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Research Article

MOLECULAR DOCKING OF SPEARMINT PHYTOCOMPOUNDS AGAINST CYP21A2: IMPLICATIONS FOR PCOS THERAPY

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ABSTRACT

Objectives: In this study, phytocompounds of spearmint that is known to have anti-androgenic activity are docked against a protein CYP21A2. This protein is also known as progesterone complex, one of the member cytochrome P450 enzymes; mutations in the genes encoding these proteins are causative factors of polycystic ovarian syndrome (PCOS).

Methods: The study was based on computations using different phytochemicals of spearmint docking to a target protein CYP21A2 which causes hormonal imbalance leading to PCOS and hirsutism. Molecular docking was conducted using PyRx-virtual screening tool and Biovia discovery studio 2.0 to determine binding affinities of different phytochemicals to target protein.

Results: The docking result revealed that bicyclogermacrene, cubebol, (-)-beta-bourbonene, alpha-bourbonene, and spathulenol showed highest binding affinities between –8.1 and –8.5 kcal/mol. Further, absorption, distribution, metabolism, excretion, and toxicity properties of these compounds are explored mainly to understand the possibility of developing potential drugs for PCOS.

Conclusion: These bioactive compounds can be considered as potential agents that can be used with polyherbal plant extract to reduce the androgen levels in women suffering from PCOS.

Keywords: Polycystic ovarian syndrome, Hirsutism, Bioactive compounds, Docking, Binding affinity, Phytocompounds.

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INTRODUCTION

Polycystic ovarian syndrome (PCOS) can be considered the epidemic endocrine disorder of the 21st century [1,2]. It is affecting women of reproductive age, around the age group between 18 and 44. PCOS is also linked with irregularity in menstrual cycles, hyperandrogenism, alopecia, hirsutism, obesity, insulin (INS) resistance, anovulation, oligomenorrhea, and pre-diabetes [3,4]. It is characterized by elevated androgen levels (hyperandrogenemia) leading to anovulation, microcysts in ovaries (polycystic ovaries) and can cause inhibition of follicular development. PCOS condition is diagnosed based on the National Institutes of Health consensus or Rotterdam criteria [5]. PCOS is a multifactorial condition that is a combination of genetic and environmental factors, the latter factor predominantly involves poor dietary choices, increased stress levels among working women, and lack of physical activity [6].

PCOS disorder is polygenic in nature, and multiple genes and biochemical pathways disrupt ovulation and impairment of androgen. Some of genes and their associated biochemical pathway leading to PCOS are steroidogenesis (e.g., CYP17A1, CYP11A1, CYP19A1), chronic inflammation (e.g., tumor necrosis factor-alpha, interleukin 6), INS secretion (e.g., INS, insulin receptor [INSR], insulin receptor substrate-1), cancer (e.g., matrix metalloproteinase, INS, androgen receptor type 1), complement and coagulation cascade (e.g., von Willebrand factor), and signaling (e.g., luteinizing hormone/human chorionic gonadotropin receptor [LHCGR], INS, and adiponectin gene, anti-Mullerian hormone [AMH]) pathways [6-10]. One gene associated with the manifestation of PCOS is CYP21A2, and it is one among the cytochrome P450 (CYPs) enzymes involved in steroidogenesis pathways [11, 12]. Steroidogenesis in ovaries starts with converting cholesterol to androgen, estrogen, and progestin, all of which act as substrates for synthesizing steroid hormones. CYP21A2 gene mutation results in limiting the 21-hydroxylase (21-OH) enzyme, which consequently leads to diminished cortisol and aldosterone production. In reverse increased production of dihydrotestosterone and testosterone can lead to symptoms of androgen excess, including hirsutism, acne, and, in severe cases, female virilization [13]. A deformed 21-OH enzyme accounts for 90% of disturbance in steroid hormone production.

Leaves of spearmint are known to have anti-androgenic properties and also reduce body weight in PCOS. Hyperinsulinemia and increased visceral adiposity result in the elevated production of androgen in ovaries. Reduction in body mass index of anovulatory obese women decreases testosterone concentration and INS resistance and reinstates ovulation. *Mentha spicata* also alleviates menstrual pain and hirsutism [14, 15].

In this study, *in silico* virtual screening of phytocompounds from *M. spicata* is docked against CYP21A2 protein (progesterone complex), to analyze their binding affinities for potential drug candidates in reducing androgen levels in PCOS women.

METHODS

Protein retrieval and preparation

The target structure should be determined experimentally by either nuclear magnetic resonance or X-ray crystallography, which can be downloaded from the protein data bank [PDB] database (https://www.rcsb.org/structure/4y8w). The three-dimensional (3D) X-ray crystallographic structure of CYP21A2–progesterone complex (PDB ID–4Y8W) solved at 2.64 Å resolution was retrieved from RCSB PDB. The proteins underwent preparation through the following procedural steps. (a) Load the protein molecule downloaded in 3D simple document format (SDF) from PDB database, (b) hetero atoms, metal ions, water molecules, and cofactors are removed, (c) bound ligands removed, (d) B and C chains of the CYP21A2 protein are removed, and (e) save the novel protein file in PDB format. The protein was subjected to Ramachandran plot And Hydropathy plot analysis by employing the

PDBsum (https://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/GetPage.pl) and ExPASy respectively (https://web.expasy.org/protscale/).

Ligand selection and preparation

A total of 25 phytocompounds listed in Table 1 are isolated from *M. spicata* plant that has been reported to have anti-androgenic properties. These phytocompounds are obtained from Indian Medicinal Plants, Phytochemistry, and Therapeutic (IMPPAT) database (https://cb.imsc.res.in/imppat/formulations/Mentha%20spicata) and were selected for virtual screening and molecular docking. The 3D structures of these phytocompounds were obtained from the PubChem database in SDF.

Protein-ligand docking

Molecular docking protocol was accomplished through a flexible docking protocol. PyRx-Virtual Screening Tool is utilized for docking procedure with the following steps: (a) Load the protein molecule which is devoid of other ligands and hetero atom which was achieved through Biovia discovery studio, (b) convert the protein file from PDB to PDBQT format, (c) upload the 25 phytochemicals in the control panel of PyRx software, which were downloaded from the PubChem database, (d) minimize energy of ligands and convert all the ligands to AutoDock PDBQT format, (e) grid box is generated, ligands of interest are docked in this grid. Hence, it is important to maximize the grid box to cover most of the protein surface, and (f) docking results are obtained in excel sheet format consisting of values of binding affinities and root mean square deviation.

Screening of phytocompounds

Swiss absorption, distribution, metabolism, excretion, and toxicity (ADME) software of Swiss Institute of Bioinformatics was accessed on a web server that displays the submission page of Swiss ADME. This web tool was used to evaluate ADME behaviors of the phytoconstituents from *M. spicata*. The list of canonical Simplified Molecular Input Line Entry System (SMILES) of phytocompounds is made and the results are presented for every molecule in tables and Excel spreadsheets. An important segment of drug development is drug-likeness analysis which is used to identify the biological properties of drug candidates.

RESULTS

Protein preparation and analysis

The 3D structure of target protein (4Y8W) was retrieved from the PDB database. Later, by utilizing the software tool Biovia Discovery Studio, protein is subjected structural analysis and trimming protein with only one amino acid chain devoid of ligands and heteroatoms (Fig. 1).

The Ramachandran plot of 4Y8W depicted that most of the residues are congregated in favored regions with few irregularities for all the drug targets. The protein CYP21A2 (4Y8W) structures were solved by X-ray crystallography to resolution at 2.0Å and the protein had a normal G-factor score of 0.6 (Fig. 2).

The hydropathy plot indicates hydrophobic and hydrophilic tendencies of an amino acid sequence of A-chain of CYP21A2 protein (Fig. 3).

Selected phytocompounds of spearmint

Table 1 presents the name of 25 phytocompounds of *M. spicata*, which are identified using the IMPPAT database and their PubChem ID along with their canonical SMILES obtained from PubChem database.

Molecular Docking

The molecular docking of 25 phytocompounds Table 1 of *M. spicata* was docked against CYP21A2 protein (A chain of protein) also known as progesterone complex (PDB ID–4Y8W) using PyRx-virtual screening tool. After analyzing the active phytocompounds by binding free energy score and molecular interaction profile, out of 25 phytocompounds, only 5 (Bicyclogermacrene, Cubebol, (-)-beta-Bourbonene, alpha-Bourbonene, and Spathulenol) displayed the best binding affinity (ranging from –8.1 to –8.5 kcal/mol) and molecular interactions (Figs. 4-8). The specific target site for the receptor was set using the grid box with dimensions (61.5935 × 51.8689 × 64.7989) Å. The phytocompounds that have high binding affinity to target protein are called hit compounds due to their high-affinity scores. Thus, this filtered list of 5 phytocompounds was designated as druggable and was subsequently used for further studies (Table 2).

Table 3 presents the physicochemical properties of phytocompounds bicyclogermacrene, cubebol, (-)-beta-bourbonene, alpha-bourbonene, and spathulenol, such as their molecular weight, number of atoms, fraction CSP3, number of rotatable bonds, molar refractivity, and

Table 1: Phytocompounds selected for docking studies

S. No.	Plant part	Phytochemical name	PubChem ID	Canonical SMILES
1.	Aerial	Myrcenol	10975	CC (C)(CCCC(=C) C=C) O
2.	Aerial	Beta-bisabolene	10104370	CC1=CCC (CC1) C(=C) CCC=C (C) C
3.	Aerial	Carvacrol	10364	CC1=C (C=C (C=C1) C (C) C) O
4.	Aerial	Jasmone	1549018	CCC=CCC1=C (CCC1=O) C
5.	Aerial	3-octanol	11527	CCCCCC (CC) O
6.	Aerial	Pinocarvone	121719	CC1(C2CC1C(=C) C(=O) C2) C
7.	Aerial	Piperitenone	381152	CC1=CC(=O) C(=C (C) C) CC1
8.	Aerial	Myrcene	31253	CC(=CCCC(=C) C=C) C
9.	Aerial	Bicyclogermacrene	13894537	CC1=CCCC(=CC2C (C2(C) C) CC1) C
10.	Aerial	Cubebol	11276107	CC1CCC (C2C13C2C (CC3)(C) O) C (C) C
11.	Aerial	(-)-beta-Bourbonene	62566	CC (C) C1CCC2(C1C3C2CCC3=C) C
12.	Aerial	Gamma-terpinene	7461	CC1=CCC(=CC1) C (C) C
13.	Aerial	Dihydrocarvyl acetate	30248	CC1CCC (CC1OC(=O) C) C(=C) C
14.	Aerial	Verbenone	29025	CC1=CC(=O) C2CC1C2(C) C
15.	Aerial	Neomenthyl acetate	75699	CC1CCC (C (C1) OC(=O) C) C (C) C
16.	Aerial	3-Octyl acetate	521238	CCCCCC (CC) OC(=O) C
17.	Aerial	Alpha-bourbonene	530816	CC1=CCC2C1C3C2(CCC3C (C) C) C
18.	Aerial	p-Cymene	7463	CC1=CC=C (C=C1) C (C) C
19.	Aerial	Hedycaryol	6432240	CC1=CCCC(=CCC (CC1) C (C)(C) O) C
20.	Aerial	Thymol	6989	CC1=CC(=C (C=C1) C (C) C) O
21.	Aerial	Linalyl acetate	8294	CC(=CCCC (C)(C=C) OC(=O) C) C
22.	Aerial	Beta-cubebene	93081	CC1CCC (C2C13C2C(=C) CC3) C (C) C
23.	Aerial	Spathulenol	92231	CC1(C2C1C3C (CCC3(C) O) C(=C) CC2) C
24.	Aerial	Carvone oxide	442462	CC(=C) C1CC2C (O2)(C(=O) C1) C
25.	Aerial	Eucalyptol	2758	CC1(C2CCC (O1)(CC2) C) C

SMILES: Simplified Molecular Input Line Entry System

topological polar surface area. The molecular weight and number of atoms were less than 500 and 20, but molar refractivity and polar surface area were more than 50 and 20°A^2 representing poor oral bioavailability 5 hit compounds.

Table 4 demonstrates lipophilicity of bicyclogermacrene, cubebol, (-)-beta-bourbonene, alpha-bourbonene, and spathulenol. Lipophilicity is the partition coefficient between water (log $P_{o/w}$) and *n*-octanol. From Table 5, hit compound values fall within the permissible range of –0.4 to +5.6, implying a good lipophilic compound. Lipophilicity is an important factor for pharmacokinetics drug discovery.

Table 5 represents hydrophilicity values of 5 hit compounds. As indicated in table, values of bicyclogermacrene and spathulenol are mostly soluble in aqueous medium as log S values were <–4.0. Other molecules, cubebol, (-)-beta-bourbonene, and alpha-bourbonene, are moderately soluble in aqueous medium. The high water-soluble molecules vastly enhance drug development and formulation.

Table 6 demonstrates pharmacokinetic values. According to results, cubebol and spathulenol showed high gastrointestinal absorption compared to other molecules. Except for bicyclogermacrene, all other compounds, cubebol, (-)-beta-bourbonene, alpha-bourbonene, and spathulenol, could cross the blood–brain barrier. All the 5 phytocompounds affected the liver CYPs enzymes such as CYP2C19, CYP2C9 (except for spathulenol), and CYP1A2 (except for bicyclogermacrene and cubebol). None of the phytocompounds affected CYP2D6 and CYP3A4 CYPs enzymes. However, skin penetration was better for all phytocompounds.

Based on Table 7, all 5 hit compounds obeyed the druglikeness filters, Ghose, Veber, and Egan. Cubebol and spathulenol obeyed Lipinski's rule of 5 and with one violation of Lipinski's filter of bicyclogermacrene, (-)-beta-bourbonene, and alpha-bourbonene. All 5 phytocompounds have one violation of Muegge filter. All compounds have a good bioavailability score of 0.55 which is more than 0.10 that is required for compound to be considered a potential drug candidate.

Table 8 demonstrates that the filters for leadlikeness such as Pains filter and Brenk filter were obeyed by all 5 hit compounds bicyclogermacrene, cubebol, (-)-beta-bourbonene, alpha-bourbonene, and spathulenol (except for, cubebol violated Brenk filter). Synthetic accessibility values are moderate and can be considered for Investigational New Drug.

Table 2: Binding affinities of phytocompounds from *Mentha spicata* when docked against CYP21A2 protein

S. No.	Phytocompounds	Binding affinity (kcal/mol)
1.	Myrcenol	–5.5
2.	Beta-Bisabolene	–7.6
3.	Carvacrol	–6.9
4.	Jasmone	–6.8
5.	3-Octanol	–5.2
6.	Pinocarvone	–7.4
7.	Piperitenone	–6.9
8.	Myrcene	–5.8
9.	Bicyclogermacrene	–8.5
10.	Cubebol	–8.3
11.	(-)-beta-Bourbonene	–8.5
12.	Gamma-terpinene	–7
13.	Dihydrocarvyl acetate	–7.5
14.	Verbenone	–7.3
15.	Neomenthyl acetate	–6.9
16.	3-Octyl acetate	–5.6
17.	Alpha-bourbonene	–8.1
18.	p-Cymene	–7
19.	Hedycaryol	–7.8
20.	Thymol	–6.6
21.	Linalyl acetate	–6.2
22.	Beta-cubebene	–7.9
23.	Spathulenol	–8.1
24.	Carvone oxide	–6.6
25.	Eucalyptol	–6.8

DISCUSSION

PCOS is associated with obesity, hyperglycemia, and INS resistance which correlate with elevated oxidative stress that comprises hyperandrogenemia environment in the ovary. In one study conducted in Turkey, men reported that consuming herbal tea of *M. spicata* or *M. piperita* caused diminished libido [15]. This may be due to antiandrogenic properties of spearmint and peppermint. Studies show that spearmint decreases the oxidative stress and reduces cholesterol in type 2 diabetes. Leaf extract of spearmint contains phenolic compounds that remarkably enhance the antioxidant defence system and reduce levels of glucose and cholesterol in diabetic male rat [14]. PCOS is an oligogenic disorder, and it seems that the genes thymocyte selection-associated HMG BOX 33, DENN domain containing 1A, AMH, LHCGR, thyroid adenoma-associated gene, AMH receptor type 2, and INSR are important genes in the susceptibility of PCOS [16,17]. The occurrence of modification in the genes homeobox 11 (gene codes for DNA-binding nuclear transcription factor) and homeobox A10 (protein coding gene) in women with PCOS affects the endometrial reception leading to infertility with implantation failure. Few studies also shown that polymorphism in FSHR gene is significantly associated with PCOS [16,18].

Many studies have validated that hyperandrogenism is one of the most distinguishable clinical features reported in patients with PCOS associated with heterozygous phenotypes with diverse genetic variants. These situations, involving deficiency of enzymes in the steroidogenesis pathway, are considered a prediction for PCOS [19]. A subgroup of CYP genes encodes for enzymes involved in the steroidogenesis biosynthesis pathway. Biosynthesis of steroid hormones, inclusive of glucocorticoids, mineralocorticoids, androgens, progestins, and estrogen, are regulated by the enzymes, i.e., steroidogenic enzymes that comprise steroid hydroxysteroid dehydrogenases and certain CYPs enzymes [5,6,11,16]. In ovary steroidogenesis starts with alteration of cholesterol progresses sequentially to progestin, androgen, and estrogen, all of which are essential for subsequent synthesizing steroid hormones. These hormones are ultimately transported into blood circulation, where they

Table 3: Physicochemical properties of 5 hit compounds

Physicochemical properties	Bicyclogermacrene	Cubebol	(-)-beta-bourbonene	Alpha-bourbonene	Spathulenol
Molecular weight (g/moL)	204.35	222.37	204.35	204.35	220.35
Num. heavy atoms	15	16	15	15	16
Num. arom. heavy atoms	0	0	0	0	0
Fraction CSP3	0.73	1	0.87	0.87	0.87
Num. rotatable bonds	0	1	1	1	0
Num. H-bond acceptors	0	1	0	0	1
Num. H-bond donors	0	1	0	0	1
Molar refractivity	68.78	68.82	67.14	67.14	68.34
TPSA(°A^2)	0	20.23	0	0	20.23

exert their reaction on both central nervous system [6] and peripheral nervous system.

Majority of research is focused on CYP11A1, CYP17A1, and CYP19A1 genes. The family of enzymes consisting of P450 enzymes is involved in the synthesis of cholesterol, steroids, lipids, and metabolism of drugs. CYP proteins are located in the network of endoplasmic reticulum, and these proteins catalyze the last step of steroid biosynthesis. Mutation in this gene may increase or decrease aromatase activity. In one study of letrozole, the drug was found to be an aromatase inhibitor, and it was approved for patients with hormone-responsive breast cancer. However, it has been studied for the induction of ovulation in women suffering from PCOS. The efficacy of drugs letrozole and anastrozole was studied for induction of ovulation, and there is no statistically significant difference in pregnancy rates. Mutation associated with CYP17A1 gene results in adrenal hyperplasia 17 alpha-hydroxylase deficiency also pseudohermaphroditism [6, 11]. Gene CYP11A1 codes for members of the CYPs family of enzymes. This protein is located in the inner membrane of mitochondria and it catalyzes the first step, conversion of cholesterol to pregnenolone in steroid hormone synthesis. The Tenascin X protein, retinitis pigmentosa, complement factor 4 (C4), and CYP21A2 genes are adjacent structurally, forming a genetic module of the RCCX complex. Mutation in CYP21A2 gene consequently results in 21-OH deficiency and also results in variable copy count of C4 gene, and the C4 gene encodes similar protein involved in classic complement activation [11,16].

The study of heritable changes in gene expression which is not encoded in the nucleotide sequence of DNA is known as epigenetics. Subjection to certain chemicals in environment (e.g., dietary substances, endocrine disruptors, and heavy metals) also behavioral exposures during early growth (e.g., child abuse) can lead to epigenetic modification. From many studies of cancers, there is conclusive evidence suggesting the link between human diseases and epigenetic dysregulation [20]. Data

Table 4: Lipophilicity values of 5 hit compounds

Name of the phytocompounds	Consensus log $P_{o/w}$
Bicyclogermacrene	4.15
Cubebol	3.5
(-)-beta-bourbonene	4.4
Alpha-bourbonene	4.29
Spathulenol	3.26

Table 5: Hydrophilicity values of 5 hit compounds

Hydrophilicity properties	Bicyclogermacrene	Cubebol	(-)-beta-bourbonene	Alpha-bourbonene	Spathulenol
Log S (ESOL)	−3.72	−3.62	−4.01	−3.86	−3.17
Solubility (mol/L)	1.93E-04	2.39E-04	9.81E-05	1.39E-04	6.83E-04
Class	**Soluble**	**Soluble**	**Moderately soluble**	**Soluble**	**Soluble**
Log S (Ali)	−3.85	−4.04	−4.44	−4.19	−3.2
Solubility (mol/L)	1.42E-04	9.04E-05	3.64E-05	6.46E-05	6.26E-04
Log S_w (SILICOS-IT)	−3.52	−2.73	−3.32	−3.07	−2.96
Solubility (mol/L)	3.03E-04	1.85E-03	4.81E-04	8.51E-04	1.09E-03
Class	Soluble	Soluble	Soluble	Soluble	Soluble

Table 6: Pharmacokinetic properties of 5 hit compounds

Pharmacokinetics properties	Bicyclogermacrene	Cubebol	(-)-beta-Bourbonene	Alpha-Bourbonene	Spathulenol
GI absorption	Low	High	Low	Low	High
BBB permeability	No	Yes	Yes	Yes	Yes
P-gp substrate	No	No	No	No	No
CYP1A2 inhibitor	No	No	Yes	Yes	No
CYP2C19 inhibitor	Yes	Yes	Yes	Yes	Yes
CYP2C9 inhibitor	Yes	Yes	Yes	Yes	No
CYP2D6 inhibitor	No	No	No	No	No
CYP3A4 inhibitor	No	No	No	No	No
Log KP (skin permeation cm/s)	−4.61	−4.87	−4.2	−4.37	−5.44

BBB: Blood brain barrier, GI: Gastrointestinal

Table 7: Druglikeness values of 5 hit compounds

Name of phytocompounds	Lipinski	Ghose	Veber	Egan	Muegge	Bioavailability score
Bicyclogermacrene	1	0	0	0	1	0.55
Cubebol	0	0	0	0	1	0.55
(-)-beta-bourbonene	1	0	0	0	1	0.55
Alpha-bourbonene	1	0	0	0	1	0.55
Spathulenol	0	0	0	0	1	0.55

Table 8: Leadlikeness values of 5 hit compounds

Name of phytocompound	Pains	Brenk	Leadlikeness	Synthetic accessibility
Bicyclogermacrene	0	1	2	4.34
Cubebol	0	0	2	4.13
(-)-beta-Bourbonene	0	1	2	3.94
Alpha-Bourbonene	0	1	2	5.04
Spathulenol	0	1	1	3.78

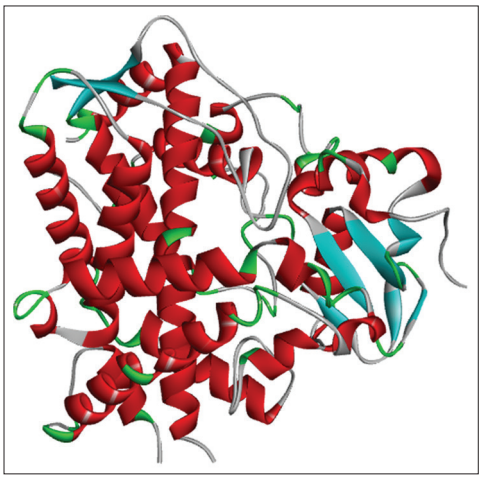

Fig. 1: 3-dimensional structure of A-chain of protein CYP21A2, devoid of ligands, and heteroatoms

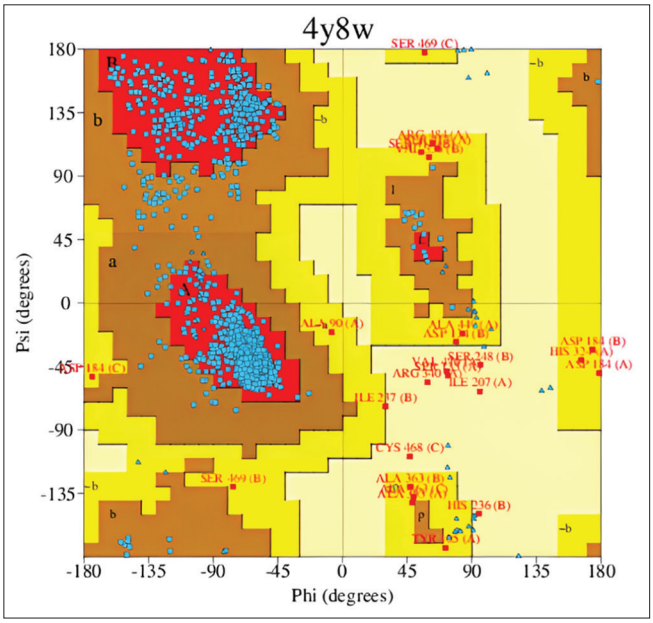

Fig. 2: Ramachandran plot of protein 4Y8W

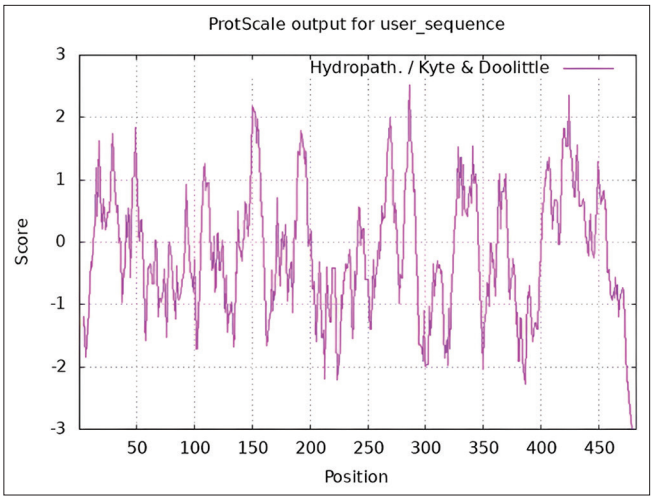

Fig. 3: Hydropathy plot of A-chain of protein CYP21A2

mining analyses suggest that abnormal DNA methylation patterns are connected with various disorders, including obesity, anemia, type 2 diabetes, cardiovascular disease, and numerous neurodevelopmental disorders indicating the importance of epigenetic regulation in the development of human diseases. Obesity and type 2 diabetes INS resistance is one of the main reasons for the development of PCOS.

Adiponectin is a protein hormone produced by adipocytes, and it has been reported that women with PCOS have decreased adiponectin. Insufficient levels of adiponectin are associated with INS resistance in murine models of obesity. One study concludes that increase in adiponectin is linked to weight loss rather than changes in macronutrient balance. Different macronutrient ratios through diet do not significantly alter adiponectin levels; instead hyperandrogenism is the main cause of adipose tissue dysfunction [21]. Hence, it becomes an important factor to maintain androgen levels in the body of women with PCOS to regulate healthy hormonal balance in the body. Most of the symptoms of PCOS such as hirsutism, INS resistance, and irregular periods can be managed by lifestyle changes such as exercise and better healthier choices of plant-based food but it is not sufficient to battle the root cause of PCOS which remains unclear. PCOS women are more prone to mental health disorders such as high rates of depression and anxiety. Manifestation of physical and mental health disturbances reduces the quality of life [22].

It is important to understand and learn about antiandrogens to fight against hyperandrogenism in PCOS conditions. Many of the drugs are administered for this purpose that includes antidiabetic drugs to reduce INS resistance (e.g., metformin, sulfonylurea, biguanide, and clomiphene), aromatase inhibitors (e.g., letrozole), antiandrogens (e.g., spironolactone, flutamide, and finasteride), and oral contraceptives to restore ovulation in women who do not wish to become pregnant [1]. These pharmaceutical drugs also come with side effects (bloating, liver inflammation, hypoglycemia, and allergic skin reactions) associated with them so now the focus has been shifting to herbal remedies such as herbal teas, essential oils, and polyherbal capsules.

Plant-derived antiandrogens are bioactive phenolic compounds that act as antagonist to androgens. Licorice (*Glycyrrhiza glabra*) is known to drop testosterone levels to normal which affects free testosterone in the body. Licorice root also known as sweet root has compound which is 50 times sweeter than sugar, it also contains phytoestrogens and other compounds that are thought to have endocrine effects. Licorice root also contains glycyrrhetinic acid and glycyrrhizin is known to have slight anti-androgen effect. Green tea (*Camellia sinensis*) comprises epigallocatechin which limits the conversion of 5-alpha reductase to testosterone to dihydrotestosterone. White peony (*Paeonia lactiflora*) has compound paeoniflorin which inhibits synthesis of testosterone and promoting the aromatase enzyme activity in the conversion of testosterone to estrogen [23]. Other plants that are studied for their anti-androgenic effect are Reishi, Black cohosh, Chaste tree, and Saw palmetto.

Spearmint (*M. spicata*) has anti-androgenic properties which accounts for the reduction in free testosterone in blood. It is mainly used as an herbal remedy for women suffering from hirsutism. Spearmint does not affect the total dehydroepiandrosterone sulfate and total testosterone levels. In one clinical study, consumption of spearmint tea twice a day for a month resulted in decreased levels of androgens and gonadotropins in plasma [15]. Bioactive compounds are also present in the food one consumes; these compounds are biological activities. Mainly bioactive compounds include flavonoids, isoflavonoids, polyphenols, phytoestrogens, glucosinolates, tannins, lycopene, lignin, and phytosterols [24]. These phytocompounds promote good health and have anti-oxidant, anti-diabetic, and anti-cancerous properties. Focusing on innovative and cost-effective extraction procedures of bioactive compounds from food waste by incorporating microbiome

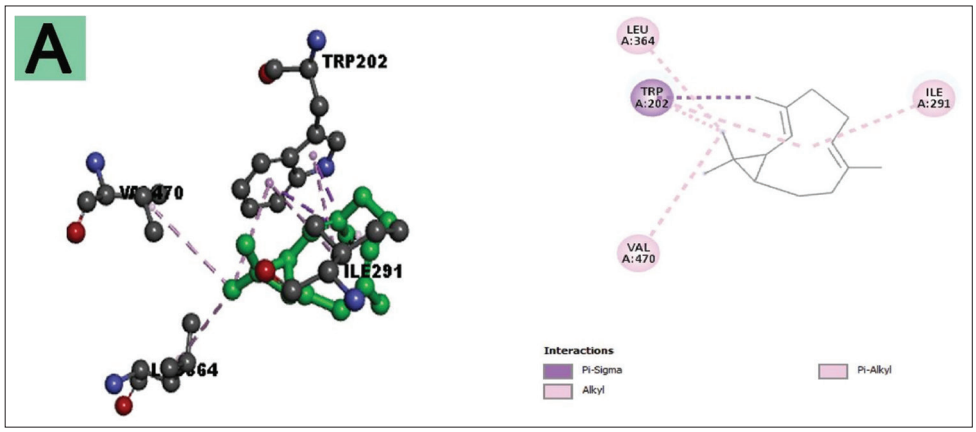

Fig. 4: 3-dimensional (3D) and 2-dimensional (2D) binding mode of bicyclogermacrene to A-chain of protein CYP21A2. From the 3D representation, ligands are colored in green and amino acid residues are colored in gray. Spheres and gray sticks represent amino acid residues and ligands, respectively, in 2D presentation

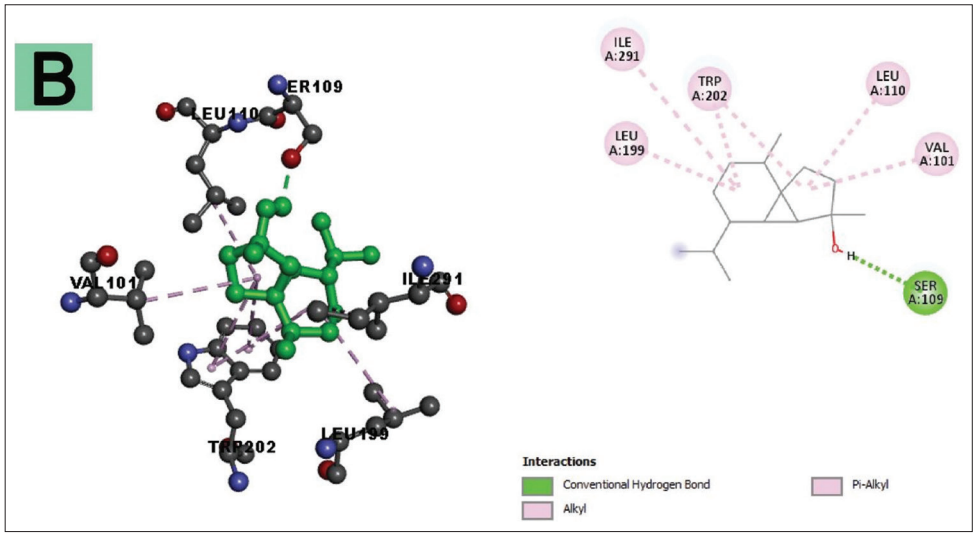

Fig. 5: 3-dimensional (3D) and 2-dimensional (2D) binding mode of cubebol to A-chain of protein CYP21A2. From 3D representation, ligands are colored in green and amino acid residues are colored in gray. Spheres and gray sticks represent amino acid residues and ligands, respectively, in 2D presentation

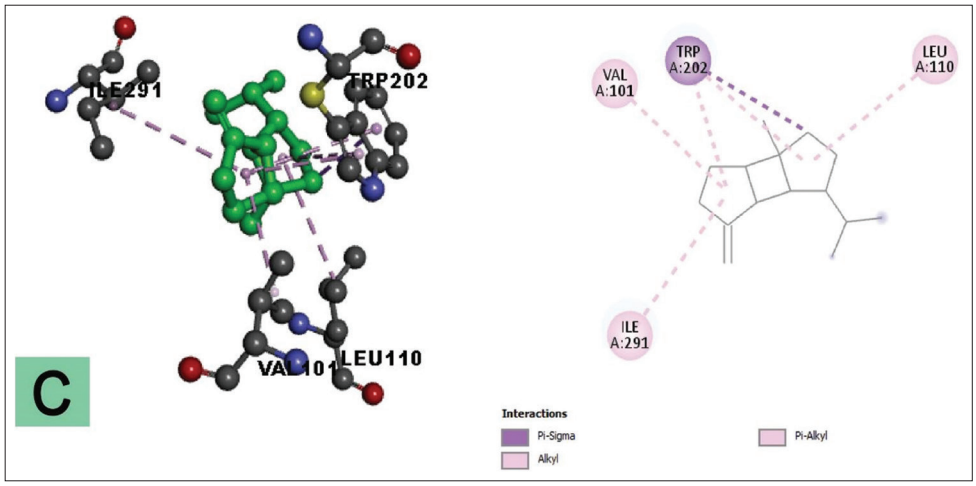

Fig. 6: 3-dimensional (3D) and 2-dimensional (2D) binding mode of (-)-beta-bourbonene to A-chain of protein CYP21A2. From 3D representation, ligands are colored in green and amino acid residues are colored in gray. Spheres and gray sticks represent amino acid residues and ligands, respectively, in 2D presentation

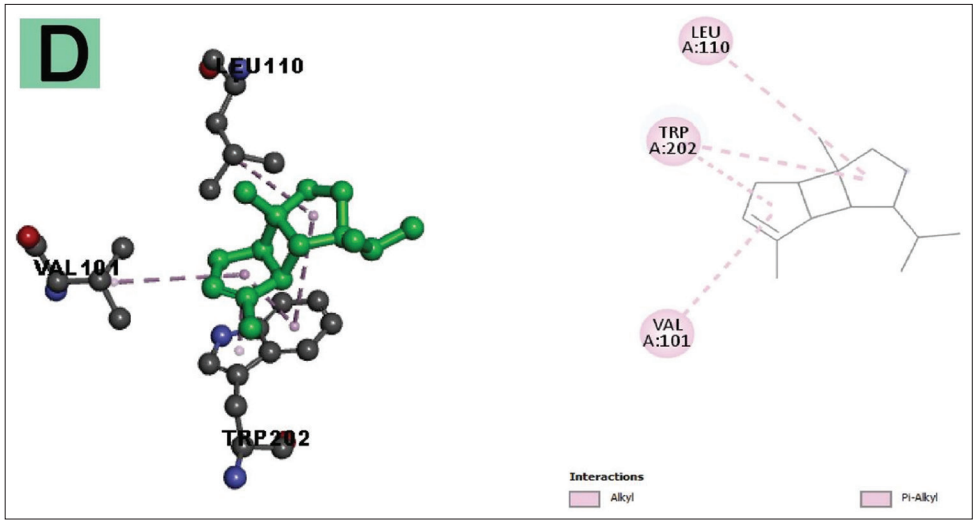

Fig. 7: 3-dimensional (3D) and 2-dimensional (2D) binding mode of alpha-bourbonene to A-chain of protein CYP21A2. From 3D representation, ligands are colored in green and amino acid residues are colored in gray. Spheres and gray sticks represent amino acid residues and ligands, respectively, in 2D presentation

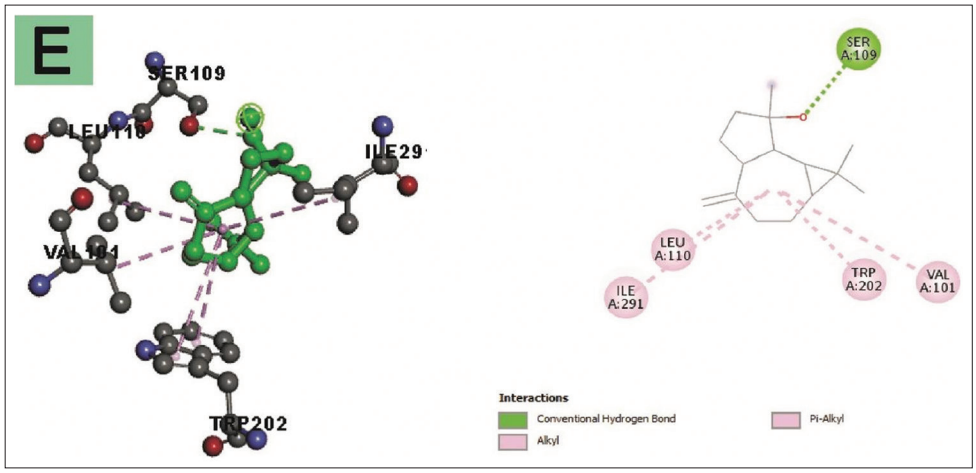

Fig. 8: 3-dimensional (3D) and 2-dimensional (2D) binding mode of spathulenol to A-chain of protein CYP21A2. From 3D representation, ligands are colored in green and amino acid residues are colored in gray. Spheres and gray sticks represent amino acid residues and ligands, respectively, in 2D presentation

to help women suffering from PCOS, areas have been researched further. More research has to be done on herbal remedies of Ayurveda and plants from other parts of the world to create sustainable use of products that could lead to abatement of PCOS.

CONCLUSION

Overall, the favorable results from binding affinities and absorption, distribution, metabolism, excretion, and toxicity profile of 5 phytocompounds (bicyclogermacrene, cubebol, (-)-beta-bourbonene, alpha-bourbonene, and spathulenol) isolated from *M. spicata* plants could be explored as potential anti-androgenic agents to reduce symptoms of PCOS. However, *in vitro* and *in vivo* studies have to be performed to confirm findings of this study. Apart from spearmint, many other plants (such as licorice, chaste tree, and white peonies) are also known to have anti-androgenic effects. These plants and their extract have been studied and explored in detail to formulate polyherbal preparation as potential drugs for the treatment of PCOS. There is a saying "you are what you eat" first quoted in 1826 by French author Jean Anthelme Brillant-Savarin, and certain nutrients affect health differently also gut microbiome varies depending on what one eats. It is important to make a lifestyle change along with these herbal remedies as a speedier elimination of PCOS.

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AUTHORS CONTRIBUTION

The contribution of the author to the manuscript has been clearly stated.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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REFERENCES

1. Ndefo UA, Eaton A, Green MR. Polycystic ovary syndrome: A review of treatment options with a focus on pharmacological approaches. P T 2013;38:336-55.

2. Chang S, Dunaif A. Diagnosis of polycystic ovary syndrome: Which criteria to use and when? Endocrinol Metab Clin North Am 2021;50:11-23.
3. Jiang NX, Li XL. The disorders of endometrial receptivity in PCOS and Its mechanisms. Reprod Sci 2022;29:2465-76.
4. Zeng X, Xie YJ, Liu YT, Long SL, Mo ZC. Polycystic ovarian syndrome: Correlation between hyperandrogenism, insulin resistance and obesity. Clin Chim Acta 2020;502:214-21.
5. Rasquin LI, Anastasopoulou C, Mayrin JV. Polycystic ovarian disease. In: StatPearls. Treasure Island, FL: StatPearls Publishing; 2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK459251 [Last accessed on 2022 Nov 15].
6. De Leo V, Musacchio MC, Cappelli V, Massaro MG, Morgante G, Petraglia F. Genetic, hormonal and metabolic aspects of PCOS: An update. Reprod Biol Endocrinol 2016;14:38.
7. Heidarzadehpilehrood R, Pirhoushiaran M, Abdollahzadeh R, Binti Osman M, Sakinah M, Nordin N, et al. A review on CYP11A1, CYP17A1, and CYP19A1 polymorphism studies: Candidate susceptibility Genes for polycystic ovary syndrome (PCOS) and infertility. Genes (Basel) 2022;13:302.
8. Stangler Herodež Š, Fijavž L, Zagradišnik B, Kokalj Vokač N. Detection of mutations in the CYP21A2 gene: genotype-phenotype correlation in Slovenian couples with conceiving problems. Balkan J Med Genet 2016;18:25-32.
9. Seyed Abutorabi E, Hossein Rashidi B, Irani S, Haghollahi F, Bagheri M. Investigation of the FSHR, CYP11, and INSR mutations and polymorphisms in Iranian infertile women with polycystic ovary syndrome (PCOS). Rep Biochem Mol Biol 2021;9:470-7.
10. Kaur R, Kaur T, Sudhir N, Kaur A. Association analysis of CYP11A1 variants with polycystic ovary syndrome: A case-control study from north India. Reprod Sci 2021;28:2951-60.
11. Zhang J, Yang M, Luan P, Jia W, Liu Q, Ma Z, et al. Associations between cytochrome P450 (CYP) gene single-nucleotide polymorphisms and second-to-fourth digit ratio in Chinese university students. Med Sci Monit 2021;27:e930591.
12. Wang M, Strand MJ, Lanser BJ, Santos C, Bendelja K, Fish J, et al. Expression and activation of the steroidogenic enzyme CYP11A1 is associated with IL-13 production in T cells from peanut allergic children. PLoS One 2020;15:e0233563.
13. Xia J, Liu F, Wu J, Xia Y, Zhao Z, Zhao Y, et al. Clinical and genetic characteristics of 17 α-hydroxylase/17, 20-lyase deficiency: C.985_987delTACinsAA mutation of CYP17A1 prevalent in the Chinese han population. Endocr Pract 2021;27:137-45.
14. Sadeghi Ataabadi M, Alaee S, Bagheri MJ, Bahmanpoor S. Role of essential oil of Mentha spicata (spearmint) in addressing reverse hormonal and folliculogenesis disturbances in a polycystic ovarian syndrome in a rat model. Adv Pharm Bull 2017;7:651-4.
15. Akdoğan M, Tamer MN, Cüre E, Cüre MC, Köroğlu BK, Delibaş N. Effect of spearmint (Mentha spicata Labiatae) teas on androgen levels in women with hirsutism. Phytother Res 2007;21:444-7.
16. Chaudhary H, Patel J, Jain NK, Joshi R. The role of polymorphism in various potential genes on polycystic ovary syndrome susceptibility and pathogenesis. J Ovarian Res 2021;14:125.
17. Otto-Buczkowska E, Grzyb K, Jainta N. Polycystic ovary syndrome (PCOS) and the accompanying disorders of glucose homeostasis among girls at the time of puberty. Pediatr Endocrinol Diabetes Metab 2018;24:40-4.
18. Kara M, Ozcan SS, Aran T, Kara O, Yilmaz N. Evaluation of endometrial receptivity by measuring HOXA-10, HOXA-11, and leukemia inhibitory factor expression in patients with polycystic ovary syndrome. Gynecol Minim Invasive Ther 2019;8:118-22.
19. Rosenfield RL, Ehrmann DA. The pathogenesis of polycystic ovary syndrome (PCOS): The hypothesis of PCOS as functional ovarian hyperandrogenism revisited. Endocr Rev 2016;37:467-520.
20. Olden K, Freudenberg N, Dowd J, Shields AE. Discovering how environmental exposures alter genes could lead to new treatments for chronic illnesses. Health Aff (Millwood) 2011;30:833-41.
21. Rodriguez Paris V, Solon-Biet SM, Senior AM, Edwards MC, Desai R, Tedla N, et al. Defining the impact of dietary macronutrient balance on PCOS traits. Nat Commun 2020;11:5262.
22. Karsten MDA, Wekker V, Groen H, Painter RC, Mol BWJ, Laan ETM, et al. The role of PCOS in mental health and sexual function in women with obesity and a history of infertility. Hum Reprod Open 2021;2021:hoab038.
23. Grant P, Ramasamy S. An update on plant derived anti-androgens. Int J Endocrinol Metab 2012;10:497-502.
24. Sorrenti V, Burò I, Consoli V, Vanella L. Recent advances in health benefits of bioactive compounds from food wastes and by-products: Biochemical ASPECTS. Int J Mol Sci 2023;24:2019.