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# Elucidation of drug-like properties in metabolites of *Curcuma angustifolia* Roxb.

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The plant, *Curcuma angustifolia* Roxb., belonging to the family Zingiberaceae was subjected to a pot experiment to study the effects of arbuscular mycorrhizal fungi (AMF) on its metabolome. The experiment consisted of two treatments: Mycorrhizal (M), *C. angustifolia* plants inoculated with a consortium of AMF and non-mycorrhizal control plants (NM), plants not inoculated with AMF. GC-MS analyses revealed that AMF colonization modulated the composition and concentration of metabolites in different plant parts, leaves, rhizomes and roots as compared to their respective NM counterparts. However, n-hexadecanoic acid, 5-Hydroxymethylfurfural, 9, 12-octadecadienoic acid and terpineol were observed in all the parts in higher percentages. Cis-vaccenic acid was found in the highest concentration in rhizomes while roots showed a high concentration of coronarin E. These metabolites were found to possess good characteristics of being therapeutic drugs and their predicted targets are involved in different diseases such as obesity, diabetes mellitus, hypertension, cancers, etc. They also have many structurally similar compounds to the metabolites which are active against various protein targets involved in different diseases. Among them, terpineol has the Androgen receptor (AR) as the highest probable target whose roles have been reported in several cancers, especially prostate cancer. AR showed a thermodynamically favourable binding affinity with terpineol (ΔG = -6.2 kcal/mol). Similarly, other metabolites also showed binding affinities with their predicted targets. Thus, in the study, we report various metabolites of *C. angustifolia* and their potential characteristic drug-like properties, which may serve as a green alternative for better health care to mankind.

**Keywords**: Arbuscular mycorrhizal fungi, *Curcuma angustifolia*, Drugs, Metabolites, Therapeutic potential

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## Introduction

*Curcuma angustifolia* Roxb., commonly known as East Indian Arrowroot or white turmeric is a rhizomatous plant, belonging to the family, Zingiberaceae. The plant is prevalent in India and other South Asian countries, Bangladesh, Laos, Myanmar Nepal, Pakistan and Thailand[1]. The rhizome and flower of the plant are consumed as food. The starch obtained from the rhizome of the plant, *Tikhur* or *Tugaksheeree*, is a preferred food for babies as it is easily digestible[2]. It is also used for the preparation of sweets[3]. Tikhur is also an important component of Ayurvedic medicines. It is thus, not surprising that the rhizomes were used to treat many illnesses or diseases such as the treatment of bone fractures, inflammation and intestinal diseases, peptic ulcers, colitis, diarrhoea and dysentery, cough and bronchitis, diabetes etc[4-6]. The leaves of the plant are also endowed with antimicrobial and antioxidant activities[7,8]. These medicinal properties of the plant have been attributed to the presence of a multitude of bioactive compounds. Shrivastava *et al.*[8], identified 81 and 87 volatile components from the rhizome of *C. angustifolia* plants collected from the Central and Southern parts of India, respectively. Jena *et al.* [7], identified 32 and 35 essential oils in the leaves and rhizomes of the plant, respectively. They also showed that essential oils present in the leaves were more efficient in scavenging reactive oxygen species than those found in rhizome[7]. These findings indicate that the plant has enormous therapeutic potential. However, literature surveys revealed relatively lesser studies on C. *angustifolia* than its more popular relative *Curcuma longa.* Thus, profiling the metabolome present in the whole plant and presenting their drug-likeness would aid in tapping the therapeutic potential to its optimum level.

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Supplementary table is available online only.

Correspondingly, *C. angustifolia* is gaining importance in the medicinal plant world and is now mass cultivated in Chhattisgarh, Madhya Pradesh and Jharkhand. The application of organic manure has shown positive results in improving the growth and biomass of the plant[9]. Thus, it provides a reason to explore more alternatives of natural sources of plant growth promoters. In this regard, arbuscular mycorrhizal fungi (AMF) can serve as a potential biofertilizer. AMF are soil-borne symbiotic fungi of the phylum Glomeromycota. AMF inoculation is synonymous with improved plant growth, biomass and secondary metabolite concentration in many plants[10] including members of Zingiberaceae, such as *C. longa*[11,12] and *C. xanthorrhiza*[13]. Thus, with this background, the study was conducted to determine the influence of AMF on growth, biomass and metabolite concentrations in different plant parts, and analyze the therapeutic potentials of the metabolites present in them.

## Materials and Methods

### Collection of AMF and plant material

*Curcuma angustifolia* rhizomes along with the rhizosphere soil were collected from the wild from Khurkhul Makha Leikai (24°54'45.0"N 93°51'36.9"E), Imphal West district, Manipur in 2016 and brought to the laboratory. To ensure the correct identification of the plant, the rhizomes were collected during the flowering season. The soil was air-dried for further investigations and the rhizome was used for raising a nursery.

### Identification of AMF and preparation of AMF inoculum

The rhizosphere soil was initiated for trap culture using *Trigonella foenum-graecum* L. and *Zea mays* L. in autoclaved sand: soil mixture. The plants were watered regularly with autoclaved tap water. AMF spores were isolated from the culture using the wet-sieving and decanting technique[14]. The spores were identified morphologically by consulting the identification guides available at INVAM (International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi). The identified AMF were *Sclerocystis rubiformis, Claroideoglomus claroideum, C. etunicatum*, *Glomus multicaule, Acaulospora dilatata, Acaulospora laevia* and *Glomus aureum.* The AMF inoculum was then prepared by trap culturing the spores as a consortium following the procedure described above. Ten grams of the inoculum consisted of 70 spores along with chopped AMF-colonized roots of trap plants.

### Raising nursery

A nursery of *C. angustifolia* plants was raised using the rhizomes of these plants on autoclaved soil: sand (1:1) mixture. The soil was collected from the Botanical Garden, Rajiv Gandhi University, Doimukh, Arunachal Pradesh (India) and had the following characteristics: pH 5.282, electrical conductivity 0.072 mS, soil organic carbon 11.82%, total nitrogen 0.180%, phosphorus 0.098% and calcium 0.736%. The plants were watered with autoclaved tap water and allowed to grow under natural conditions of temperature, light and humidity.

### Pot experiment

A pot experiment was carried out in the Department of Botany, Rajiv Gandhi University under natural conditions of temperature, humidity and light. The experiment consisted of two treatments, namely, i) Non-mycorrhizal, NM (control plants without AMF) and ii) Mycorrhizal, M plants (inoculated with AMF). Each treatment was replicated four times. Before transplantation, M plants received 10 g of soil inoculum while the NM plants received a filtrate of the soil inoculum to allow uniform microbial population. Uniform-sized rhizomes from the nursery were selected and transplanted into pots (14’x13’’) containing 13 kg of autoclaved soil: sand (1:1) mixture. The plants were watered twice a week with autoclaved tap water.

### Parameters studied

#### *AMF colonization and plant biomass*

The plants were harvested after three months and separated into roots and shoots. The lengths and fresh weights of roots and shoots were measured immediately. Dry weights were measured after drying the plant parts in a hot air oven at 70°C for 72 h. The AMF-colonization was evaluated according to Philips and Hayman[15].

#### *Profiling of metabolites*

The different plant parts, leaf, rhizome and root were harvested, washed thoroughly and dried under shade. The dried samples were then pulverized using a pestle and mortar. The phytochemicals present in these plant parts were extracted in methanol using a cold extraction method. Two grams of the powdered sample were taken in a conical flask and soaked with 4 mL methanol overnight. The extract was then filtered using Whatman paper No.1. The filtrate was then injected with autoinjector AOC-20i into GCMS Shimadzu QP 2010 Plus with Thermal Desorption

System TD 20 with helium as the carrier. The column used was the Restek GC column, Rxi ®-R Sil MS. The column oven temperature was kept at 60°C and the injection temperature at 260°C. The metabolites detected on the chromatogram were identified using the MS library search (WILEY and NIST14)[16] and their quantities were calculated based on the peak area occupied in the chromatogram.

***Analysis for drug-like characteristics***

Drug likenesses of the metabolites were investigated on those metabolites which were found in the highest concentrations, irrespective of the plant parts as well as the treatment. The chemical structures and canonical smiles formulae of the compounds were retrieved from PubChem[17] and analyzed for their drug likeness using SwissADME software[18]. The drug-likeness properties considered were molecular weight, number of hydrogen bond donor atoms, number of hydrogen bond acceptor atoms, rotatable bonds, solubility, absorption in the gastro-intestinal route, skin, blood-brain barrier etc. and violations of Lipinski rule of five[19].

***Target predictions***

As the drug likenesses of the compounds were confirmed, their potential protein targets were determined using SwissTargetPrediction tool[20]. The highest probable and statistically significant target proteins were then selected and their classes/family e.g., enzyme, G-protein coupled receptors, oxidoreductase, phosphatase, nuclear receptors, proteases, kinases etc. were determined.

***Pathways and disease association studies of the predicted targets***

The top 100 predicted potential targets were selected and the pathways and diseases associated with them were analyzed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database[21] and DisGenNet[22] databases, respectively. Further, the data obtained was incorporated using GeneCodis4[23]. The pathways and diseases which showed statistically significant association were plotted for their numbers of genes (proteins) associated against their -log (adjusted p-value).

***Molecular docking analyses of the metabolite with their respective most probable target protein***

To validate the findings on predicted potential targets, molecular docking analyses of the compounds were conducted with their respective highest probable predicted targets. For the analyses, the three-dimensional (3D) protein structures of the predicted targets were retrieved from the protein data bank, RCSB PDB[24] and the 3D conformer structures of the compounds were retrieved from PubChem[17]. The PDB IDs of the proteins used in the molecular dockings were 5TWO (Peroxisome proliferator-activated receptor gamma, PPARG), 7PLS (Alpha-L-fucosidase I, FUCA1), 1HMR (Fatty acid binding protein-adipocyte, FABP4), 1E3G (Androgen Receptor, AR), 1I7G (Peroxisome proliferator-activated receptor alpha, PPARA) and 1XU7 (11-beta-hydroxysteroid dehydrogenase 1, HSD11B1)[24-27]. In the proteins, ligands for docking were prepared using Chimera[28] and blind dockings were performed using AutodockVina docking software[28-29]. The binding affinities which were recorded as Gibbs free energy (ΔG) and thermodynamically favourable bindings were finally analyzed. The ligand binding with the proteins having the most thermodynamically favourable poses were plotted as a 2D ligand-protein interaction diagram using Discovery Studio Visualizer[30].

## Results

**AMF colonization and plant biomass**

Plants inoculated with the AMF consortium showed the presence of hyphae, arbuscules and vesicles in the root. The per cent AMF colonization was 58±5.91%. The control plants showed no AMF colonization (Table 1). AMF colonization resulted in better growth and higher biomass in *C. angustifolia* plants than the control plants. The lengths as well as fresh and dry weights of shoot and root were significantly higher in AM plants than in the control plants. The AM plants also exhibited significantly more shoots than the control plants (Table 1).

Table 1 — Growth parameters in AMF inoculated and control plants of *Curcuma angustifolia*. Data presented are average of four replicates with a standard deviation

| Parameters | Treatment | | Significance |
|---|---|---|---|
| | Control | AM plants | |
| AMF Colonization | Nil | 58.5±5.91 | *** |
| Shoot length (cm) | 38±6.68 | 39.5±8.1 | * |
| Shoot fresh weight (g) | 47.75±19.78 | 49.28±18.91 | * |
| Shoot dry weight (g) | 8.52±1.38 | 8.75±1.23 | * |
| Number of shoots | 5±1.41 | 7.5±1.29 | ** |
| Root length (cm) | 23.25±6.84 | 30±5.47 | * |
| Root fresh weight (g) | 71.25±10.43 | 124.04±11.80 | *** |
| Root dry weight (g) | 9.56±1.96 | 11.135±3.52 | ** |

* significance at $P$ <0.5; ** significance at $P$ <0.05; *** significance at $P$ >0.005

**Analysis of metabolite profile**

The leaf, rhizome and root of non-mycorrhizal *C. angustifolia* plants contained 92, 89 and 102 compounds, respectively. On the other hand, the mycorrhizal plants showed 87, 101 and 74 compounds in the leaf, rhizome and root, respectively. The compounds with the highest concentration are shown in Table 2. However, the composition of the compounds varied with AMF colonization as well as part of the plant. Of the many compounds, 9, 12-octadecadienoic acid, 5-hydroxymethylfurfural, terpineol and n-hexadecanoic acid were observed in all the plant parts. While cis-vaccenic acid is the major component in the rhizome, in roots, the principal component is coronarin E.

AMF colonization was found to increase the concentration of 5-hydroxymethylfurfural in leaf and rhizome by 74 and 131%, respectively, over the NM counterparts. However, 5-hydroxymethylfurfural concentration was lower in the root of M plants as compared to NM plants. Alternatively, the concentration of n-hexadecanoic acid was lower in the leaf and rhizome but higher in the root as compared to control plants. In rhizomes, AMF colonization showed more than a 30% increase in the concentration of cis-vaccenic acid. In the root, AMF colonization resulted in a decrease in the concentration of Coronarin E, Terpineol and 5-hydroxymethylfurfural while increasing the concentration of n-hexadecanoic acid, 2-oxabicyclo[2,2,2]octane,1,3,3-trimethyl-, (E)-15,16-Dinorlabda-8(17),12-dien-14-al and cis-vaccenic acid.

**Drug-like properties of the *C. angustifolia* metabolites**

The compounds, 9, 12-octadecadienoic acid, 5-Hydroxymethylfurfural, n-Hexadecanoid acid, Terpineol, Cis-vaccenic acid and Coronarin E occurring in highest concentrations in the plant parts (Table 2) were found to possess drug-like physicochemical characteristics obeying Lipinsky's rule of five[19] (Supplementary Table 1). The compounds, 9,12-Octadecadienoic acid, Palmitic Acid, Cis-vaccenic acid and Coronarin E showed one violation each since one of the derivatives of lipophilicity measurements, MLOGP>4.15 but other characteristics viz, molecular weights, number of hydrogen bond acceptors, donors, lipophilicity and solubility were favourable (Supplementary Table 1). All the metabolites showed high Gastrointestinal (GI) absorptions except Coronarin E and high synthetic accessibility apart from the other fundamental physicochemical properties (Supplementary Table 1). These characteristics of the metabolites expressed their potential drug-like properties.

***Potential targets, their classes, associated diseases and docking analyses with metabolites***

The predicted target proteins of the metabolites are presented in Table 3. Pathway and disease association

Table 2 — Phytochemical compounds with the highest concentration in leaf, rhizome and root of M and NM *C. angustifolia* plants. Percentages are indicated in parentheses

| S. No | NM (control) | M |
|---|---|---|
| | Leaf | |
| 1 | n-hexadecanoic acid (15.73%) | 5-hydroxymethylfurfural (19.86%) |
| 2 | 5-hydroxymethylfurfural (11.36%) | n-hexadecanoic acid (11.84%) |
| 3 | (-)-5-OXATRICYCLO[8.2.0.0(4,6)]DODECANE,, 12-TRIMETHYL-9-METHYLENE-, [1R (12.81%) | (-)-5-OXATRICYCLO[8.2.0.0(4,6)]DODECANE,,12-TRIMETHYL-9-METHYLENE-, [1R (9.87%) |
| 4 | BICYCLO[7.2.0]UNDEC-4-ENE,4,11,11-TRIMETHYL-8-METHYLENE-,[1R-(1R*,4E,9S (7.60%) | 9,12-Octadecadienoic acid (Z,Z)- (9.10%) |
| | | 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (6.58%) |
| | Rhizome | |
| 5 | Cis-vaccenic (29.49%) | Cis-vaccenic acid (38.83%) |
| 6 | n-hexadecanoic acid (24.34%) | 5-hydroxymethylfurfural (18.98%) |
| 7 | 5-hydroxymethylfurfural (8.20%) | n-hexadecanoic acid (12.05%) |
| 8 | Terpineol (3.47%) | Octadecanoic acid (3.27%) |
| 9 | | 1,5-ANHYDRO-6-DEOXYHEXO-2,3-DIULOSE (3.07%) |
| | Root | |
| 10 | Coronarin E (18.93%) | Coronarin E (16.81%) |
| 11 | 5-hydroxymethylfurfural (10.72%) | n-hexadecanoic acid (10.21%) |
| 12 | Terpineol (10.54%) | 2-OXABICYCLo[2,2,2]OCTANE,1,3,3-TRIMETHYL- (9.91%) |
| 13 | 2-OXABICYCLo[2,2,2]OCTANE,1,3,3-TRIMETHYL- (9.80%) | Terpineol (8.96%) |
| 14 | n-hexadecanoic acid (8.47%) | (E)-15,16-Dinorlabda-8(17),12-dien-14-al (8.24%) |
| 15 | | Cis-vaccenic acid (6.49%) |

Table 3 — Predicted top 15 highest probable targets of the compounds identified from *C. angustifolia* Roxb

| S. No. | Metabolites | | | | | |
|---|---|---|---|---|---|---|
| | 9,12-octadecadienoic acid | 5-Hydroxymethyl furfural | n-Hexadecanoid acid (Palmitic acid) | Terpineol | Cis-vaccenic acid | Coronarin E |
| 1 | PPARG | FUCA1 | FABP4 | AR | PPARA | HSD11B1 |
| 2 | PPARA | PIM1 | PPARA | CYP19A1 | PPARD | FKBP1A |
| 3 | PPARD | GBA | FABP3 | CA2 | FABP4 | CXCR3 |
| 4 | FFAR1 | HEXA | FABP5 | CA1 | FABP3 | SLC6A2 |
| 5 | FABP4 | HEXB | PPARD | CA4 | FABP5 | SLC6A4 |
| 6 | FABP3 | OGA | FABP2 | TRPM8 | SCD | SLC6A3 |
| 7 | PTGS1 | CDA | FFAR1 | CHRM2 | FAAH | C5AR1 |
| 8 | SCD | ADA | SLC22A6 | SLC6A4 | PPARG | TRPA1 |
| 9 | FABP5 | CA9 | CDC25A | NR1H3 | TERT | CHRM2 |
| 10 | FAAH | CA12 | HSD11B1 | PTPN1 | FABP1 | CHRM1 |
| 11 | TERT | HSP90AA1 | AKR1B10 | NR1I3 | PTPN1 | P2RX7 |
| 12 | FABP1 | KDM4E | POLB | SREBF2 | FFAR1 | BRD4 |
| 13 | CNR1 | CA2 | VDR | NPC1L1 | PTPN2 | BRD2 |
| 14 | ALOX5 | CA1 | NR1H4 | BCHE | PTGS1 | BRD3 |
| 15 | PTPN1 | TYR | PHF8 | ESR1 | HMGCR | SRD5A1 |

Table 4 — Molecular docking binding affinities (ΔG) of the metabolites of *C. angustifolia* with their respective highest probable targets.

| S. No. | Molecule | Target protein | Binding affinity (ΔG) kcal/mol |
|---|---|---|---|
| 1 | 9,12-octadecadienoic acid | PPARG | -6.3 |
| 2 | 5-Hydroxymethylfurfural | FUCA1 | -4.4 |
| 3 | n-Hexadecanoid acid (Palmitic acid) | FABP4 | -5.8 |
| 4 | Terpineol | AR | -6.2 |
| 5 | Cis-vaccenic acid | PPARA | -5.3 |
| 6 | Coronarin E | HSD11B1 | -8.5 |

analyses of the highest probable 15 target proteins of the compounds revealed their involvement in important metabolic pathways and association with different diseases. Molecular interaction analyses between the six metabolites with their respective highest probable targets by molecular docking also endorse the target predictions. The compounds showed thermodynamically stable interactions in the protein-ligand adducts (Table 4). The binding affinity between the proteins and the ligands was facilitated by forming hydrogen bonds, Van der Waal's interactions, alkyl and pi-alkyl bonds. The compounds, their target proteins and their interactions are described below:

***9, 12-Octadecadienoic acid*:** The potential targets of 9, 12-Octadecadienoic acid belong to different categories and examination of the top 15% target genes (proteins) showed that they were mainly fatty acid binding proteins and nuclear receptors (Fig. 1a). Enrichment analysis of the targets of 9, 12-Octadecadienoic acid showed enrichment of PPAR signaling, Insulin resistance, lipid metabolism, AMPK signaling pathways etc (Fig. 2a). In addition, disease association analysis of the targets of 9, 12-Octadecadienoic acid showed that reperfusion injury, obesity, lipoidosis, kidney necrosis, liver carcinoma, insulin resistance etc., were significantly associated (Fig. 3a). The interaction between 9, 12-octadecadienoic acid and the highest probable target, Peroxisome proliferator-activated receptor gamma (PPARG) showed two hydrogen bonds with Glycine 284 and Serine 342 with the binding affinity of ΔG, -6.3 (Fig. 4a).

***5-Hydroxymethylfurfural*:** The majority of its highest probable targets of the compound were enzymes, lyases and hydrolases (Fig. 1b) and Alpha-L-fucosidase I (FUCA1) was its most probable target (Table 3). Nitrogen metabolism, glucan metabolism, glycosphingolipid metabolism, amino acid and nucleotide metabolisms were significantly enriched among the targets of the compound (Fig. 2b). These targets were found to be associated with many diseases including Gaucher disease, fucosidase deficiency disease, hexosaminidase A deficiency, pyelonephritis, Sandhoff disease and infantile Sandhoff disease (Fig. 3b). 5-Hydroxymethylfurfural formed thermodynamically stable interactions with two hydrogen bonds with Arginine 259 and Tryptophan 71 with its highest probable target protein, Alpha-L-fucosidase I (FUCA1) (Fig. 4b).

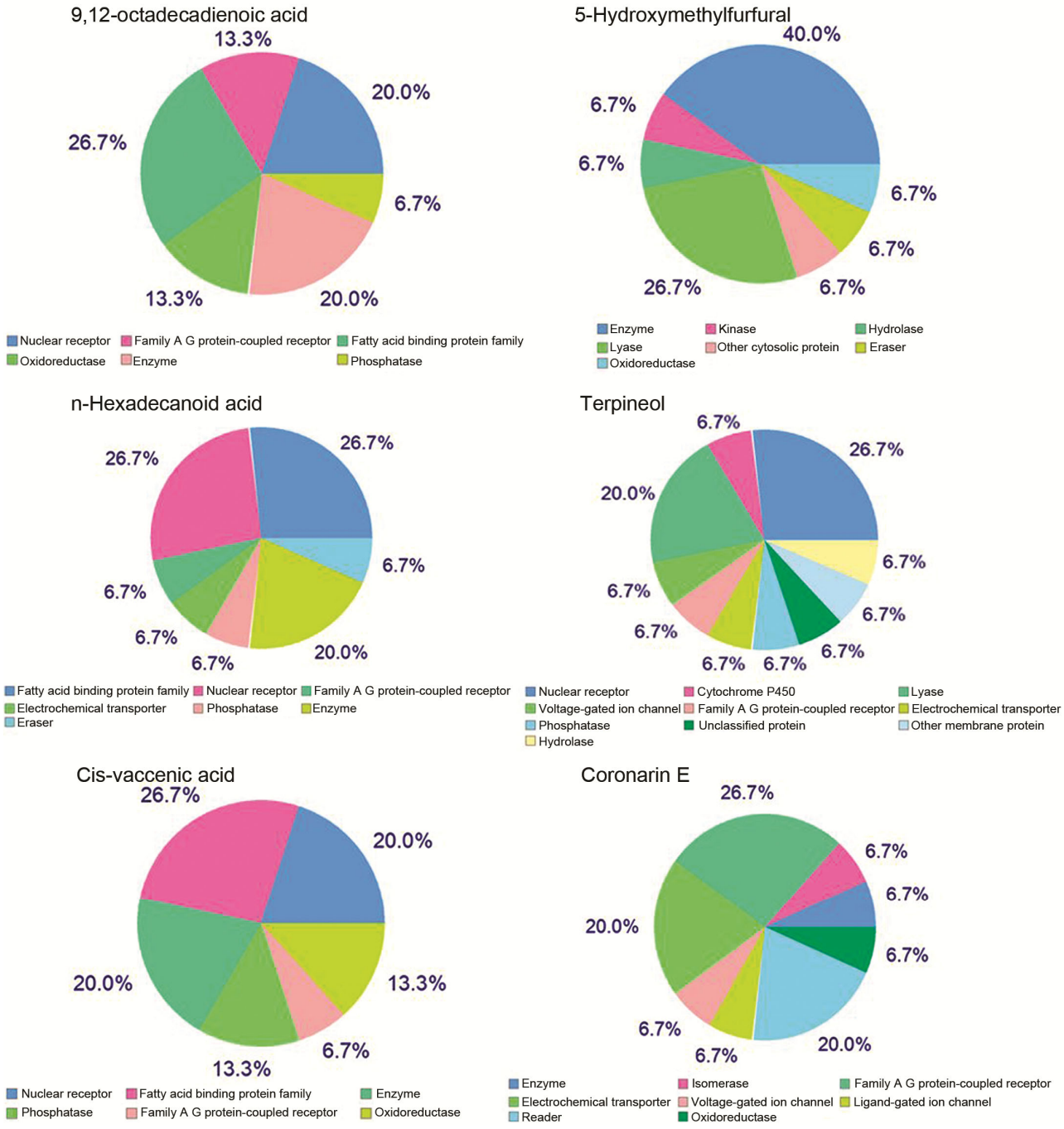


Fig. 1 — Class of the top 15% targets of the six metabolites.

***n-hexadecanoic acid*:** Fatty acid binding proteins and nuclear receptors constitute the top 15% potential targets of n-hexadecanoic acid (Fig. 1c). Fatty acid binding protein adipocyte (FABP4) was the highest probable target of the metabolite (Table 3). PPAR signaling, chemical carcinogenesis, carbohydrate and lipid metabolisms etc. were highly enriched among the metabolite targets (Fig 2c). The targets of the metabolite were also significantly associated with neoplasms, fatty liver diseases, steatohepatitis, myopathy, Crohn's disease, cholestasis etc. (Fig. 3c). Interaction between the metabolite and its most probable target, Fatty acid binding protein-adipocyte (FABP4) showed thermodynamically stable three hydrogen bonds between Glutamine 95 and Arginine 78 of FABP4 (Fig. 4c).

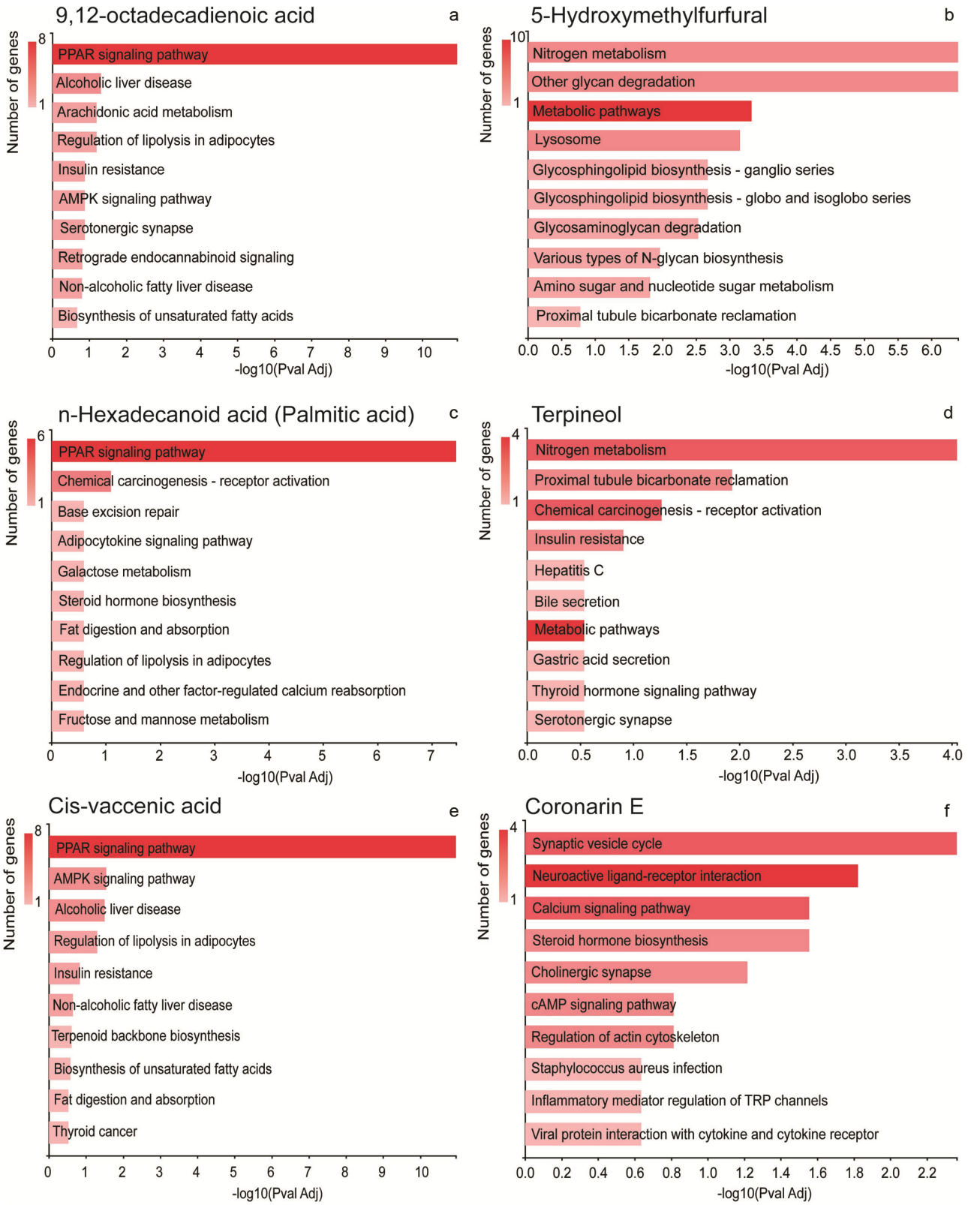


Fig. 2 — Enriched pathways of the top 15 predicted targets of the six metabolites.

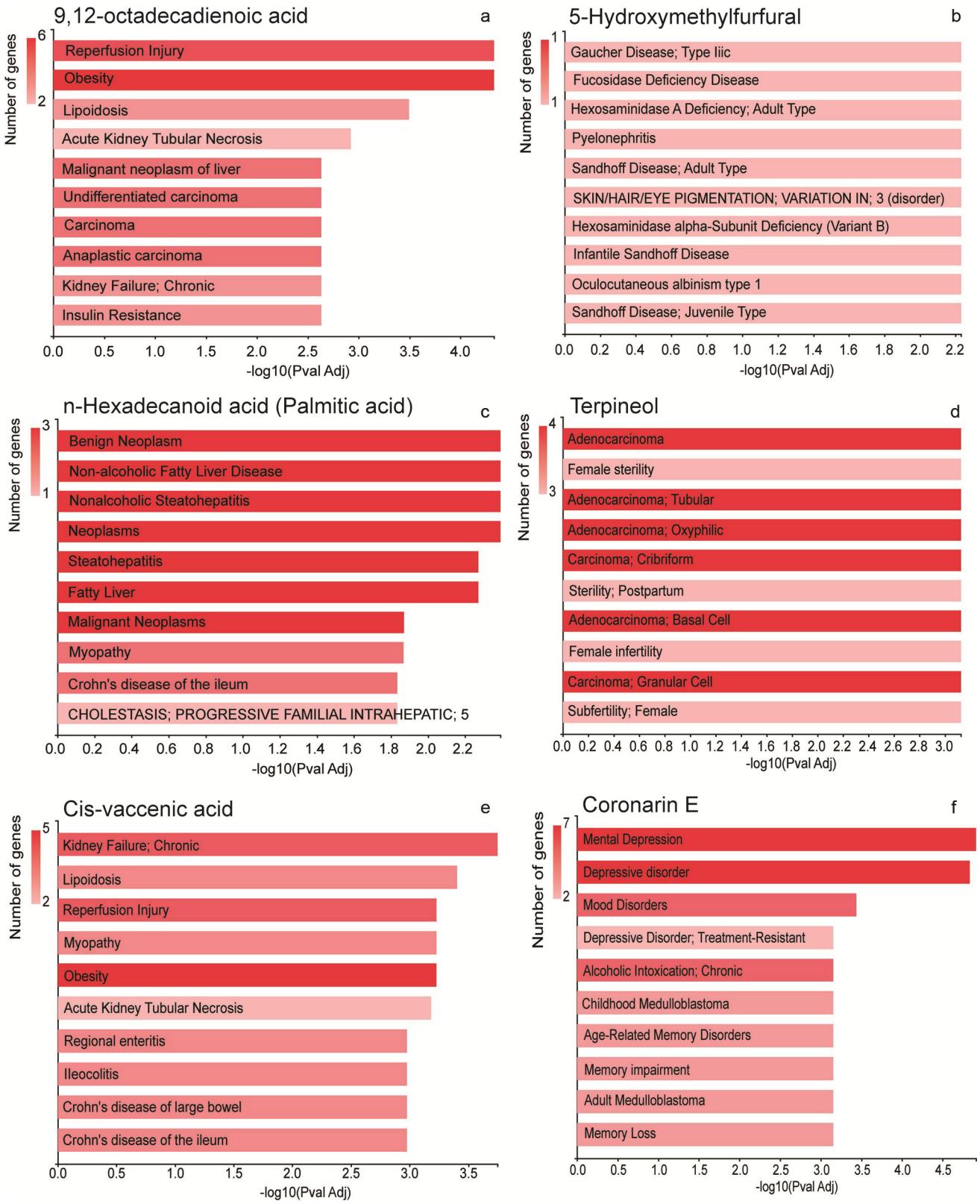


Fig. 3 — Diseases associated with the top 15 predicted targets of the six metabolites.

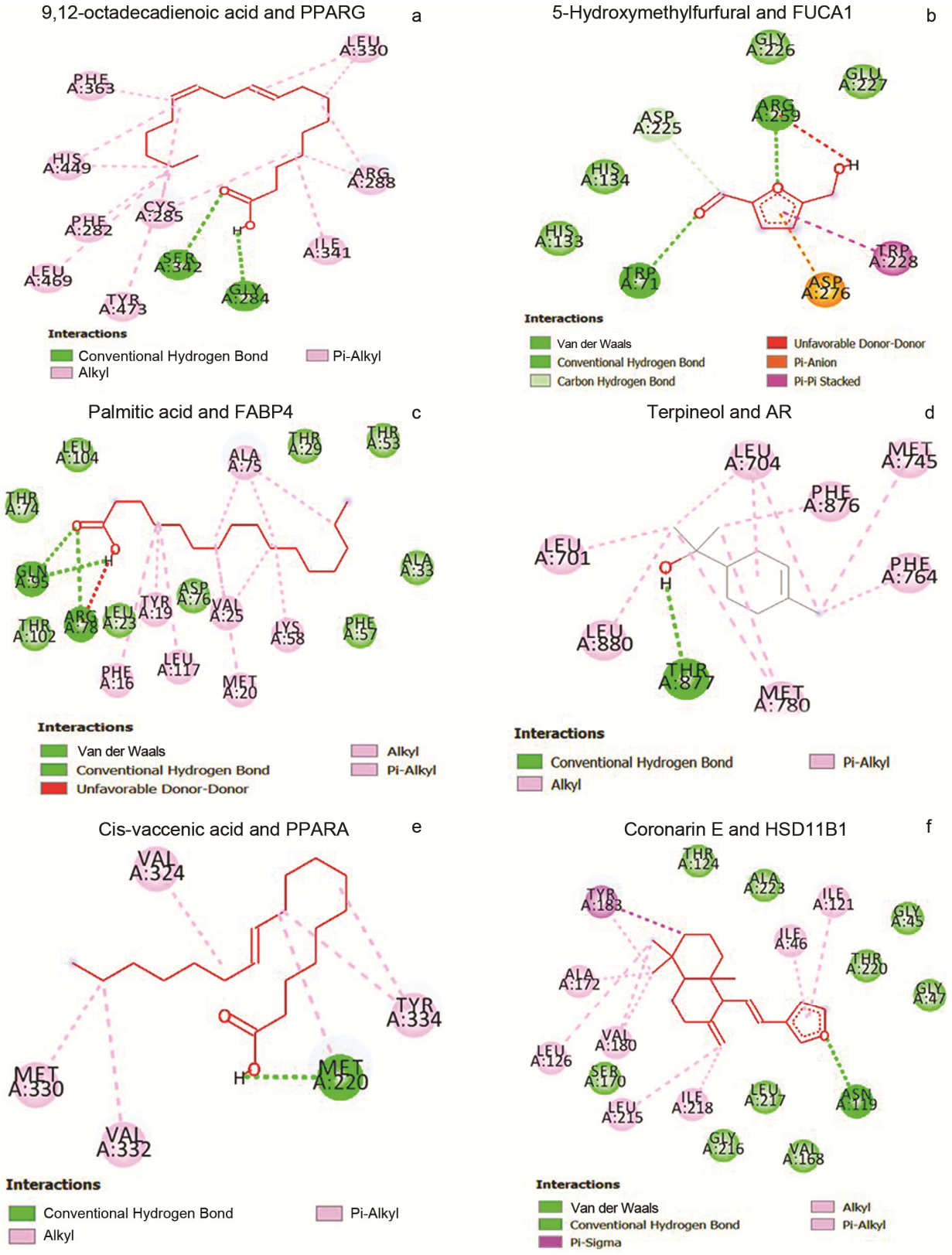


Fig. 4 — Interactions between the metabolites and their respective target genes.

***Terpineol***: Androgen Receptor (AR) was the most probable target of terpineol (Table 3) and its top 15% probable targets include nuclear receptor proteins and lyase enzymes (Fig. 1d). Nitrogen metabolism, proximal tubule bicarbonate reclamation, insulin resistance, Hepatitis C, gastric and bile secretion, thyroid hormone signaling pathways etc. were the significantly enriched pathways of the predicted targets of Terpineol (Fig. 2d). These targets were associated with tubular, oxyphilic, basal cell adenocarcinomas, cibriform and granular cell carcinomas, postpartum sterility and female infertility (Fig. 3d). Terpineol formed adducts with their highest probable targets, Androgen Receptor (AR) with one hydrogen bond. Formation of hydrogen bonds and other bonds, especially Van der Waal's interactions between these adducts exhibited their stable complexes (Fig. 4d).

***Cis-vaccenic acid***: The potential targets of the metabolite belong to different categories and examination of the top 15% target genes (proteins) of Cis-vaccenic acid showed that they were mainly fatty acid binding proteins and nuclear receptors (Table 3, Fig. 1e). Peroxisome proliferator-activated receptor alpha (PPARA) was the most probable target of cis-vaccenic acid. PPAR signaling, AMPK signaling, alcoholic liver disease, lipolysis, insulin resistance, non-alcoholic fatty liver, fat metabolisms, thyroid cancer etc. were the pathways enriched among the targets of the compound (Fig. 2e). The major diseases associated with the targets of the compound include chronic kidney failure, lipidosis, reperfusion injury, myopathy, obesity, acute kidney tubular necrosis, regional enteritis, Crohn's disease etc. (Fig. 3e). Cis-vaccenic acid formed adducts with its highest probable target, Peroxisome proliferator-activated receptor alpha (PPARA) with one hydrogen bond indicating a stable complex (Fig. 4e).

***Coronarin E***: The compound was found to target Family A G protein-coupled receptor proteins and electrochemical transporter proteins mostly (Table 3, Fig. 1f). 11-beta-hydroxysteroid dehydrogenase 1 (HSD11B1) was its highest probable target (Table 3). Synaptic vesicle cycle, neuroactive ligand-receptor interaction, calcium signaling pathway, cAMP signaling pathway, inflammatory mediator regulation of TRP channels, steroid hormone biosynthesis, viral protein interaction with cytokine and cytokine receptor etc. were enriched among the targets of Coronarin E (Fig. 2f). The targets of Coronarin E were found to be strongly associated with diseases viz, mental depression, chronic alcoholic intoxication, childhood and adult medulloblastoma, age-related memory disorders, etc. (Fig. 3f). Coronarin E formed adducts with its highest probable target, 11-beta-hydroxysteroid dehydrogenase 1 (HSD11B1) with one hydrogen bond each indicating stable complex (Fig. 4f).

## Discussion

In the study, it was observed that inoculation of *C. angustifolia* plants with AMF increased plant growth and biomass. This observation is in accordance with earlier reports on other species of *Curcuma*, such as *C. longa*[11,12] and *C. xanthorrhiza*[13], as well as plants belonging to other families[10]. This may be attributed to the AMF's ability to explore and uptake more water and nutrients, often increasing biomass and their quality[31].

AMF colonization was found to influence the composition as well as the concentration of metabolites in *C. angustifolia* plants. The number of metabolites observed varied with different plant parts and treatments. The concentration of a specific metabolite varied with the plant parts. Our findings are similar to earlier findings wherein AMF colonization was reported to have a direct relationship with the accumulation of metabolites, such as carotenoids, flavonoids, polyphenols, and terpenes in medicinal plants[10]. Our study also demonstrates the feasibility of focusing on a specific plant part for the extraction of a specific compound. Our findings clearly indicate that AMF inoculation can increase the concentration of bioactive compounds by increasing the biomass (roots, rhizomes and shoots) of *C. angustifolia* plants.

Traditionally, *C. angustifolia* rhizomes have been consumed as food as well as used in the treatment of diseases. These medicinal attributes may be related to volatile components and essential oils present in rhizomes and leaves[7,8]. However, extensive scientific research on their medicinal properties is not yet reported. The present study reports the metabolome of the whole plant, *C. angustifolia* for the first time revealing that six metabolites viz, 9, 12-octadecadienoic acid, 5-Hydroxymethylfurfural, n-Hexadecanoid acid, Terpineol, Cis-vaccenic acid and Coronarin E are the major constituents. Physicochemical analyses of these metabolites also revealed their drug-like properties obeying Lipinski's

rule along with other rules of drug-likeness. They also have affinities with different proteins and prediction of their potential revealed important target proteins involved in different diseases.

9, 12-Octadecadienoic acid is an unsaturated fatty acid, prevalent in plant glycosides. It is used in the biosynthesis of prostaglandins and cell membranes in mammals[32]. Also known as alpha-linoleic acid, it is part of common natural diets of people but being an unsaturated fatty acid, its potential in increasing lipolysis, reducing obesity and lowering the risk of type 2 diabetes mellitus (T2DM) has been reported[33]. The most probable target of the compound was PPARG. It is a transcription factor belonging to the nuclear receptor family and is involved in glucose metabolism[34]. Activation of PPARG by agonists was reported to increase glucose absorption in skeletal muscles in hyperglycemic conditions in diabetes[35].

5-Hydroxymethylfurfural is known to possess antioxidant properties, block immune-mediated allergic reactions as well as adverse effects of hypoxia and help in preventing sickle haemoglobin by downregulating xanthine oxidase[36]. It is also present in numerous dietary sources and it is generally not toxic and has many health benefits[37]. But its metabolites, especially 5-sulfoxymethylfurfural (SMF) is carcinogenic and could also cause different other pathologic conditions other than cancers[38]. Our study revealed that Alpha-L-Fucosidase 1 (FUCA1) is the most probable target of 5-Hydroxymethylfurfural. FUCA1 encodes a lysosomal enzyme involved in the metabolisms of glycolipids and glycoproteins[26]. Mutation in FUCA1 can cause fucosidosis, a neurodegenerative lysosomal storage disorder resulting in psychomotor retardation, neurological disorders, angiokeratoma, dysostosis multiplex etc. among human[26].

Terpineol is known for its many biological functions. The compound has cytotoxic and insecticidal properties and the ability to scavenge reactive oxygen species (ROS). It can also be used as an antiepileptic, antiulcer, antihypertensive, and anti-nociceptive compound[39]. The study revealed that the androgen receptor (AR) is the most probable target of terpineol. AR is a steroid hormone nuclear receptor which mediates the actions of androgens (testosterone and dihydrotestosterone)[40] Mutation of AR and deregulation of it's signaling pathways have been associated with different disorders and diseases especially, prostate cancer[40-42].

N-hexadecanoic acid has the potential to act as an antioxidant, anticancerous, hypercholesteromic, nematicide, and pesticide[43]. FABP4 is the highest probable target protein of palmitic acid, is involved in fatty acid metabolism in adipocytes, and is associated with liposarcoma of bone and familial partial lipodystrophy[44].

Cis-vaccenic acid is a long-chain fatty acid. Its most probable target is PPARA, a steroid hormone receptor which mediates the action of peroxisome proliferators involved in the metabolism of lipids and cholesterol in peroxisomes[45]. The target protein is mainly associated with liver diseases and recently its involvement in innate immune modulation has been reported[45,46].

Coronarin E is a diterpenoid and its most probable target is Hydroxysteroid 11-Beta Dehydrogenase 1 (HSD11B1), a microsomal enzyme which catalyzes the reversible interconversion of glucocorticoids such as cortisol to cortisone and vice versa[47]. HSD11B1 has been associated with diseases such as obesity, insulin resistance, cortisone reductase deficiency 2, diabetes mellitus type 2 and hyperandrogenism etc[47].

## Conclusion

AMF colonization improved the growth and biomass of *C. angustifolia* plants indicating its potential as a biofertilizer for the cultivation of the plant. This reaffirms the benefits of using an eco-friendly sustainable means of a plant growth stimulant in AMF. Colonization with AMF also influenced the composition and concentration of metabolites of *C. angustifolia*, as revealed by GCMS analyses. *In silico* analyses of the metabolites with their most probable targets validate their affinities towards each other. These findings indicated that these metabolites have the potential to be investigated for their characteristic properties which could be further developed into drugs targeting the above important pathways and diseases.

## Conflict of interest

There are no conflicts of interest among the authors.

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