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Isolation And Identification Of Chemical Constituents From Herbal Syrup In Treating Fibroid

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Abstract
Purpose:
Fibroid is one of the reasons for infertility as well as heavy bleeding in women. The main source of fibroid growth is estrogen. Down-regulation of oestrogen helps to control fibroid growth. Various conventional medicines are prescribed to treat this fibroid. The present study aims to identify components from the syrup by combining two or three ingredients mixed together and analysing the components for their therapeutic activity in treating fibroid both *in vitro* and *insilico.* The syrup we prepared from *Ficus carica* bark powder, *Prosopis juliflora* bark powder, and *Eujenia jambolana* bark powder samples was equally mixed with methanol

Methods and Material: The following studies were performed, which are as follows: DPPH Assay, FTIR, GC-MS, and a computational docking study were used to Antioxidant activity studied using DPPH assay since free radical elevate the level of oesterogen, identify the components, and also investigate the efficacy of the components present in the syrup in controlling oestrogen regulation respectively.

Results: DPPH assay showed the herbal extract's maximum antioxidant activity at a concentration of 80 g/ml. The existence of O-H, C-C, C=O, NH3+ in amino acids, OH in carboxylic acids, C-N, C-OH in amino acids, C-O, and CH3 functional groups was validate by FT-IR analysis. Dodecanoic acid, Undecane, 1-Tetradecanol, Tetradecanoic acid, (+)-Cycloisolongifol-5-ol, Tridecanoic acid etc, compounds were identified using GC-MS analysis.
Conclusions: Based on the docking scores and hydrogen bond interactions, identified compounds bound well to the selected target proteins(aromatase enzyme, acetylcholine esterase, Beta Catenin, and progesterone receptor) which help to control oestrogen regulation

Key-words: Fibroid, FTIR, GC-MS, Docking, Protein-ligand.

INTRODUCTION

Menopause is the stage where a woman cannot produce fertilizable eggs. This stage is common for all women all over the world who are crossing the age of 45 plus[1]. During this time interval, heavy bleeding was observed in most of the cases Fibroid is one of the reasons for heavy bleeding during the menopause stage. Fibroids or polyps are benign growths within the uterine wall that resemble tumours but are not cancerous. Fibroids are

generally not dangerous; however, they can lead to discomfort or might result in difficulty such as a decrease in red blood cells (anaemia), which causes tiredness, as a result of severe blood loss[2]. Rarely, a transfusion is needed due to blood loss. Estrogen is one of the reasons for developing fibroids. Various contraceptive pills and vitamin tablets are prescribed for treating fibroids. There are various herbs studied to control the growth of fibroids[3,4]. However, there were no studies performed for fibroidal activity with the chosen herbs (*Ficus carica* bark powder, *Prosopis juliflora* bark powder, and *Eujenia jambolana* bark powder). Based on various pathways (MAPK, ROS, etc.,) that influence oestrogen regulation, four proteins (aromatase enzyme, acetylcholine esterase, Beta Catenin, and progesterone receptor) were selected to perform a computational docking study to determine whether the prepared extract components have the ability to control oestrogen regulation[5].

Ficus carica bark powder[6], *Prosopis juliflora* bark powder[7], and *Eujenia jambolana* bark powder[8] were the selected herbs to perform this study. Various medicinal values are associated with the selected herbs; there was no specific study found to relate to anti-fibroid activity.

Target proteins which are as follows aromatase enzyme, acetylcholine esterase, Beta Catenin, and progesterone receptor selected to perform computational investigation. To study whether the prepared extract components has the ability to inhibit the selected proteins.

Aromatase enzyme is a key enzyme which synthesis estrogen in our body. Aromatase enzyme converts androgens into estrogens[9]. Various commercially using aromatase inhibitors employed to control the estrogen production thereby help to shrink the fibroid[10,11].

Acetylcholine esterase is an enzyme that aids in the conversion of acetylcholine to choline. Choline is the stress hormone; stress is one of the factors in fibroid growth, and choline also stimulates oestrogen regulation. There are various inhibitors used to inhibit acetylcholine in controlling various disease states[12,13].β Catenin is the main controller in Wnt signaling pathway. Beta catenin is the key regulator of estrogen, β catenin reduction found in the reduction of ERα expression it was a novel pathway found in the breast cancer patients[14,15].

Progesterone Receptor is the one which produce progesterone, some clinical studies shows that the progesterone does decrease the quantity of estrogen by interfering with the replenishment of R_{α} and that this decrease results in a reduced sensitivity of uterine tissue to estrogen[16,17].

In this present study aims to find out the possible pathways to control the estrogen regulation with the prepared herbal extracts using computational methods as well as to study the antioxidant activity using *invitro* analysis since the ROS, stress hormones influencing the growth of fibroid. That the prepared extracts were significantly produced positive results.

Subjects and Methods:

Materials and Methods

Sample Preparation

Samples (*Prosopis juliflora* bark, *Ficus carica* bark & *Prosopis juliflora* bark) were collected and dried using hot air oven to remove the excess moisture. Equal amount (25g) of each sample mixed together with 500ml of methanol. Syrup prepared using soxhlet apparatus and the resultant extract dried to get the powder using hot air oven.

Antioxidant Activity

DPPH Activity

The ability of the extracts to rummage free radicals has been tested by DPPH radical scavenging test. Plant solvents' ability to give hydrogen atoms was determined by neutralising a methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). In the presence of antioxidants, DPPH tends to produce a purple/violet colour in methanol solution before fading to yellow shades. 0.1 mM DPPH solution in methanol has been prepared, and 2.4 mL of the sample was blended with 1.6 mL of methanol take out at varying doses (20-100g/mL). The sample solution was mixed thoroughly and stored in the dark at room temperature for 30 minutes. A spectrophotometer was used to measure the absorbance of the mixture at 517 nm. Ascorbic acid served as a benchmark. The percentage DPPH radical scavenging activity was calculated using the equation below: in which A0 would be the absorbance of the control and A1 would be the absorbance of the extractives/standard. The chart was then used to calculate the IC50 by plotting the inhibition percentage against concentration.[18]

Identification of Compounds

FTIR Analysis

FTIR analysis performed to analyze the bonds presence in the prepared extract. c. Every plant specimen's finely ground sample were loaded into an FTIR Spectroscope 400 to 4000 cm^{-1} range and $4cm^{-1}$ resolution.

GC-MS

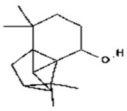
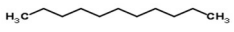
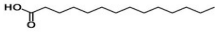
In this study shimadzu GCMS QP 2020 were used, in this fused silica column loaded by SH-Rxi-5Sil MS (0.25 mm 30 m df ID 250m) then detached the components by As a carrier gas, helium with a continuous flow rate of 1 ml/min. During the chromatographic run, the temperature of the injector were maintained by 280°C. The oven temperature was as follows for the 1L of extract specimen injected into the instrument: 40 degrees Celsius for 120 seconds, accompanied by 280 °C at a rate of 10 °C min1 and 280 degrees Celsius for 180 seconds. The mass detector settings was as follows: transfer line temperature 240 °C, ion source temperature 240 °C, ionisation mode electron impact at 70 eV, scan time 0.2 sec, also scan interval 0.1 sec. 40 to 550 Da was the fragments span by size . The component spectrums have been matched to an index of identified component spectrums saved in the GC-MS NIST (2017) archive[19].

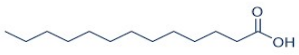
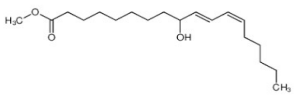
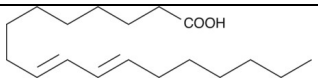
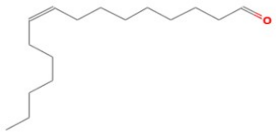
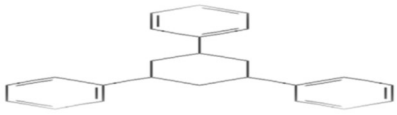
Computational Docking Study

Ligand Selection

The structures of the ligands be downloaded from the Pubchem server based on the GC-MS results (Tabel1).

Table 1: Ligand Name and their Structures

S.No	Ligand Name	Structure
1	Dodecanoic acid	
2	(+)-Cycloisolongifol-5-ol	
3	Undecane	
4	1-Tetradecanol	
5	Tetradecanoic acid	

6	Tridecanoic acid	
7	Methyl 10-trans,12-cis-octadecadienoate	
8	9(E),11(E)-Conjugated linoleic acid	
9	cis-9-Hexadecenal	
10	Cyclohexane, 1,3,5-triphenyl-	

***Insilico* ADME/TOX Property**

The webserver was used to evaluate the drug-likeness of identified ligand molecules http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp[20].

Target proteins Preparation

Aromatase inhibitor[21] (Fig.1a) (3EQM), Acetylcholine esterase[22] (Fig.1b) (4EY7), Beta Catenin[23] (Fig.1c) (1JDH), Progestrone receptor[24] (Fig.1d) (1A28). Identified target proteins have been downloaded from the website www.rcsb.org.

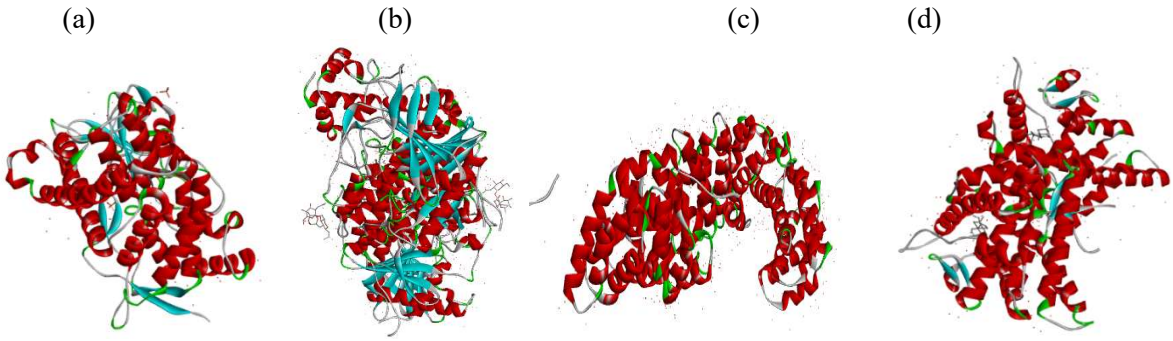


Figure 1 (a) PDB ID 3EQM Crystal structure of human placental aromatase cytochrome P450 in complex with androstenedione (b) PDBID 4EY7Crystal Structure of Recombinant Human Acetylcholinesterase in Complex with Donepezil (c) PDBID 1JDH Crystal Structure of Beta-Catenin and Htcf-4 (d) PDBID 1A28 Hormone-Bound Human Progesterone Receptor Ligand-Binding Domain.

Molecular Docking

The structure of protein were download and make ready by autodock, which added polar hydrogen and Kollman charges to the structure also changed it to.pdbqt arrangement. The ligand were created by identifying tiltable bonds as well as establishing the aromatic criterion, and the ligand structure of ligand was modified to. pdbqt. Additional docking research will necessitate the creation of a Grid parameter file. Grid created through adjusting size of the grid. The grid size aids the ligand in locating its binding pocket. Docking parameter files must be ready for docking analyses through first selecting a macromolecule, then a ligand file, lastly a docking parameter file written with the Lamrickan method.

Results:

Antioxidant Activity

Table 2a Absorbance of prepared extract at different Concentrations

S.No	Sample	Concentraion	Wavelength (nm)	Absorbance	% Scavenging
1	S1	20 µg/ml	512	0.381	33.85
2	S2	40 µg/ml	512	0.301	47.74
3	S3	80 µg/ml	512	0.202	64.93
4	Control		512	0.576	69.44

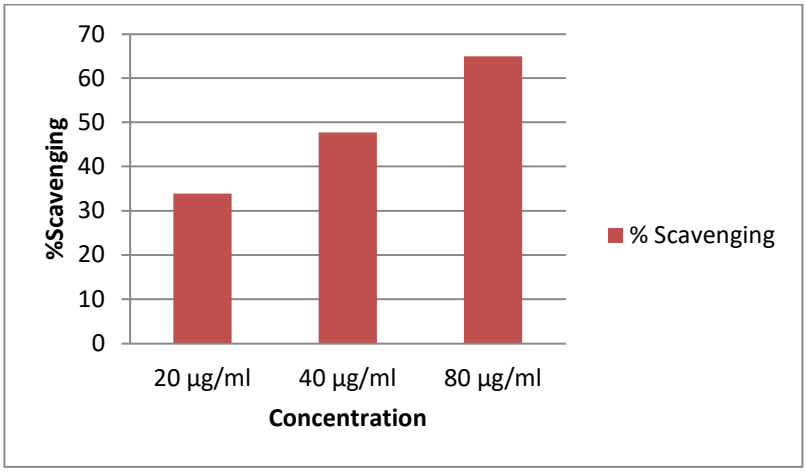


Figure 2 DPPH scavenging activity and IC_{50} value of the prepared herbal syrup.

FTIR Analysis

Table 3 FTIR Peak value of methanolic extract of herbal syrup

S.No	Peak Value	Functional group
1	3445.26	O-H
2	2079.05	**C≡C**
3	1651.25	(***C***=O)
4	1496.93	NH_3^+ in amino acids

5	1439.29	OH in carboxylic acids
6	1108.53	C-N
7	665.27	C-OH

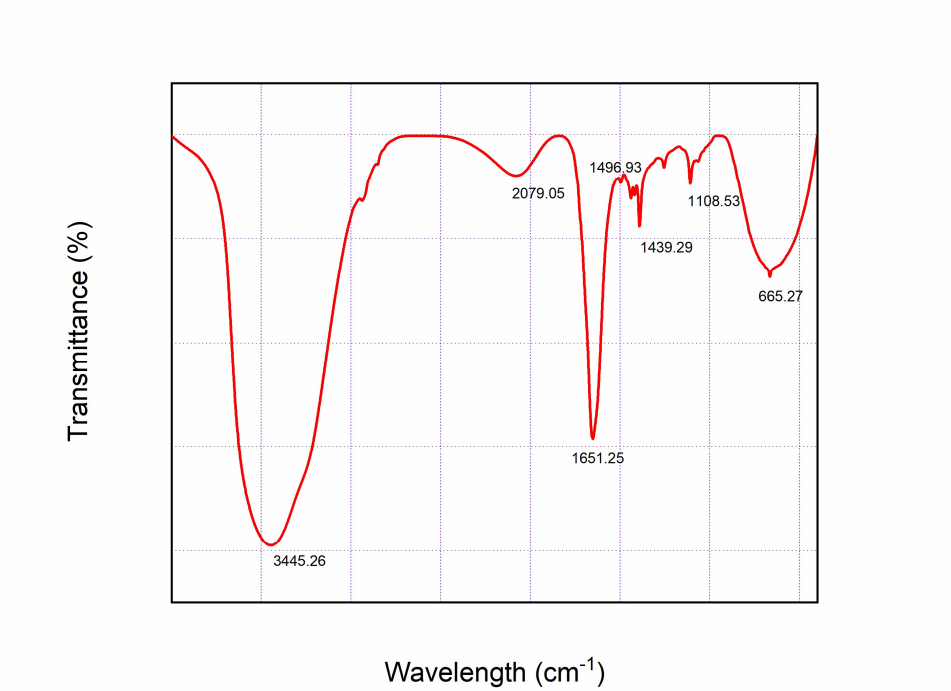


Figure 3 FTIR analysis of prepared herbal syrup extract.

GC-MS Analysis

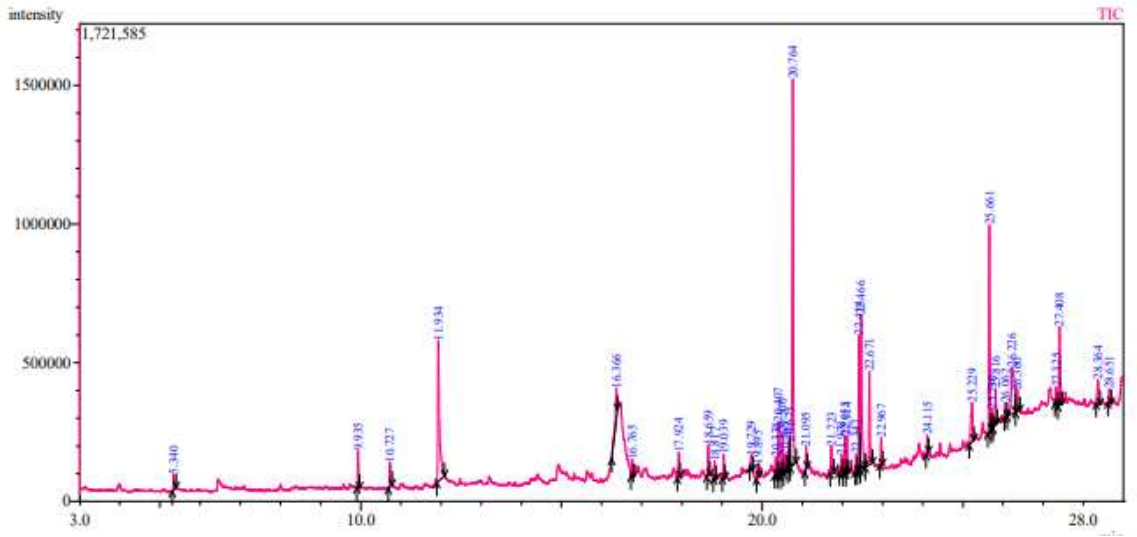
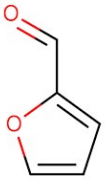
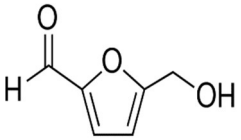
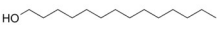
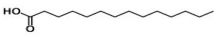
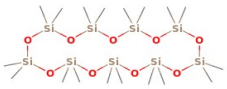
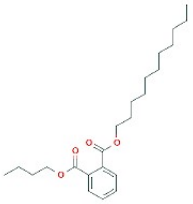
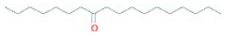
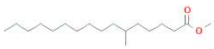
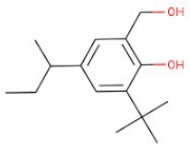
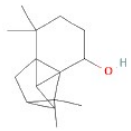
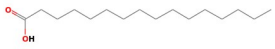
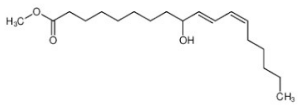
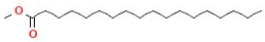
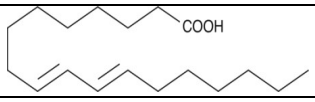


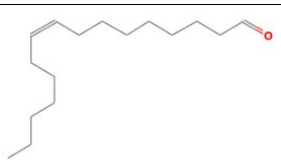
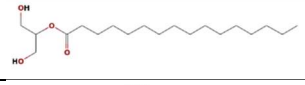
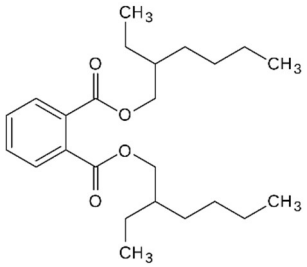
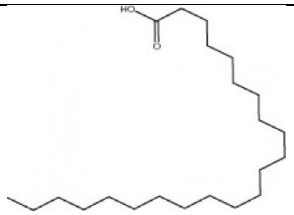
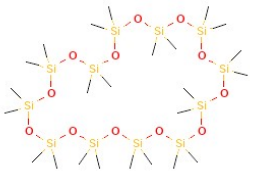
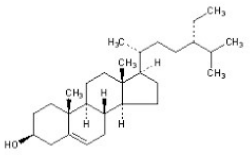
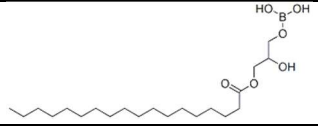
Figure 4 GC-MS analysis of methanolic extract of herbal syrup

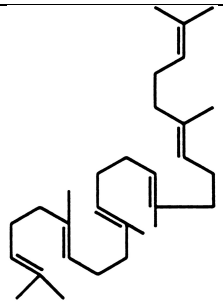
Table 4- Identified compounds with its Structure

S. No	Name	Structure
1	Dodecanoic acid	
2	Undecane	
3	2-Furancarboxaldehyde	
4	5-Hydroxymethylfurfural	
5	1-Tetradecanol	
6	Tetradecanoic acid	

7	Cyclononasiloxane, octadecamethyl-	
8	1-Hexadecanol	
9	Phthalic acid, butyl undecyl ester	
10	8-Octadecanone	
11	Hexadecanoic acid, methyl ester	
12	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hyd	
13	Oxirane, hexadecyl-	

14	(+)-Cycloisolongifol-5-ol	
15	N-hexadecanoic acid	
16	1-Octadecene	
17	Tridecanoic acid	
16	Methyl 10-trans,12-cis-octadecadienoate	
17	6-Octadecenoic acid, methyl ester, (Z)-	
18	Methyl stearate	
19	9(E),11(E)-Conjugated linoleic acid	

20	Cis-9-Hexadecenal	
21	Octadecanoic acid	
22	Hexadecanoic acid 2-hydroxy-1-(hydroxymethyl)ethyl ester	
23	Bis(2-ethylhexyl) phthalate	
24	Docosanoic acid	
25	Cyclododecasiloxane, tetracosamethyl-	
26	Gamma-sitosterol	
27	Octadecanoic acid, 2,3-dihydroxypropyl ester	

28	Squalene	

Computational Docking Study
***Insilico* ADME/TOX Property**

Table 5 ADME/TOX results

S. No	Chemical compound	Mass <500	Hydrogen bond donor <5	Hydrogen bond acceptor <10	LOGP <5	Molar refractivity 40-130
1	Dodecanoic acid	312.000000	5	6	-0.053101	77.145782
2	Undecane	156.000000	0	0	3.846189	61.040981
3	1-Tetradecanol	214.000000	0	0	4.909350	80.354980
4	Tetradecanoic acid	228.000000	0	0	4.744639	81.483978
5	(+)-Cycloisolongifol-5-ol	220.000000	0	1	3.310009	79.201782
6	Tridecanoic acid	214.000000	0	0	4.417349	76.126984
7	Methyl 10-trans,12-cis-octadecadienoate	294.000000	0	1	-0.688780	77.233002
8	9(E),11(E)-Conjugated linoleic acid	280.000000	0	0	4.696539	106.514961
9	cis-9-Hexadecenal	238.000000	0	1	4.270449	85.677979
10	Octadecanoic acid	284.000000	1	1	3.502719	96.480972
11	Cyclohexane, 1,3,5-triphenyl-	312.000000	0	0	4.039829	103.415962

PROTEIN-LIGAND INTERACTIONS

Discussion:

Antioxidant Assay

DPPH assay was performed at different concentrations to study the ability of the prepared extract antioxidant activity based on the decolorizing the DPPH solution (Table 2). Figure 2 displays the herbal extract's maximum antioxidant activity at a concentration of 80 g/ml.

Identification of Chemical Components

FTIR Analysis

Depending on the peaks values in the IR radiation region, the FT-IR spectrum were also applied to pick out the functional groups of the active components present in a sample. Functional groups of the components have been isolated using the FT-IR after the extract was passed through it. The existence of O-H, C-C, C=O, NH3+ in amino acids, OH in carboxylic acids, C-N, C-OH in amino acids, C-O, and CH3 functional groups was validate by FT-IR analysis (Fig.3 and Table 3). FTIR spectroscopy has been demonstrated as dependable as well as delicate way aimed at detecting composition of bio molecular.

GC-MS

Prepared extract analyzed using GC-MS (Fig 4). Identified components were listed in the Table 4.

Computational Docking Study

***Insilico* ADME/TOX Property**

The drug-resembelence of chosen ligands were investigated in order to assure oral bioavailability inside the human system. From findings, every single ligands followed the rule of five. The outcomes demonstrated that the identified ligands shows the desired possessions for the human system (Table 5).

Protein – Ligand Interactions

All the ligand screened ligand compounds (Table1) were docked against certain target proteins (Figure1) to control the regulation of estrogen. All the ligands were bound well with the target proteins. Top ligand scored complex were listed (Table 6) for all three target proteins and their top ligand docked scores and interactions[25,27] (Fig.5).

Conclusion

Samples (Prosopis juliflora bark, Ficus carica bark & Prosopis juliflora bark) were collected and mixed together and extract prepared, analysed for its antioxidant activity and the herbal extract showed better antioxidant activity since the free radical mediated oxidative stress also influence the oestrogen regulation[26]. Prepared extract was analysed using FTIR & GC-MS to identify the constituents existing in the extract. Then identified compounds was analysed by computational docking studies against the selected target proteins (Aromatase inhibitor (3EQM), Acetylcholine esterase (4EY7), Beta Catenin (1JDH), Progestrone receptor (1A28) to control the growth of fibroid.

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