitation gravimetric technique), flavonoids (by Aluminum chloride colorimetric), tannins (by titrimetric indigosulphonic acid assay), coumarins (by spectrophotometer), and phenols (using Folin-Ciocalteau method).

Separation techniques*:* By thin layer chromatography (TLC) using alumina silica gel, for detecting the presence or absence of specific compounds in the *L- sativum* methanol extract by using suitable solvents, then visualize the spots under UV lamp at 254 nm and 366 nm, and spray with Dragendorff's reagent. Preparative silica gel thin layer chromatography (TLC), silica gel 60 GF 254 was used. Visualize the TLC using the UV lamp (254 and 365 nm).[14] Then scratch out the interest band from the other separated bands.

Spectroscopic techniques

Absorbance spectra: absorbance spectra were measured using a SPECORD® 200 plus UV-visible spectrophotometer, using quartz cells of 1 cm path length, Tris buffer (0.01M Tris, 0.1 M sodium chloride (NaCl) at PH 7.4), water (distilled deionized), the spectra of UV-visible absorbance were recorded in the 200-800 nm range.

Fluorescence spectra: fluorescence excitation and emission spectra were measured using a Jasco FP-6200 spectrofluorometer. The cuvette used in this method was a 4-sided quartz fluorescence of 1 cm path length. Tris's buffer (0.01M Tris, 0.1 M NaCl at PH 7.4), water (distilled deionized) was used, and the spectra of fluorescence emission were recorded in the 285-550 nm range

Fourier transform infrared (FTIR) spectra: FTIR spectra were recorded on a Cary 630 FTIR spectrometer, over the IR absorption spectral range 400-4000 cm^{-1}.

Molecular modeling study: AutoDock and AutoDock Vina in PyRx virtual screening software -Python prescription version 0.8 (2008-2010), were used to set up and perform docking calculations between various constituents of *L.sativum* with α and β human estrogen receptor. The crystal structures of human estrogen receptor in a complex with a transition-state analogue were downloaded from the Protein Data Bank of α estrogen (https: //www. rcsb.org/structure/1X7E,1X7R), and β estrogen (https: //www. rcsb.org/structure/1U9E,1U3S,1X7J,1X7B). which were used for the docking studies with the *L.sativum* constituents. 17β-estradiol, the known estrogenic compound was used as positive controls to compare molecular docking energies with the herbal phytochemicals of *L-sativum seeds*, The structure of the protein was prepared using BIOVIA Discovery Studio Visualizer (version 4.5, 2021), *L.sativum* constituent's chemical structures were drawn and optimized using ChemDraw Ultra (version 8.0, Cambridgesoft Com., USA). The Discovery Studio Visualizer (2021) was used for analyzing the possible type of binding and interaction between the ligands and receptors.[15,16]

RESULTS AND DISCUSSIONS

Phytochemical screening

The preliminary phytochemical tests were done, and

these tests revealed that alkaloids are present in the methanol extract of the seeds, carbohydrates are found in methanol extract more than in ethyl acetate extract and it is not found in hexane extract, glycosides, saponins, flavonoids, protein, amino acids, phenols, tannins, and resin are present in methanol extract only, the phytosterols were found in hexane extract more than ethyl acetate and methanol extract, coumarins are found in all extracts. There is a correlation between these results and the previous work that was done in December 2011.[4] In this study the extraction yield for cold maceration of ground dry seeds powder of the plant hexane extract: % yield = 17.23% (highest yield), ethyl acetate extraction**:** % yield= 10.57% (lowest yield), and for methanol extraction: % yield = 15.90%, which is different from the previous work done by. Chatoui,2016[17] Whereas the methanol extract was the highest percentage yield. Quantitative estimation indicates that *L. sativum* seeds have total flavonoids of 10.812 mg/g, the total alkaloids 7.633%, the % total tannins 6.842%, and a higher quantity of total phenols content 317.64 mg/g. These higher phenol content results agree with those expressed by. El-salam, 2019 [18] But with different values. Other studies indicate the plant seeds contain a higher percentage of flavonoids and minimal alkaloids and total phenols percentage.[4]

TLC profiling: In the current study, methanol extract for *L-sativum* seeds was spotted on alumina TLC and runed by mobile phase then sprayed by Dragendorff's reagent, an orange color was detected, with retardation factor (Rf) value equal to 0.56, thus, the suspected compounds may be alkaloids, but it needs further investigations by other analytical techniques like NMR estimation.

For Preparative silica gel TLC: The method was performed on 10*20 cm glass TLC plates coated with silica GF 254, as shown in Figure 1, then after running the solvent system, the TLC bands were visualized using the UV lamp.

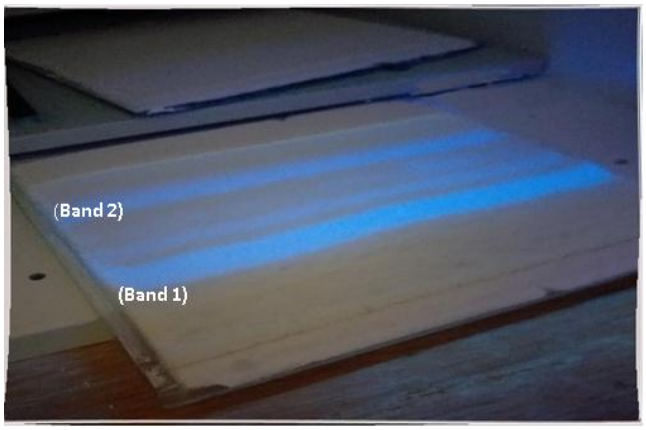

Figure 1: Bands of TLC silica gel plate under UV lamp (366 nm).

Spectroscopic techniques: The spectra of UV-visible spectroscopy showed characteristic peaks for proteins in both hexane and ethyl acetate extracts, whereas the methanol and water extracts spectra illustrate the alkaloids, flavonoids, and phenols compounds.[19] The fluorescence spectroscopy results of four different extracts (hexane, ethyl acetate, methanol, and water) of *Lepidium sativum* seeds showed that each extract has a different fluorescence emission wavelength, as shown in Figure 2. These results indicate that different solvents have different effects on the fluorescence properties of the *Lepidium sativum* seed extracts.

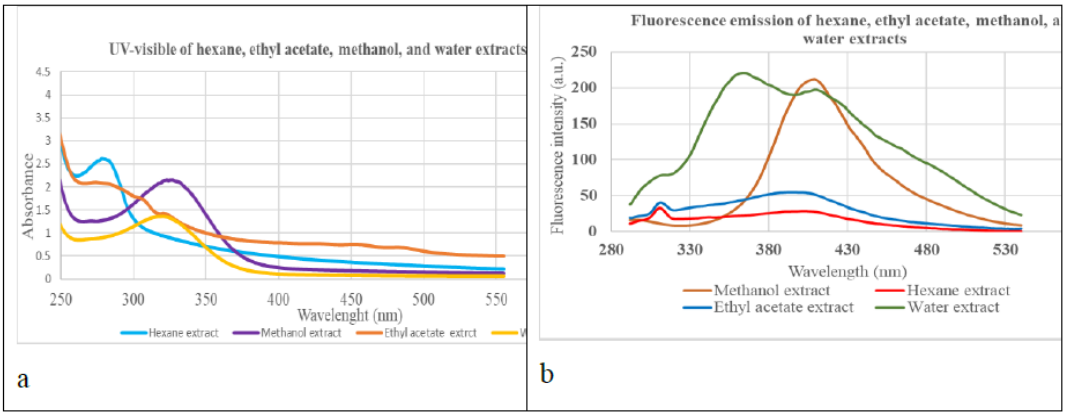

Figure 2: a- Ultraviolet-visible absorption spectra of hexane, ethyl acetate, methanol, and water extracts of *L-sativum* seeds vs. wavelength from 250-550 nm. b- Plot of fluorescence emission of hexane, ethyl acetate, methanol and water extracts of *L. sativum* seeds vs. wavelength from 292-540 nm using excitation of λ266 nm.

The difference in FTIR spectra between the samples obtained from preparative TLC and raw *L-sativum* seeds suggests that the samples have different chemical compositions and phytochemical compounds (Figure 3). In summary, the FTIR spectra of *Lepidium sativum* seeds suggest the presence of various functional groups such as hydroxyl, nitrile, aromatic ring, conjugated double bond, carbonyl group, amine group, alcoholic group, ester group, and alkane.

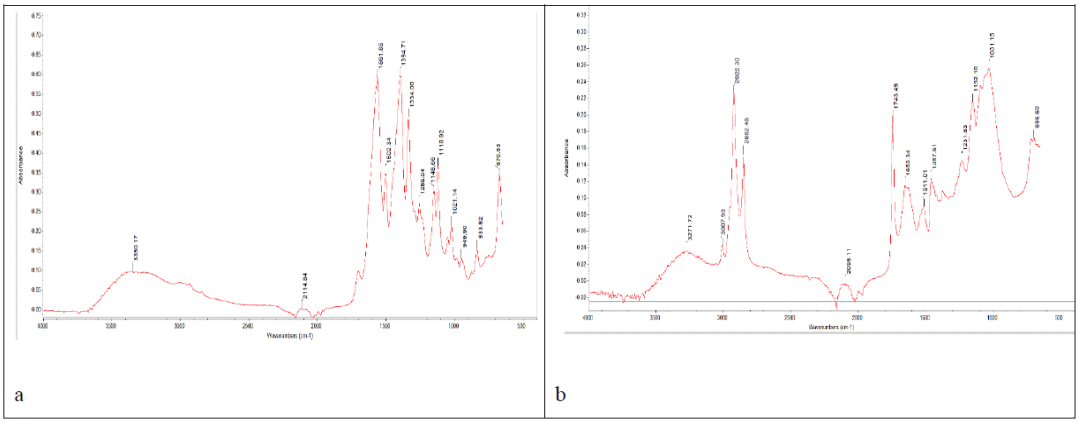

Figure 3: FTIR spectra of *Lepidium sativum* seed by preparative TLC (band 1), b- FTIR spectra of raw *Lepidium sativum* seed powder. The spectra were drawn by Omnic 9 software.

Molecular modeling

Molecular docking energies of ligands with α and β estrogen receptors were presented in the table 1,2.

Table 1: Docking results of ligands with α estrogen receptors.

Compounds	1x7E	1x7R
	Binding energy (kcal/mol)	Binding energy (kcal/mol)
17β- Estradiol	-8.83	3.24
Genisten (5,7-dihydroxy-3-(4- hydroxyphenyl) chromen-4-one)	---------	-0.06
Way44 [5-hydroxy-2-(4-hydroxyphenyl)- 1-benzofuran-7-yl] acetonitrile.	-7.83	---------
Sitosterol	-0.06	109.98
Campesterol	-7.58	68.0
Avenasterol	-6.99	68.97
Cholesterol	-6.6	62.95
Stigmasterol	1.91	109.47
24,25-Dihydrolansterol	-1.33	101.86
β -Amyrin	15.07	180.69
Semilepidinoside A	-6.75	0.07
Semilepidinoside B	-4.91	13.69
Lepidine B	-2.87	16.67
Lepidine C	-3.7	2.65
Lepidine D	-7.4	3.28
Lepidine F	-6.29	19.48
Gallic Acid	-4.41	-4.41
P-Coumaric Acid	-4.67	-4.99
Ferulic Acid	-5.31	-5.15
Sinapic Acid	-4.42	-4.8
Glutamic Acid	-4.2	-3.37
Quinic Acid	-5.71	-4.33
5,3dihydroxy-7,8,4-Trimethoxy Flavonone	-3.92	0.63
Linolinic Acid	-4.99	-3.55
Caffeic Acid	-5.07	-4.96
Protocatechuic Acid	-4.67	-4.99
Quercetin	-7.19	2.51
Kaempferol	-7.02	2.0

Table 2: Docking results of ligands with β estrogen receptors.

Compounds	1U3S	1U9E	1x7J	1x7B
17β- Estradiol	-6.9	-7.3	-7.55	-7.22
Genisten (5,7-dihydroxy-3-(4- hydroxyphenyl) chromen-4- one)	---------	---------	-6.02	---------
3-(6-hydroxy-naphthalen-2- yl)-benzo[d]isooxazol-6-ol	-4.91	---------	---------	---------
4-hydroxy-phenyl) benzofuran-5-ol	---------	-6.11	---------	---------
2-(3-fluoro-4-hydroxyphenyl)- 7-vinyl-1,3-benzoxazol-5-ol	---------	---------	---------	-5.92
Sitosterol	54.94	54.94	14.6	3.26
Campesterol	27.05	2.9	-4.65	13.22
Avenasterol	45.94	19.02	5.89	25.89
Cholesterol	9.83	9.14	1.5	48.57
Stigmasterol	54.94	1.58	-5.08	9.97
24,25-Dihydrolansterol	81.06	59.27	51.08	74.52
β -Amyrin	205.35	118.62	84.72	184.82
Semilepidinoside A	-3.5	-3.88	-3.54	-2.16
Semilepidinoside B	10.89	-0.65	-4.91	-0.17
Lepidine B	8.49	0.61	3.08	7.24
Lepidine C	1.74	3.14	1.63	4.38
Lepidine D	0.25	1.35	2.34	8.49
Lepidine F	-0.2	-4.49	-7.16	-5.16
Gallic Acid	-4.19	-4.45	-3.81	-4.03
P-Coumaric Acid	-4.97	-5.11	-4.88	-5.06
Ferulic Acid	-5.23	-5.27	-5.03	-5.22
Sinapic Acid	-4.83	-5.14	-4.9	-5.19
Glutamic Acid	-3.76	-4.81	-3.39	-4.71
Quinic Acid	-5.16	-4.65	-4.61	-5.25
5,3dihydroxy-7,8,4- Trimethoxy Flavonone	-3.49	-3.95	-4.2	-3.36
Linolinic Acid	-2.68	-5.06	-2.61	-2.72
Caffeic Acid	-5.05	-5.38	-5.11	-5.23
Protocatechuic Acid	-5.22	-5.53	-4.93	-4.9
Quercetin	-4.11	-4.51	-4.4	-3.86
Kaemferol	-4.37	-5.04	-4.67	-4.75

The docking results in this study as shown in table 1,2, revealed eight strongly docking compounds binding to human estrogen receptor α1x7E (campesterol, avenasterol, cholesterol, lepidine D, lepidine F, Semilepidinoside A, quercetin and kaempferol), in which campesterol gave the highest negative binding energy (-7.58 kcal/mol) followed by lepidine D (-7.4 kcal/mol) compared to standard estradiol (-8.83 kcal/mol), they are strongly docking with α1x7E estrogen receptor and their docking binding energies closely near to the co-crystalline ligand Way 44. Regarding the second investigated estrogen receptor β1x7J, there were two strongly docking compounds binding to human estrogen receptor β1x7J (lepidine F, and caffeic acid), in which lepidine F gave high negative binding energy (-7.16 kcal/mol) compared to standard estradiol (-7.55 kcal/mol) followed by caffeic acid (-5.11 kcal/mol), these results are nearly equal to the co-crystalline ligand genistein (-6.02 kcal/mol) as shown in figure 4. Other *L-sativum* phytochemicals, which gave negative binding energies with the active sites of α1x7E and β1x7J estrogen receptors, showed lower binding energies (weak docking); other types of estrogen receptors (1x7R, 1x7B,1U3S, 1U9E) showed a weak docking result. The molecular modeling studies showed that there is hydrogen bonding and Vander Waals interactions between all tested ligands of *L.sativum* seeds with α1x7E and β1x7J estrogen receptors, The combination of these two types of interactions is likely to be important in determining the overall strength and specificity of the binding between the compounds and the α1x7E and β1x7J estrogen receptors.

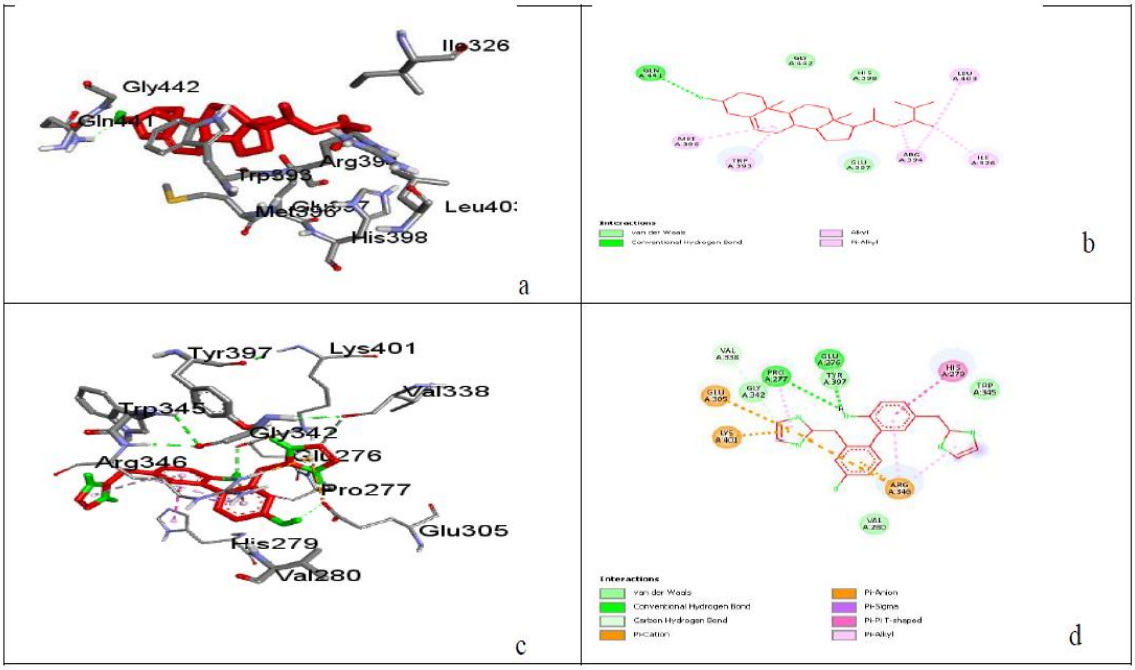

Figure 4: a- The interaction model of campesterol (red color) at the 1x7E α estrogen receptor active site. b- 2D diagram interaction model of campesterol at the 1x7E α estrogen receptor active. c- The interaction model of lepidine F (red color) at the 1x7J β estrogen receptor active site. d- 2D diagram interaction model of lepidine F at the 1x7J β estrogen receptor active site.

There is a known correlation between estrogen signaling and functional ovarian cysts. Estrogen stimulates the growth and development of ovarian follicles, which can lead to the formation of functional cysts. Modulators of estrogen signaling, such as the compounds identified in this molecular modeling study, may be potential therapeutic agents for functional ovarian cysts. Therefore, they can modulate some women's reproductive disorders like menstrual disorders, pain, and bloating. The docking study has only observed docking of natural ligands and does not show in vivo hydrolysis.

CONCLUSION

The results of the current study showed that *Lepidium sativum* seeds have an important content of phytochemicals, phenolic, flavonoids, alkaloids, tannins, saponins, minerals, and other components, which play roles in the treatment of many diseases. Thin layer chromatography is commonly done for the identification of the bioactive components and the separation of compounds from a mixture. in the present study, TLC profiling further confirmed the presence of some active secondary metabolites of *Lepidium sativum* seeds, but further identification is needed to confirm this. Comparing the RF value of separated spots with that of the standard is a very important step.

phytochemical analysis, UV-visible spectroscopy, fluorescence spectroscopy, and FTIR spectroscopy, are all techniques used to study the molecular properties and composition of the plant, each of these techniques provides different information about the sample, and they can be used in combination to provide a more comprehensive understanding of the plant's properties and composition. Based on the molecular docking results of this research, it can be concluded that *Lepidium sativum* seeds contain phytochemical components that bind to human estrogen receptors (α1x7E and β1x7J), and reveal estrogen modulation, which aids as a useful tool in relieving functional ovarian cysts. These were related to phytoestrogen content and other classes of phytochemical compounds in the seeds.

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