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RESEARCH ARTICLE

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

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ABSTRACT

Objective*:* In this study phytocompounds of spearmint, that are known to have anti-androgenic activity are docked against a protein CYP21A2. This protein is also known as progesterone complex one of member cytochrome P450 enzymes, mutations in the genes encoding these proteins are causative factors of PCOS. ***Methods****:* The study was based on a computational 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 Biocyclogermacrene, cubebol, (-)-beta-Bpurbonene, alpha-bourbonene and spathulenol showed highest binding affinities between -8.1 to -8.5 kcal/mol. Further ADMET properties of these compounds are explored mainly to understand the possibility developing potential drug for PCOS. ***Conclusion:*** These bioactive compounds can be considered as potential agents that can used with polyherbal plant extract to reduce the androgen levels in women suffering from PCOS.

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INTRODUCTION

Polycystic ovarian syndrome (PCOS) can be considered as the epidemic endocrine disorder of 21st century. It is affecting women of reproductive age, around age group between 18-44. PCOS is also linked with irregularity in menstrual cycles, hyperandrogenism, alopecia, hirsutism, obesity, insulin resistance, anovulation, oligomenorrhea and prediabetes. Stein and Leventhal first described about PCOS in 1935 (7). 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 (NIH) National Institutes of Health consensus or Rotterdam criteria (8). PCOS is a multifactorial condition that is a combination of genetic and environmental factors, the latter factor predominantly involves poor dietary choices, increased stress level among working women & lack of physical activity. PCOS disorder is polygenic in nature , multiple genes and biochemical pathways disrupt ovulation & 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., TNF-alpha, IL-6), insulin secretion (e.g., INS, INSR, IRS-1), cancer (e.g., MMP, INS, AR1), complement and coagulation cascade (e.g., VWF), and signalling (e.g., LHCGR, INS, ADIPOQ, AMH) pathways (12). One gene associated in manifestation of PCOS is CYP21A2, it is one among the cytochrome P450 enzymes(CYPs) involved in steroidogenesis pathways (5). Steroidogenesis in ovaries starts with converting cholesterol to androgen, oestrogen and progestin, all of which acts as substrates for synthesizing steroid hormones. CYP21A2 gene mutation results in limiting the 21- hydroxylase (21-OH) enzyme, which consequently leads to diminished cortysole and aldosterone production. In reverse increased production of dihydrotestosterone and testosterone (6). 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, resulting in the elevated production of androgen in ovaries. Reduction in BMI of anovulatory obese women decreases testosterone concentration and insulin resistance and reinstates ovulation. *Mentha spicata* also alleviates menstrual pain and hirsutism.

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

METHODOLOGY

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 PDB database (https://www.rcsb.org/structure/4y8w). The 3-dimensional (3D) X-ray crystallographic structure of CYP21A2 – progesterone complex (PDB ID – 4Y8W) solved at 2.64 Å resolution was retrieved from RCSB Protein Data Bank (PDB). The was prepared for docking by the following steps using software tool Biovia Discovery Studio (a) Load the protein molecule downloaded in 3D SDF format 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. (e) Save the novel protein file in PDB format. Ramachandran plot CYP21A2 and Hydropathy plot of A-chain of CYP21A2 protein for evaluating the accuracy of predicted protein structure using tools PDBsum (https://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/GetPage.pl) and ExPASy (https://web.expasy.org/protscale/) .

Ligand selection and preparation: A total of 25 phytocompounds listed in Table:1 are isolated from *Mentha spicata* plant that have been reported to have anti-androgenic properties. These phytocompounds are obtained from 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 simple document format (SDF).

Protein-ligand docking: Molecular docking protocol was accomplished via a flexible docking protocol. PyRx-Virtual Screening Tool is utilized for docking procedure with 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 control panel of PyRx software, which were downloaded from PubChem database. (d) Minimize energy of ligands and covert all the ligand to autodockpdbqt format. (e) Grid box is generated, ligands of interest are docked in this grid. Hence its important to maximize the grid box to cover most of protein surface. (f) Docking results are obtained in excel sheet format consisting of values of binding affinities and RMSD. Ramachandran plot of 4Y8W protein and Hydropathy plot of A-chain of CYP21A2 protein for evaluating the accuracy of predicted protein

Screening of phytocompounds: Swiss ADME software of Swiss Institute of Bioinformatics was accessed in a web server that displays the submission page of Swiss ADME. This web tool was used to evaluate ADME behaviours of the phytoconstituents from *M.spicata*. The list of canonical SMILES (simplified-molecular-input-line-entry-system) of phytocompounds is made and the results are presented for every molecule in tables and excel spreadsheet.

A 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 PDB database. Later, by utilizing the software tool Biovia Discovery Studio protein is subjected structural analysis and trimming protein to with only one amino acid chain devoid of ligands and heteroatoms. The Ramachandran plot of 4Y8W depicted that most of residues are congregated in favoured regions with few irregularity for all the drug targets.

Figure 1. 3D structure of A-chain of protein CYP21A2, devoid of ligands and heteroatoms

The protein CYP21A2 (4Y8W) structures solved by X-ray crystallography to resolution at 2.0Å and protein had a normal G-factor score of 0.6.

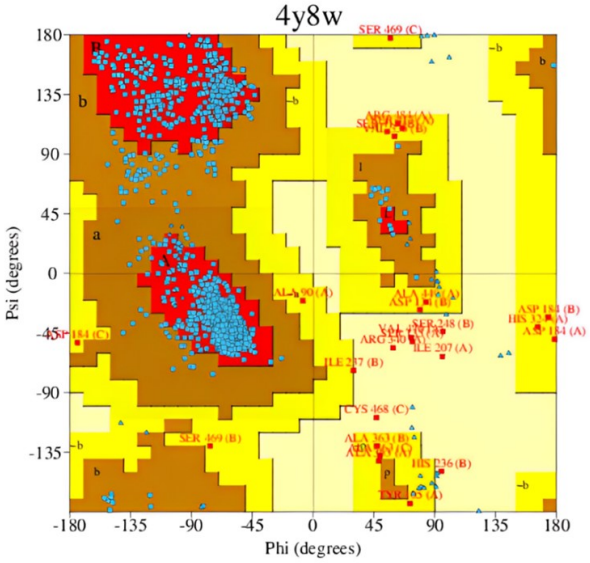

Figure 2. Ramachandran plot of protein 4Y8W

The hydropathy plot indicates hydrophobic and hydrophilic tendencies of an amino acid sequence of A-chain of CYP21A2 protein.Table:1 presents the name of 25 phytocompounds of *M.spicata*, which are identified using IMPPAT database and their PubChem ID along with their canonicalSMILES obtained from PubChem database.

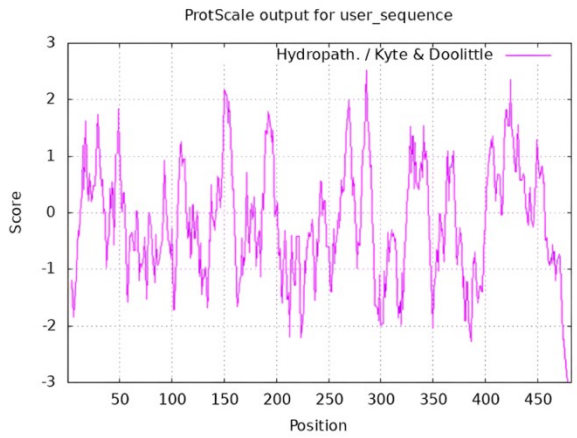

Figure 3. Hydropathy plot of A-chain of protein CYP21A2

Molecular Docking: The molecular docking of 25 phytocompounds Table:1 of *M.spicata* were docked against CYP21A2 protein (A chain of protein) also known as progesterone complex (PDB ID – 4Y8W) using PyRx- Virtual Screening Tool. After analysing the active phytocompounds by binding free energy score and molecular interaction profile, out of 25 phytocompounds only 5 (Biocyclogermacrene, Cubebol, (-)-beta-Bourbonene, alpha-Bourbonene and Spathulenol) displayed the best binding affinity (ranging from -8.1 to -8.5 kcal/mol) and molecular interactions. 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 as 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.

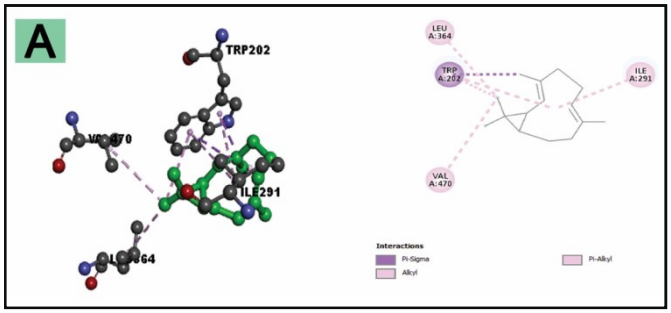

Figure 4. 3D and 2D binding mode of biocyclogermacrene to A-chain of protein CYP21A2. From 3D representation, ligands are colored in green and amino acid residues are color in gray. Spheres and gray sticks represent amino acid residues and ligands respectively in 2D presentation

Table 3, presents the physicochemical properties of phytocompounds biocyclogermacrene, cubebol, (-)-beta-bourbonene, alpha-bourbonene and spathulenol, such as their molecular weight, number of atoms, fraction CSP3, number of rotatable bonds, molar refractivity and 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 $^{o}A^{2}$ representing poor oral bioavailability 5 hit compounds.Table 4, demonstrates lipophilicity of biocyclogermacrene, cubebol, (-)-beta-bourbonene, alpha-bourbonene and spathulenol. Lipophilicity is partition coefficient between water (log $P_{o/w}$) and *n*-octanol. From the table 5 hit compounds values falls within permissible range of -0.4 to +5.6, implying a good lipophilic compounds.

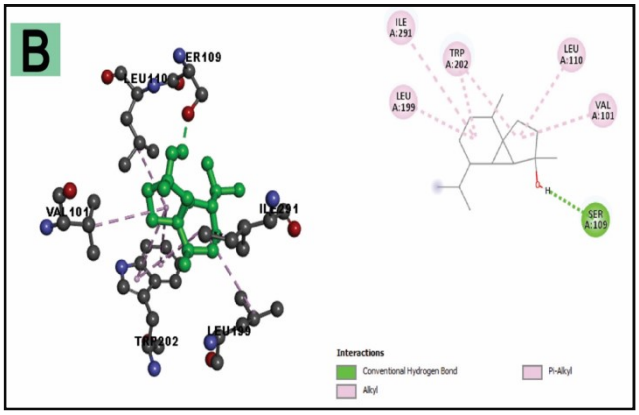

Figure 4. 3D and 2D binding mode of cubebol to A-chain of protein CYP21A2. From 3D representation, ligands are colored in green and amino acid residues are colored in gray. Spheres and gray sticks represent amino acid residues and ligands respectively in 2D presentation

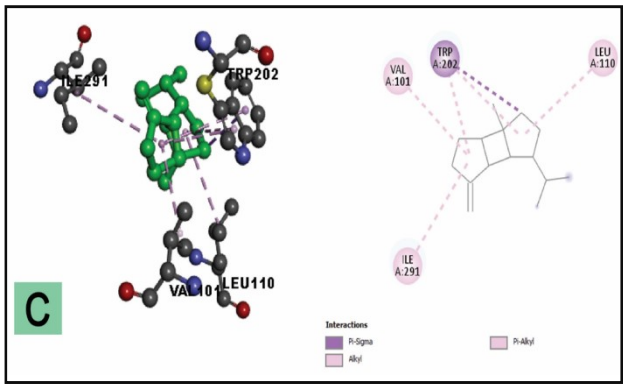

Figure 5. 3D and 2D binding mode of (-)-beta-bourbonene to A-chain of protein CYP21A2. From 3D representation, ligands are colored in green and amino acid residues are colored in gray. Spheres and gray sticks represent amino acid residues and ligands respectively in 2D presentation

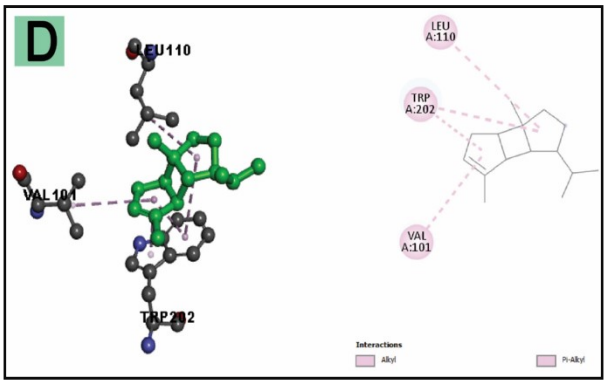

Figure 6. 3D and 2D binding mode of alpha-bourbone to A-chain of protein CYP21A2. From 3D representation, ligands are colored in green and amino acid residues are colored in gray. Spheres and gray sticks represent amino acid residues and ligands respectively in 2D presentation

Lipophilicity is a important factor for pharmacokinetics drug discovery (26). Table 5, represents hydrophilicity values of 5 hit compounds. As indicated in table values of biocyclogermacrene and spathulenolare mostly soluble in aqueous medium as log S values were less than -4.0. Other molecules cubebol, (-)-beta-bourbonene and alpha-bourbonene are moderately soluble in aqueous medium. The high water soluble molecules vastly enhances drug development and formulation.

Table 1. Phytocompounds selected for docking studies

S\N	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

Table 2. Binding affinities of phytocompounds from M. spicata when docked against CYP21A2 protein

S\N	Phytocompounds	Binding affinity (kcal/mo
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	Biocyclogermacrene	-8.5
10	Cubebol	-8.3
11	(-)-beta-Bourbonene	-8.5
12	Gamma-terpine	-7
13	Dihydrocarvyl acetate	-7.5
14	Verbenone	-7.3
15	Neomenthyl acetate	-6.9
16	3-Octyl acetate	-5.6
17	Alpha-boubonene	-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

Table 3. Physicochemical properties of 5 hit compounds

Physicochemical properties	Biocyclogermacrene	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($^{o}A^{2}$)	0	20.23	0	0	20.23

Table 4: Lipophilicity values of 5 hit compounds

Name of the phytocompounds	Consensus Log $P_{O/W}$
Biocyclogermacrene	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	Biocyclogermacrene	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
Class	Soluble	Moderately soluble	Moderately soluble	Moderately soluble	Soluble
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	Biocyclogermacrene	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 K_P (skin Permeation cm/s)	-4.61	-4.87	-4.2	-4.37	-5.44

Table 7. Druglikeness values of 5 hit compounds

Name of phytocompounds	Lipinski	Ghose	Veber	Egan	Muegge	Bioavailability score
Biocyclogermacrene	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
Biocyclogermacrene	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

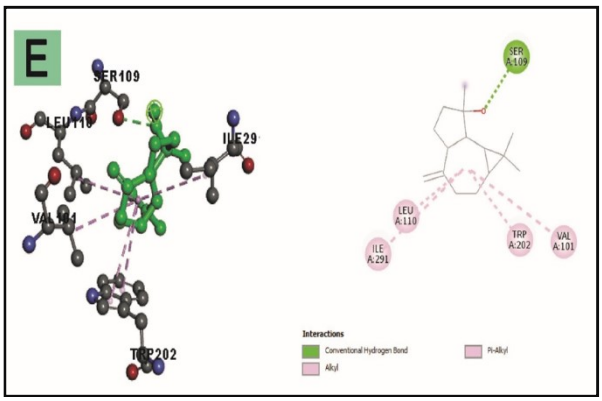

Figure 7. 3D and 2D binding mode of spathulenol to A-chain of protein cYP21A2. From 3D representation, ligands are colored in green and amino acid residues are colored in gray. Spheres and gray sticks represent amino acid residues and ligands respectively in 2D presentation

Table 6, demomstratespharmacokinetic values. According to results cubebol and spathulenol showed high gastrointestinal absorption compared to other molecules. Except for biocyclogermacrene all other compounds, cubebol, (-)-beta-bourbonene, alpha-bourbonene and spathulenol could cross blood brain barrier. All the 5 phytocompounds affected the liver cytochrome P450 enzymes such as CYP2C19, CYP2C9 (except for spathulenol) and CYP1A2(except for biocyclogermacrene and cubebol) . None of the phytocompounds affected CYP2D6 and CYP3A4 cytochrome P450 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 spathulenolobeyed the Lipinski's rule of 5 and with one violation of Lipinski's filter of biocyclogermacrene, (-)-beta-bourbonene and alpha-bourbonene. All 5 phytocompounds are with one violatation of Muegge filter. All compounds have good bioavailibity score of 0.55 which is

more than 0.10 that is required for compound to be considered a potential drug candidate. (26-32). Table 8: demonstrates the filters for leadlikeness, like Pains filter and Brenk filter were obeyed by all 5 hit compondsbiocyclogermacrene, cubebol, (-)-beta-bourbonene, alpha-bourbonene and spathulenol (except for, cubebol violated Brenk filter). Synthetic accessibility values are moderate can be considered for Investigational New Drug (IND) (33, 34).

DISCUSSION

PCOS is associated with obesity, hyperglycemia and insulin resistance which correlate with elevated oxidative stress, that comprises of hyperandrogenemic 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 (3). This may be due to anti-androgenic properties of spearmint and peppermint. Studies show that spearmint decreases the oxidative stress and reduce 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 (1). PCOS is a oligogenic disorder, it seems that the genes TOX3, DENND1A, AMH, LHCGR, THADA, AMHR2 and INSR are important genes in the susceptibility of PCOS (5). The occurrence of modification in the genes HOX-11 and HOXA-10 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 (10, 11)

Many studies have validated that hyperandrogenism is one of the most distinguishable clinical features reported in patients with PCOS associated with heterogenous phenotypes with diverse genetic variants. These situations, that involves deficiency of enzyme in the steroidogenesis pathway, is considered a prediction for PCOS. A subgroup of CYP genes encodes for enzymes involved in steroidogenesis biosynthesis pathway. Biosynthesis of steroid hormones, inclusive of glucocorticoids, mineralocorticoids, androgens, progestins and oestrogen, are regulated by the enzymes i.e., steroidogenic enzymes that comprise steroid hydroxysteroid dehydrogenases (HSDs) and certain cytochrome P450 enzymes (CYPs) (14). In ovary steroidogenesis starts with alteration of cholesterol progresses towards sequentially to progestin, androgen and oestrogen, all of which essential for subsequent synthesizing steroid hormones. These hormones are ultimately transported into blood circulation, where they exert their reaction on both central nervous system (CNS) (15) and peripheral nervous system (PNS). Majority of research is focused on CYP11A1, CYP17A1 and CYP19A1 genes. The family of enzymes consisting of P450 enzymes are involved in synthesis of cholesterol, steroids, lipids and metabolism of drugs. Cytochrome P450 proteins are located on network of endoplasmic reticulum, these protein catalyse last step of steroid biosynthesis. Mutation in this gene may increase or decrease aromatase activity.In one study of letrozole drug was found to be aromatase inhibitor, it was approved for patients with hormone responsive breast cancer. But it has been studied for induction of ovulation in women suffering from PCOS. Efficacy of drugs letrozole and anastrozole was studied for induction of ovulation, no statistically significant difference in pregnancy rates (2). Mutation associated with CYP17A1 gene results in adrenal hyperplasia 17 alpha- hydroxylase deficiency also psuedohermaphroditism. Gene CYP11A1 codes for member of cytochrome P450 family of enzymes.This protein is located in the inner membrane of mitochondria and it catalyses first step, conversion of cholesterol to pregnenolone in steroid hormone synthesis. The TNX, RP, C4 and CYP21A2 genes are adjacent structurally, forming a genetic module of RCCX complex. Mutation in CYP21A2 gene consequently results in 21-OH deficiency also results in variable copy count of C4 gene, the C4 gene encodes similar protein involved in classic complement activation (16-20).

The study of heritable changes in gene expression which are 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 behavioural exposures during early growth (e.g., child abuse) can lead to epigenetic modification. From many studies of cancers there is a conclusive evidence suggesting the link between human diseases and epigenetic dysregulation (23). Data mining analyses suggests abnormal DNA methylation patterns are connected with various disorders, including obesity, anemia, type 2 diabetes, cardiovascular disease numerous neurodevelopmental disorders indicating the importance of epigenetic regulation in the development of human diseases. Obesity and type 2 diabetes insulin resistance is one of main reason in development of PCOS. Adiponectin, is a protein hormone produced by adipocytes, it is been reported that women with PCOS have decreased adiponectin. Insufficient levels of adiponectin is associated with insulin 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 the adiponectin levels instead hyperandrogenism is the main cause of adipose tissue dysfunction (21). Hence it becomes a important factor to maintain androgen levels in body of women with PCOS to regulate healthy hormonal balance in body. Most of the symptoms of PCOS such as hirsutism, insulin resistance, irregular periods etc, 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 (24).

It is important to understand and learn about antiandrogens to fight against hyperandrogenism in PCOS condition. Many of the drugs are administered for this purpose that includes, antidiabetic drugs to reduce insulin 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 (2). These pharmaceutical drugs also comes with side effects(bloating, liver inflammation, hypoglycaemia 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 anti androgens are bioactive phenolic compounds that acts as antagonist to androgens. Licorice (*Glycyrrhizaglabra*) is known to drop testosterone levels to normal which affects free testosterone in body. Licorice root is 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 endocrine effects.

Licorice root also contains glycerrhectic acid and glycyrrhizin are known to have slight anti-androgen effect. Green tea (*Camelliasinensis*) comprises of epigallocatechins which limits the conversion of 5-alpha reductase to testosterone to DHT. White peony (*Paeonialactiflora*) have compound paeoniflorin which inhibits systhesis of testosterone and promoting the aromatase enzyme activity in conversion of testosterone to estrogen (35). 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 reduction in free testosterone in blood. It is mainly used for herbal remedy for women suffering from hirsutism. Spearmint do not affect the total DHEAS and total testosterone levels. In one clinical study consumption spearmint tea twice a day for a month resulted in decreased levels of androgens and gonadotropins in plasma (3). 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, lignan and phytosterols (25). These phytocompounds promote good health, 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 to help women suffering from PCOS, areas have be researched further. More research has to be done on herbal remedies of ayurveda and plants from other parts of the world in order create sustainable use of products that could lead to abatement of PCOS.

CONCLUSION

Overall, the favourable results from binding affinities and ADMET profile of 5 phytocompounds (Biocyclogermacrene, 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, *invitro* and *invivo* studies have to performed to confirm findings of this study. Apart from spearmint many other plants(such as licorice, chaste tree, white peonies etc) are also known to have anti-androgenic effects. These plants and their extract has be studied and explored in detail to formulate polyherbal preparation as potential drugs for treatment of PCOS. There is saying “you are what you eat” first quoted in1826 by French author Jean Anthelme Brillant-Savarin, 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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ABBREVIATIONS

ADIPOQ- Adiponectin gene
ADMET- absorption, distribution, metabolism, excretion and toxicity
AMH- Anti-mullerian hormone
AMHR2-Anti-mullerian hormone receptor type 2
AR1- Androgen receptor type 1
BBB- Blood Brain Barrier
BMI- Body mass index
C4- Complement factor 4
CNS- Central nervous system
CYPs – Cytochrome P450
DENND1A- DENN domain containing 1A
DHEAS- Dehydroepiandrosterone sulphate
DHT- Dihydrotestosterone
GI absorption- Gastrointestinal absorption
HOX- 11– Homeobox 11 (gene codes for DNA-binding nuclear transcription factor)
HOXA- 10 – Homeobox A10 (protein coding gene)
HSD- Hydroxysteroid dehydrogenase
IL-6 – Interleukin 6
IMPPAT- Indian Medicinal Plants, Phytochemistry, And Therapeutics
IND- Investigational New Drug
INS- Insulin
INSR- Insulin receptor
IRS- Insulin receptor substrate 1
LHCGR- Luteinizing hormone/human chorionic gonadotropin receptor
MMP- Matrix metalloproteinase
NIH-National Institute of Health
PCOS-Polcystic ovarian syndrome
PDB- Protein Data Bank
RMSD- Root mean square deviation
RP- Retinitis pigmentosa
SMILES- Simplified Molecular Input Line Entry System
SDF- Simple Document Format
THADA- Thyroid adenoma associated gene
TNF- Tumor necrosis factor
TNX- Tenascin X protein
TOX- Thymocyte selection-associated HMG BOX
VWF- von Willebrand factor

REFERENCES

1. Sadeghi Ataabadi M, Alaee S, Bagheri MJ, Bahmanpoor S. 2017. 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 Dec;7(4):651-654. doi: 10.15171/apb.2017.078. Epub Dec 31. PMID: 29399556; PMCID: PMC5788221.
2. Ndefo UA, Eaton A, Green MR. 2013. Polycystic ovary syndrome: a review of treatment options with a focus on pharmacological approaches. P T. Jun;38(6):336-55. PMID: 23946629; PMCID: PMC3737989.
3. Akdoğan M, Tamer MN, Cüre E, Cüre MC, Köroğlu BK, Delibaş N. 2007. Effect of spearmint (Mentha spicata

Labiatae) teas on androgen levels in women with hirsutism. Phytother Res. May;21(5):444-7. doi: 10.1002/ptr.2074. PMID: 17310494.

4. Pan JX, Zhang JY, Ke ZH, Wang FF, Barry JA, Hardiman PJ, Qu F. 2015. Androgens as double-edged swords: Induction and suppression of follicular development. Hormones (Athens). Apr-Jun;14(2):190-200. doi: 10.14310/horm.2002.1580. PMID: 26158651.
5. Heidarzadehpilehrood R, Pirhoushiaran M, Abdollahzadeh R, Binti Osman M, Sakinah M, Nordin N, Abdul Hamid H. 2022. A Review on *CYP11A1*, *CYP17A1*, and *CYP19A1* Polymorphism Studies: Candidate Susceptibility Genes for Polycystic Ovary Syndrome (PCOS) and Infertility. Genes (Basel). Feb 5;13(2):302. doi: 10.3390/genes13020302. PMID: 35205347; PMCID: PMC8871850.
6. Stangler Herodež Š, Fijavž L, Zagradišnik B, Kokalj Vokač N. 2016. Detection of mutations in the *CYP21A2* gene: genotype-phenotype correlation in Slovenian couples with conceiving problems. Balkan J Med Genet. Jul 9;18(2):25-32. doi: 10.1515/bjmg-2015-0082. PMID: 27785393; PMCID: PMC5026265.
7. Rasquin LI, Anastasopoulou C, Mayrin JV. 2022. Polycystic Ovarian Disease. (Updated 2022 Nov 15). In: StatPearls (Internet). Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/ books/ NBK459251/
8. Chang S, Dunaif A. 2021. Diagnosis of Polycystic Ovary Syndrome: Which Criteria to Use and When? Endocrinol Metab Clin North Am. Mar;50(1):11-23. doi: 10.1016/j.ecl.2020.10.002. PMID: 33518179; PMCID: PMC7860982.
9. Jiang NX, Li XL. 2022. The Disorders of Endometrial Receptivity in PCOS and Its Mechanisms. Reprod Sci. Sep;29(9):2465-2476. doi: 10.1007/s43032-021-00629-9. Epub 2021 May 27. PMID: 34046867. (PubMed)
10. Kara M, Ozcan SS, Aran T, Kara O, Yilmaz N. 2019. 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. Aug 29;8(3):118-122. doi: 10.4103/GMIT.GMIT_112_18. PMID: 31544022; PMCID: PMC6743234.
11. Seyed Abutorabi E, Hossein Rashidi B, Irani S, Haghollahi F, Bagheri M. 2021. Investigation of the *FSHR, CYP11*, and *INSR* Mutations and Polymorphisms in Iranian Infertile Women with Polycystic Ovary Syndrome (PCOS). *Rep Biochem Mol Biol.,* Jan;9(4):470-477. doi: 10.52547/rbmb.9.4.470. PMID: 33969141; PMCID: PMC8068450.
12. Chaudhary H, Patel J, Jain NK, Joshi R. 2021. The role of polymorphism in various potential genes on polycystic ovary syndrome susceptibility and pathogenesis. *J Ovarian Res.,* Sep 26;14(1):125. doi: 10.1186/s13048-021-00879-w. PMID: 34563259; PMCID: PMC8466925.
13. Kaur R, Kaur T, Sudhir N, Kaur A. 2021. Association Analysis of CYP11A1 Variants with Polycystic Ovary Syndrome: a Case-Control Study from North India. *Reprod Sci.,* Oct;28(10):2951-2960. doi: 10.1007/s43032-021-00676-2. Epub 2021 Jul 6. PMID: 34231171.
14. Zeng X, Xie YJ, Liu YT, Long SL, Mo ZC. 2020. Polycystic ovarian syndrome: Correlation between hyperandrogenism, insulin resistance and obesity. *Clin Chim Acta.,* Mar;502:214-221. doi: 10.1016/j.cca.2019.11.003. Epub 2019 Nov 13. PMID: 31733195.
15. De Leo V, Musacchio MC, Cappelli V, Massaro MG, Morgante G, Petraglia F. Genetic, hormonal and metabolic aspects of PCOS: an update. ReprodBiol Endocrinol. 2016 Jul 16;14(1):38. doi: 10.1186/s12958-016-0173-x. PMID: 27423183; PMCID: PMC4947298.
16. Zhang J, Yang M, Luan P, Jia W, Liu Q, Ma Z, Dang J, Lu H, Ma Q, Wang Y, Mu C, Huo Z. Associations Between Cytochrome P450 (CYP) Gene Single-Nucleotide Polymorphisms and Second-to-Fourth Digit Ratio in Chinese University Students. Med Sci Monit. 2021 Mar 16;27:e930591. doi: 10.12659/MSM.930591. PMID: 33723203; PMCID: PMC7980499.
17. Di Nardo G, Zhang C, Marcelli AG, Gilardi G. Molecular and Structural Evolution of Cytochrome P450 Aromatase. Int J Mol Sci. 2021 Jan 10;22(2):631. doi: 10.3390/ijms22020631. PMID: 33435208; PMCID: PMC7827799.
18. Xia J, Liu F, Wu J, Xia Y, Zhao Z, Zhao Y, Ren H, Kong X. Clinical and Genetic Characteristics of 17 α-Hydroxylase/17, 20-Lyase Deficiency: c.985_987delTACinsAA Mutation of CYP17A1 Prevalent in the Chinese Han Population. EndocrPract. 2021 Feb;27(2):137-145. doi: 10.4158/EP-2020-0478. Epub 2020 Dec 8. PMID: 33547012.
19. Wang M, Strand MJ, Lanser BJ, Santos C, Bendelja K, Fish J, Esterl EA, Ashino S, Abbott JK, Knight V, Gelfand EW. Expression and activation of the steroidogenic enzyme CYP11A1 is associated with IL-13 production in T cells from peanut allergic children. PLoS One. 2020 Jun 4;15(6):e0233563. doi: 10.1371/journal.pone.0233563. PMID: 32497050; PMCID: PMC7272076.
20. 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(1):40-44. doi: 10.18544/PEDM-24.01.0101. PMID: 30083660.
21. Rodriguez Paris V, Solon-Biet SM, Senior AM, Edwards MC, Desai R, Tedla N, Cox MJ, Ledger WL, Gilchrist RB, Simpson SJ, Handelsman DJ, Walters KA. Defining the impact of dietary macronutrient balance on PCOS traits. Nat Commun. 2020 Oct 16;11(1):5262. doi: 10.1038/s41467-020-19003-5. PMID: 33067453; PMCID: PMC7568581.
22. Rosenfield RL, Ehrmann DA. The Pathogenesis of Polycystic Ovary Syndrome (PCOS): The Hypothesis of PCOS as Functional Ovarian Hyperandrogenism Revisited. Endocr Rev. 2016 Oct;37(5):467-520. doi: 10.1210/er.2015-1104. Epub 2016 Jul 26. PMID: 27459230; PMCID: PMC5045492.
23. 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 May;30(5):833-41. doi: 10.1377/hlthaff.2011.0078. PMID: 21555469; PMCID: PMC3877678.
24. Karsten MDA, Wekker V, Groen H, Painter RC, Mol BWJ, Laan ETM, Roseboom TJ, Hoek A. The role of PCOS in mental health and sexual function in women with obesity and a history of infertility. Hum Reprod Open. 2021 Oct 22;2021(4):hoab038. doi: 10.1093/hropen/hoab038. PMID: 34877412; PMCID: PMC8643501.
25. 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 Jan 19;24(3):2019. doi: 10.3390/ijms24032019. PMID: 36768340; PMCID: PMC9916361.

26. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep. 2017 Mar 3;7:42717. doi: 10.1038/srep42717. PMID: 28256516; PMCID: PMC5335600.
27. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev. 2001 Mar 1;46(1-3):3-26. doi: 10.1016/s0169-409x(00)00129-0. PMID: 11259830.
28. Lipinski CA. Lead- and drug-like compounds: the rule-of-five revolution. Drug Discov Today Technol. 2004 Dec;1(4):337-41. doi: 10.1016/j.ddtec.2004.11.007. PMID: 24981612.
29. Veber DF, Johnson SR, Cheng HY, Smith BR, Ward KW, Kopple KD. 2002. Molecular properties that influence the oral bioavailability of drug candidates. *J Med Chem.,* Jun 6;45(12):2615-23. doi: 10.1021/jm020017n. PMID: 12036371.
30. Egan WJ, Merz KM Jr, Baldwin JJ. 2000. Prediction of drug absorption using multivariate statistics. *J Med Chem*., Oct 19;43(21):3867-77. doi: 10.1021/jm000292e. PMID: 11052792.
31. Ghose AK, Viswanadhan VN, Wendoloski JJ. 1999. A knowledge-based approach in designing combinatorial or medicinal chemistry libraries for drug discovery. 1. A qualitative and quantitative characterization of known drug databases. *J Comb Chem.,* Jan;1(1):55-68. doi: 10.1021/cc9800071. PMID: 10746014.
32. Muegge I, Heald SL, Brittelli D. 2001. Simple selection criteria for drug-like chemical matter. *J Med Chem.,* Jun 7;44(12):1841-6. doi: 10.1021/jm015507e. PMID: 11384230.
33. Baell JB, Holloway GA. New substructure filters for removal of pan assay interference compounds (PAINS) from screening libraries and for their exclusion in bioassays. *J Med Chem*., 2010 Apr 8;53(7):2719-40. doi: 10.1021/jm901137j. PMID: 20131845.
34. Brenk R, Schipani A, James D, Krasowski A, Gilbert IH, Frearson J, Wyatt PG. 2008. Lessons learnt from assembling screening libraries for drug discovery for neglected diseases. *Chem Med Chem.* Mar;3(3):435-44. doi: 10.1002/cmdc.200700139. PMID: 18064617; PMCID: PMC2628535.
35. Grant P, Ramasamy S. An update on plant derived anti-androgens. Int J Endocrinol Metab. 2012 Spring;10(2):497-502. doi: 10.5812/ijem.3644. Epub 2012 Apr 20. PMID: 23843810; PMCID: PMC3693613.

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