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Cananga odorata (Ylang-Ylang) modulate pathways involved in cancer: Gene set enrichment and network pharmacology approach

Research Article

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Abstract

Traditionally, *Cananga odorata* Hook. F. & Thomson (Ylang-Ylang) is commonly used to treat various diseases and has been assessed for anti-cancer potency in experimental animal models. However, the affinity of its phytocompounds with multiple proteins involved in the pathogenesis of cancer has not been illuminated yet. The present study was framed to elucidate the molecular mechanisms of *Cananga odorata* for its anticancer activity *via* compound-gene set pathway enrichment analysis, network pharmacology, and docking studies. Initially, phytocompounds were retrieved from herbs databases and literature. Structural information of each compound was obtained from the PubChem database. Druggable characteristics and side effects were predicted using MolSoft and ADVERpred. ADMET profile was predicted using PreADMET online server. Possible target proteins of each compound were predicted by BindingDB ($p \geq 0.7$). Compounds modulating the target proteins associated with the cancer were separated based on the successful and approved targets available in the Therapeutic Target Database. STRING and KEGG pathway database was used to analyze the molecular pathways modulated by the protein targets. The interaction between compounds, proteins, and pathways was constructed by Cytoscape 3.6.1, and docking of compounds with protein target was performed using AutoDock 4.2. Among 26 compounds, 12 phytocompounds were identified to modulate 34 pathways associated with cancer. 4-hydroxy-5,6,7-trimethoxyflavanone and Reticuline showed the maximum interactions with proteins involved in cancer. All 12 compounds obeyed the rule of five and p-methoxybenzaldehyde scored the highest drug-likeness score. Micheline A and Anonaine showed the highest binding affinity with Ubiquitin-protein ligase E3 (MDM2). The current study provides the molecular documentation of phytocompounds from *Cananga odorata* in the regulation of multi-proteins and pathways associated with progression of cancer (mainly Gastric, Melanoma, Prostate, and Breast cancer), which can be further investigated via wet-lab protocols.

Key Words: *Cananga odorata,* Cancer, Docking, Network pharmacology, *Ylang-Ylang.*

Introduction

Cancer is a polygenic condition that involves multiple proteins in its pathogenesis (1). Cancer is defined as unregulated cell growth, which starts progressing when a cell is somehow altered so that it multiplies out of control. Cells divide and grow uncontrollably, invade nearby parts of the body known as benign tumors (do not spread to other parts of the body), or forming malignant tumors (spread to other parts of the body) (2). Cancer is a leading cause of death responsible for an estimated 9.6 million deaths in 2018 (3). WHO reported that deaths due to cancer worldwide are continued to rise to over 11 million by 2030. About 1 in 6 deaths in the world are caused by cancer (4). Scientists have identified more than 277 different types of cancers but the complete treatment strategy to tackle cancer is not well understood. Several factors are known to exacerbate the disease events which involve gene mutations (5), genetic disorders caused by inheritance or hereditary factors (6), chemical compounds as a foreign body to the host cells which disturb a balance multiple mechanisms of protein molecules and causes abnormal cell growth (7). Importantly, environmental chemicals with carcinogenic properties directly or indirectly affect the cytoplasm and nuclei of cells and lead to genetic disorders and genetic mutations. Viruses, bacteria, and radiation rays are other carcinogenic factors, accounting for about 7% of all cancers (8).

In most cancer cases, activation of oncogenes and/or deactivation of tumor suppressor genes leads to an uncontrolled progression of the cell cycle and inactivation of apoptotic mechanisms. In contrast to benign tumors, malignant tumors acquire metastasis, which is partly due to the suppression of cell adhesion

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receptors necessary for tissue-specific attachment of cells to cells, and activation of receptors that increase cell motility (9). Also, the activation of membrane metalloproteases provides a physical pathway for the spread of metastatic cancer cells. There are various mechanisms by which these genetic and cellular changes occur. The canonical mechanisms are mutation, chromosomal translocation or deletion, and dysregulation of the expression or activity of signaling pathways. These events can activate genes that contribute to dysregulation of the cell cycle and/or inactivation of apoptotic pathways (10). Cancer involves multi-gene and multi-pathway interactions in its pathogenesis due to the hub gene relationship. Many attempts are made to tackle cancer but lack satisfactory treatment/management. Also, the effectiveness of conventional chemotherapy is limited due to serious side effects like myelotoxicity, renal toxicity cardiotoxicity, poor selectivity, and drug resistance (11).

In Ayurveda, traditional herbs are utilized to treat complex diseases like cancer by the local healers and physicians (12). A recently published meta-analysis of 20 randomized controlled trials showed that complementary treatment with herbs improves overall survival in patients with hepatocellular carcinoma and reduces the side effects associated with conventional treatment (13). *Cananga odorata* Hook. f. & Thomson (Annonaceae) is commonly called Ylang Ylang. It is a fast-growing evergreen tree native to much of tropical Asia, from India to Papua New Guinea, and to Queensland, Australia (14). As a traditional folk medicine, plant material is used to treat malaria, tinea, and fever. In previous studies, thirteen alkaloids and twenty terpenoids were identified from the stem (15). *C. odorata* showed a potent cytotoxic effect against hepatocellular carcinoma cancer cell lines, HepG2, and Hep2.2.15 (16), anti-cancer activity in EAC treated mice (17), and exhibited anti-inflammatory effect (18). The *in vivo* and *in vitro* evaluation of Liriodenine, an alkaloid isolated from *C. odorata* showed potent inhibitory activity against topoisomerase II (19).

In the current study, we utilized a compound-gene set pathway enrichment and network pharmacology approach to elucidate the molecular mechanism of *C. odorata*. Unlike the traditional principle of drug development "one drug-one target", network pharmacology seeks to investigate the effect or intervention of drugs on the disease as a whole, based on the synergy of multi-targeted drugs and the basis of a holistic approach at the system level (20). This approach covers "systems biology, network analysis, connectivity, redundancy, and pleiotropy" (21). Recently, a network pharmacological platform has been successfully applied to screen effective ingredients and identify pharmacological mechanisms of herbs (22, 23). The network pharmacological approach to bioactive compounds derived from Chinese traditional medicines provides a new understanding of the systemic relationship between compounds, therapeutic targets, and the disease as a whole and provides a powerful and promising tool for understanding the mechanisms of the disease at a systemic level and uncover the potential bioactive ingredients (24; 25). Figure 1 represents the workflow of the current study.

Materials and Methods

Mining of phytocompounds and drug-like property prediction

Herbs databases such as Dr. Dukes DB (26), Phytochemical interaction DB (27), ChEBI (28), and public repositories were utilized to retrieve the list of the isolated compounds from *C. Odorata*. The chemical structural information i.e. molecular weight (MW), molecular formula (MF), number of hydrogen bond donors (NHBD) and acceptors (NHBA), LogP value, canonical SMILES, and chemical identification number were retrieved from the PubChem chemical database (29). The canonical SMILES were utilized to predict the probable drug-like property by Lipinski's rule of five model in the MolSoft online server (http://molsoft.com/mprop/). Absorption (human intestinal absorption), distribution (BBB (Cbrain/ Cblood) and plasma protein binding, metabolism, excretion, carcinogenicity in rat and mice, hepatotoxicity, nephrotoxicity, cardio-toxicity, etc. profile of each compound were predicted from PreADMET (http://preadmet.bmdrc.org) and ADVERpred web servers (http://www.way2drug.com/adverpred/).

Target identification

Canonical SMILES were queried for the target prediction in BindingDB (30) at the percentage similarity of ≥70% i.e. p-value ≥0.7 with corresponding to the already existed known therapeutic drug molecules. Further, the target proteins involved in cancer were identified corresponding to the known cancer targets reported in the Therapeutic Target Database (TTD) (31). The gene ID of each protein molecule was obtained from the UniProt protein database (32).

Pathway and network analysis

STRING database (33) and KEGG pathway (https://www.genome.jp/kegg/) database were utilized to understand the protein-protein interaction and molecular pathways modulated by the cancer protein targets respectively. The network interaction between compounds, target proteins, and pathways involved in the cancer was constructed by Cytoscape v3.6.1 (34). The color scale and node size were used to interpret the entire network based on the number of edges (edge count). The node with the maximum number of edge counts was represented by a colossal node (35,36,37).

Ligand and protein preparation

Based on the network analysis, compounds with the highest edge count with respected protein targets were further studied for molecular interactions. 3D structures of each compound were retrieved from PubChem in structural data format (.sdf) and converted to a protein data bank format (.pdb) using Discovery Studio Visualizer 2019. Ligand energy was minimized by the mmff94 force field using Marvin Sketch. MDM2

(PDB ID 1T4E), Urokinase-type plasminogen (PDB ID 1EJN) activation, P-glycoprotein 1 (PDB ID 6FN4), RAC-alpha serine / threonine-protein kinase (PDB ID 1UNQ) protein x-ray crystallographic structures were obtained from the PDB (https://www.rcsb.org) and to clean the binding pocket and make calculations easier so that ligand can form satisfactory interactions with the protein, water molecules and heteroatoms contained in protein structure were removed by Discovery studio visualizer 2019. The chain was selected based on its completeness of amino acid residue and the presence of the active site region.

Docking studies

The docking study was performed by Autodock 4.2 (38). The protein was added with hydrogen atoms and Kollman charges. The grid box was set to the active site and the docking process was run using the Lamarckian Algorithm. The ligand poses with the target protein having the lowest binding energy were chosen to visualize the ligand-protein interactions in Discovery Studio 2019 (39, 40).

Results and Discussion

Understanding the mode of the action of phytocompounds present in herbal medicines against complex diseases like cancer via *in vivo* and *in vitro* is difficult. The current study used gene set enrichment, network pharmacology, and docking approach to uncover the molecular mechanisms of *C. odorata*. Twenty-six different phytocompounds were identified in *C. odorata* using herbs databases and literature (Supplementary Table 1). Among Twenty-six compounds, twenty compounds were predicted to modulate 140 protein targets (Supplementary Table 2). Among them, only twelve compounds were identified to modulate 21 target proteins associated with cancer (Supplementary Table 3). These 12 phytocompounds were identified as alkaloid, ester, sesquiterpenoid, flavanone and the targeted cancer protein molecules were identified as surface proteins and enzymes. The compounds modulating proteins involved in the cancer are shown in Table 1.

The rule of five models generated by Lipinski states that compounds have poor absorptivity, bioavailability, and non-drug-like property if their molecular weight is >500g/mol, hydrogen bond donors >5, log P value (partition coefficient) >5, and hydrogen bond acceptors >10 (36). Based on the result obtained from the target prediction, 12 compounds were accessed for their drug-like property. All 12 compounds were predicted to obey the rule of five and except Isosafrole and Isoeugenol, all the compounds showed positive drug-likeness score (DLS). p-methoxy benzaldehyde scored highest DLS i.e. 2.91. Further, compounds were predicted for their probable side effects. Among 12 compounds, 6 compounds showed non-toxic property i.e. Micheline A, Trans- Feruloyltyramine, Alpha-Cadinol, 4'-Hydroxy-5,6,7-Trimethoxyflavanone, Isoeugenol, and Rocaglamide. The drug-likeness and side effects of compounds are shown at Table 2.

Understanding the pharmacokinetics and pharmacodynamics profile of phytocompounds is essential and gives a basic idea of the systemic moment of a drug in the body (41). We predicted the probable ADME profile such as Blood-Brain Barrier, Plasma Protein Binding, Human Intestinal Permeability, etc., and toxicity profile like carcinogenicity in rats and mice, hERG Inhibition, and fish aquatic toxicity. The result showed that all 12 compounds predicted to absorb 90-100% through the human intestine, water solubility at low to high concentration, 30-90% plasma protein binding, and found to be nontoxic in rats and mice, showed low to medium hERG inhibition, predicted to have p<1 for FAT Medika and Minnow toxicity. The ADME and toxicity profile of phytocompounds are shown in Table 3 and Table 4 respectively.

Figure 2 represents the protein-protein interaction network of the 21 protein targets involved in cancer modulated by the phytocompounds from *C. odorata*. The protein-protein interaction was retrieved from the STRING database. Further, the enrichment analysis of 21 protein targets modulated by 12 phytocompounds was carried out utilizing STRING and KEGG pathways database. Results reflected, 21 targets to modulate 53 potential enriched pathways (Supplementary Table 4). A peer interpretation of the gene set enrichment analysis showed 21 targets to modulate 34 molecular pathways involved in cancer. MicroRNAs in cancer, Pathways in cancer, Proteoglycans in cancer, and Gastric cancer were identified as major key pathways regulated by the phytocompounds with the lowest false discovery rate. And also predicted to interfere with breast, colorectal, pancreatic, renal, endometrial, bladder, prostate, and hepatocellular cancer. Further, these 12 compounds are predicted to target PI3K-Akt, cGMP-PKG, Jak-STAT, Ras, Rap1, cAMP, MAPK, ErbB, NF-kappa B, T cell receptor signaling pathways, which are potentially involved in the cancer pathogenesis. These are the most important intracellular signaling pathways, known as a master regulator of cancer, which controls cell cycle, growth, survival, motility, metabolism, and angiogenesis (42) and plays a crucial role in the tumor initiation, progression, and contributing to tumor heterogeneity (43). Targeting these multiple cellular pathways utilizing multi-compounds contained *C. odorata* can result in both increased cellular death and reduced cellular proliferation. The protein molecules that are involved in these pathways which are strongly modulated by the phytocompounds from *C. odorata* are EDNRA, EDNRB, EGFR, ESR1, HGF, MDM2, TERT, AKT1, ABCB1, DNMT1, EGFR, MCL1, MDM2, and PLAU. The pathway modulated by the 12 phytocompounds is represented at Table 5.

The network of compounds-proteins (Figure 3), proteins-pathways (Figure 4), and compounds-proteins-pathways (Figure 5) are constructed by the Cytoscape 3.6.1v. The compound-protein-pathway network consisting of one hundred fifty edges, in which forty-one were compound-protein interactions and one hundred nine were protein-pathway interactions. Further, the constructed network included sixty-seven nodes representing 12 compounds, 21 targets, and 34

molecular pathways. Among all the compounds, 4–hydroxy-5,6,7-trimethoxyflavanone, Micheline A, Reticuline, Anonaine scored the highest edge count. Interaction of 4–hydroxy-5,6,7-trimethoxyflavanone was found with twelve protein molecules i.e. APP, D2R, DNMT1, EDNRA, EDNRB, ESR, HGF, NFKB, ABCB1, AKT1, TERT. Reticuline with nine protein molecules i.e. D2R, D3R, ESR, MC1, MDM 2, ABCB1, TNKS, TBXA2R, PLAU. Micheline A and Anonaine shared four common proteins i.e. D2R, D3R, MCL1, MDM2. The overall complex compound-protein-pathway network is represented in Figure 5.

Based on the network analysis, compounds having the highest edge count with their respective target proteins interaction were predicted using AutoDock 4.2 software. Among the selected compounds, Micheline A scored the lowest BE with MDM2 i.e. -6.3kcal/mol with IC_{50} of 24.09µM via forming two hydrogen bonds i.e. Gly16…O- and Val93…OH. Next to Micheline A, Anonaine scored the lowest BE with MDM2 i.e. -5.84 kcal/mol IC_{50} of 52.68µM via forming one hydrogen bond i.e. Gly16…O-. 4'-Hydroxy-5,6,7-Trimethoxyflavanone scored lowest BE with P-glycoprotein 1 i.e. -3.9kcal/mol with IC_{50} of 1,330µM. The binding affinity, inhibitory constant, and hydrogen bond interaction of each compound with an individual target are shown in Table 6. Binding of Micheline A with active site residues MDM2 binding pocket and its interactions are shown in Figure 6.

Alkaloids are the key important hits from herbs that serve as a rich reservoir in drug discovery (44). Alkaloids isolated from natural plants showed potent antiproliferative and antimetastatic effects on various types of cancer (45). Alkaloids such as vincristine and vinblastine isolated from *Catharanthus roseus* and camptothecin from *Camptotheca acuminata* have already successfully turned into anti-cancer drugs (46, 47). In the present study, twelve compounds including Cinnamyl Acetate, Isosafrole, Anonaine, Micheline A, Reticuline, Trans- Feruloyltyramine, Alpha-Cadinol, 4'-Hydroxy-5,6,7-Trimethoxyflavanone, p-methoxy-benzaldehyde, Isoeugenol, Rocaglamide were identified as potential bioactive phytocompounds against cancer pathogenesis. Among them, Reticuline, Micheline A, Anonaine alkaloids showed the potent inhibitory activity against multiple proteins involved in cancer.

The present study predicts Reticuline, Micheline A, Anonaine to be non-inhibitors of the CYP2C19 enzyme, which metabolizes various antitumor drugs (48, 49) and predicted to have high Human Intestinal Absorption and moderate plasma protein binding. Reticuline, Micheline A, and Anonaine were identified as a potent inhibitor of MDM2 protein, exerts oncogenic activity via inhibition of p53-tumor suppressor (50). Further, Reticuline showed inhibitory activity against PLAU, a gene that encodes a plasminogen urokinase activator and is highly expressed in most prostate cancer cells and cancer cell lines. DNA methylation and gene amplification may play a role in the regulation of PLAU genes in prostate cancer (51). 4'-Hydroxy-5,6,7-Trimethoxyflavanone showed potent inhibitory activity against AKT, also known as protein kinase B (PKB), which is a downstream promoter of PI3K. AKT overexpression has been proposed as a cancer-specific activity (52). AKT activates a series of downstream factors through phosphorylation and thus alters the metabolism of cells that are required in cancer cells (53).

Conclusion

This study identified alkaloids and flavonoids contained in *C. odorata* as potent anticancer agents. Pathways in cancer, proteoglycans in cancer, and microRNAs in cancer were identified as key pathways modulated by the alkaloids and flavonoids, which regulate multiple intracellular signaling pathways involved in the pathogenesis. The docking study identified Micheline A and Anonaine as a potent inhibitor of Ubiquitin-protein ligase E3. Further, the effect of alkaloids and flavonoids enriched fraction on animal models is needed to be carried out to validate the current findings.

Conflict of interest

Authors declare that they do not have any competing interests.

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Table 1: Type of compounds and probable protein targets of cancer modulated by compounds

Compound name	Pubchem CID	Compound class	Protein gene ID
Cinnamyl_acetate	5282110	Ester	CA9
Isosafrole	637796	Benzodioxoles	NFKB, ABCB1
Anonaine	160597	Alkaloid	D2R, D3R, MCL1, MDM2
Micheline_A	197018	Alkaloid	D2R, D3R, MCL1, MDM2
Isoeugenol	853433	Phenylpropanoid	APP, ESR, NFKB, ABCB1, LCK
Reticuline	439653	Alkaloid	D2R, D3R, ESR, MCL1, MDM2, ABCB1, TNKS, TBXA2R, PLAU
Trans_Feruloyltyramine	5280537	Alkaloid	EGFR
Alpha-Cadinol	10398656	Sesquiterpenoid	ESR
4 -Hydroxy-5,6,7-Trimethoxyflavanone	244387	Flavanone	APP, D2R, DNMT1, EDNRA, EDNRB, ESR, HGF, NFKB, ABCB1, AKT1, TERT
p-methoxy benzaldehyde	31244	Benzaldehydes	EGFR
Eupolauridine (Canangine)	72486	Alkaloid	TOP 1
Rocaglamide	331783	Flavaglines	NFKB

Table 2: Drug-likeness and side effects of compounds

Compound name	Lipinski rule of five						
	MF	MW (g/mol)	Log P	HBD	HBA	DLS	Side Effect(s)
Acceptable Values		< 500	< 5	< 5	< 10		
Cinnamyl Acetate	$C_{11}H_{12}O_2$	176.08	2.7	0	2	1.12	Nephrotoxicity, Myocardial infarction
Isosafrole	$C_{10}H_{10}O_2$	162.07	3.35	0	2	-1.21	Hepatotoxicity
Anonaine	$C_{17}H_{15}NO_2$	265.11	3	1	3	0.67	Hepatotoxicity
Micheline A	$C_{18}H_{17}NO_3$	295.12	2.59	1	4	0.42	NIL
Reticuline	$C_{19}H23NO_4$	329.16	2.97	2	5	1.33	Arrhythmia, Myocardial infarction
Trans- Feruloyltyramine	$C_{18}H_{19}NO_4$	313.13	3.29	3	4	0.24	NIL
Alpha-Cadinol	$C_{15}H_{26}O$	222.2	4.32	1	1	0.35	NIL
4'-Hydroxy-5,6,7-Trimethoxyflavanone	$C_{18}H_{18}O_6$	330.11	2.85	1	6	0.86	NIL
p-methoxybenzaldehyde	$C_8H_8O_2$	136.05	1.74	0	2	2.17	Hepatotoxicity, Arrhythmia
Isoeugenol	$C_{10}H_{12}O_2$	164.O8	2.91	1	2	-0.76	NIL
Rocaglamide	$C_{15}H_{15}NO_3$	257.11	1.93	1	3	0.53	NIL
Eupolauridine(Canangine)	$C_{14}H_8N_2$	204.07	3.38	0	2	0.99	Arrhythmia, Myocardial infarction, Cardiac failure, Hepatotoxicity

MF: Molecular Formula; MW: Molecular Weight; Log P: Partition co-efficient; HBD: Hydrogen Bond Donor; HBA: Hydrogen Bond Acceptor; DLS: Druglikeness score

Table 3: ADME profile of phytocompounds

Compound name	BBB (logBB)	CaCO2 p (nm/ sec)	BS (mg/ L)	CYP2C19	CYP2C9	CYP2D6	CYP3A4	HIA (%)	P-gp	SP (logKp, cm/h)	PPB (%)	Water solubility (mg/L)
Cinnamyl Acetate	1.497	43.073	3716.14	I	NI	NI	NI	100	NI	-1.364	70.695	826.784
Isosafrole	1.123	57.562	316.307	I	I	NI	I	100	NI	-1.913	83.993	37.978
Anonaine	0.984	47.681	43.459	N	N	I	NI	96.493	NI	-4.113	65.565	57.025
Micheline A	0.81	35.547	62.108	NI	NI	I	NI	95.726	NI	-4.178	43.432	58.946
Reticuline	1.008	12.256	265.893	NI	I	I	I	93.264	I	-3.403	83.959	679.934
Trans-Feruloyltyramine	1.247	21.659	1186.54	NI	NI	NI	I	90.006	NI	-3.436	82.036	231.107
Alpha-Cadinol	9.218	55.407	127.458	NI	I	NI	NI	100	I	-1.082	100	25.55
4'-Hydroxy-5,6,7-Trimethoxyflavanone	0.018	33.448	41.642	I	I	NI	I	96.415	NI	-3.453	90.269	32.985
p-methoxy benzaldehyde	1.652	31.366	21.809	I	I	NI	I	100	NI	-1.843	36.109	1691.91
Eupolauridine (Canangine)	3.901	56.009	9165.6	I	I	NI	I	100	NI	-3.571	96.064	29.365
Rocaglamide	0.013	134.46	35.595	NI	I	NI	I	95.331	I	-2.844	85.882	2.57

BBB: Blood-Brain Barrier; $CaCO_2$ p: the Predicted value of intestinal absorption through CaCO2; BS: Buffer Solubility; P-gp: P-glycoprotein; PPB: Plasma Protein Binding; HIA: Human Intestinal Permeability; SP: Skin Permeability; I=Inhibitor; NI=Non Inhibitor

Table 4: Toxicity profile of phytocompounds

Compound name	Carcinogenicity		hERG Inhibition	FAT (Medika)	FAT (Minnow)
	(Mouse)	(Rat)			
Cinnamyl Acetate	Negative	Negative	MR	0.19	0.18
Isosafrole	Positive	Negative	MR	0.103	0.0742
Anonaine	Negative	Negative	MR	0.0328	0.051
Micheline A	Negative	Negative	MR	0.0545	0.074
Reticuline	Negative	Negative	MR	0.01	0.012
Trans- Feruloyltyramine	Negative	Negative	MR	0.009	0.019
Alpha-Cadinol	Negative	Negative	LR	0.0327	0.013
4'-Hydroxy-5,6,7-	Negative	Positive	MR	0.02	0.019
p-methoxy benzaldehyde	Negative	Negative	MR	0.675	0.268
Eupolauridine (Canangine)	Positive	Negative	MR	0.0527	0.038
Rocaglamide	Negative	Negative	HR	0.005	0.026

hERG Inhibition: the Predicted result of hERG inhibition by compounds. hERG inhibition leading to QT prolongation and further cardiac risk; FAT: Fish Aqueous Toxicity; M: Mutagen; NM: Non Mutagen; MR: Medium Risk; LR: Low Risk; HR: High Risk.

Table 5: Pathways associated with cancer progression modulated by phytocompounds

Pathway ID	Pathway	Gene Count	FDR	Genes within pathway
hsa05206	MicroRNAs in cancer	6	9.54E-07	ABCB1, DNMT1, EGFR, MCL1, MDM2, PLAU
hsa05200	Pathways in cancer	8	1.41E-06	AKT1, EDNRA, EDNRB, EGFR, ESR1, HGF, MDM2, TERT
hsa05205	Proteoglycans in cancer	6	1.50E-06	AKT1, EGFR, ESR1, HGF, MDM2, PLAU
hsa05226	Gastric cancer	5	1.13E-05	ABCB1, AKT1, EGFR, HGF, TERT
hsa05218	Melanoma	4	2.34E-05	AKT1, EGFR, HGF, MDM2
hsa01522	Endocrine resistance	4	5.64E-05	AKT1, EGFR, ESR1, MDM2
hsa05215	Prostate cancer	4	5.64E-05	AKT1, EGFR, MDM2, PLAU
hsa04151	PI3K-Akt signaling pathway	5	0.00034	AKT1, EGFR, HGF, MCL1, MDM2
hsa05225	Hepatocellular carcinoma	4	0.00034	AKT1, EGFR, HGF, TERT
hsa04020	Calcium signaling pathway	4	0.00038	EDNRA, EDNRB, EGFR, TBXA2R
hsa04015	Rap1 signaling pathway	4	0.00055	AKT1, DRD2, EGFR, HGF
hsa01521	EGFR tyrosine kinase inhibitor resistance	3	0.00069	AKT1, EGFR, HGF

hsa04080	Neuroactive ligand-receptor interaction	4	0.0014	DRD2, EDNRA, EDNRB, TBXA2R
hsa05224	Breast cancer	3	0.0028	AKT1, EGFR, ESR1
hsa04022	cGMP-PKG signaling pathway	3	0.0033	AKT1, EDNRA, EDNRB
hsa04630	Jak-STAT signaling pathway	3	0.0033	AKT1, EGFR, MCL1
hsa05219	Bladder cancer	2	0.0046	EGFR, MDM2
hsa04024	cAMP signaling pathway	3	0.0052	AKT1, DRD2, EDNRA
hsa04014	Ras signaling pathway	3	0.0074	AKT1, EGFR, HGF
hsa05213	Endometrial cancer	2	0.0076	AKT1, EGFR
hsa05223	Non-small cell lung cancer	2	0.0089	AKT1, EGFR
hsa04917	Prolactin signaling pathway	2	0.009	AKT1, ESR1
hsa05211	Renal cell carcinoma	2	0.009	AKT1, HGF
hsa05212	Pancreatic cancer	2	0.0096	AKT1, EGFR
hsa05220	Chronic myeloid leukemia	2	0.0098	AKT1, MDM2
hsa04010	MAPK signaling pathway	3	0.0108	AKT1, EGFR, HGF
hsa04012	ErbB signaling pathway	2	0.011	AKT1, EGFR
hsa05210	Colorectal cancer	2	0.0112	AKT1, EGFR
hsa04064	NF-kappa B signaling pathway	2	0.0126	LCK, PLAU
hsa04660	T cell receptor signaling pathway	2	0.0136	AKT1, LCK
hsa04210	Apoptosis	2	0.021	AKT1, MCL1
hsa04072	Phospholipase D signaling pathway	2	0.0235	AKT1, EGFR
hsa04218	Cellular senescence	2	0.0265	AKT1, MDM2
hsa05202	Transcriptional misregulation in cancer	2	0.0302	MDM2, PLAU

FDR: False Discovery Rate

Table 6: Binding affinity, inhibitory constant, and hydrogen bond interaction of the compound with their protein targets

Compound name	Gene ID	Protein name	PDB ID	BE (kcal/mol)	IC_{50} (µM)	HBI (amino acid… ligand interaction)
Reticuline	MDM2	MDM2-MDMX	1T4E	-5.1	194.98µM	Phe55…OH, Gln59…O-
	PLAU	Urokinase-type plasminogen activator	1EJN	-5.4	86.51µM	Nil
Micheline A	MDM2	Ubiquitin-protein ligase E3	1T4E	-6.3	24.09µM	Gly16…O-, Val93…OH
4'-Hydroxy-5,6,7-Trimethoxyflavanone	ABCB1	P-glycoprotein 1	6FN4	-3.9	1,330µM	Asp804…OH
	AKT1	RAC-alpha serine/ threonine-protein kinase	1UNQ	-5.2	148.44µM	Lys8…=O, Lys8…O-
Anonaine	MDM2	MDM2-MDM	1T4E	-5.8	52.68µM	Gly16…O-

PDB: Protein Data Bank; BE: Binding energy; IC_{50}: Half Maximal Inhibitory Concentration; HBI: Hydrogen Bond Interactions

Figure 1. Work flow of the current study

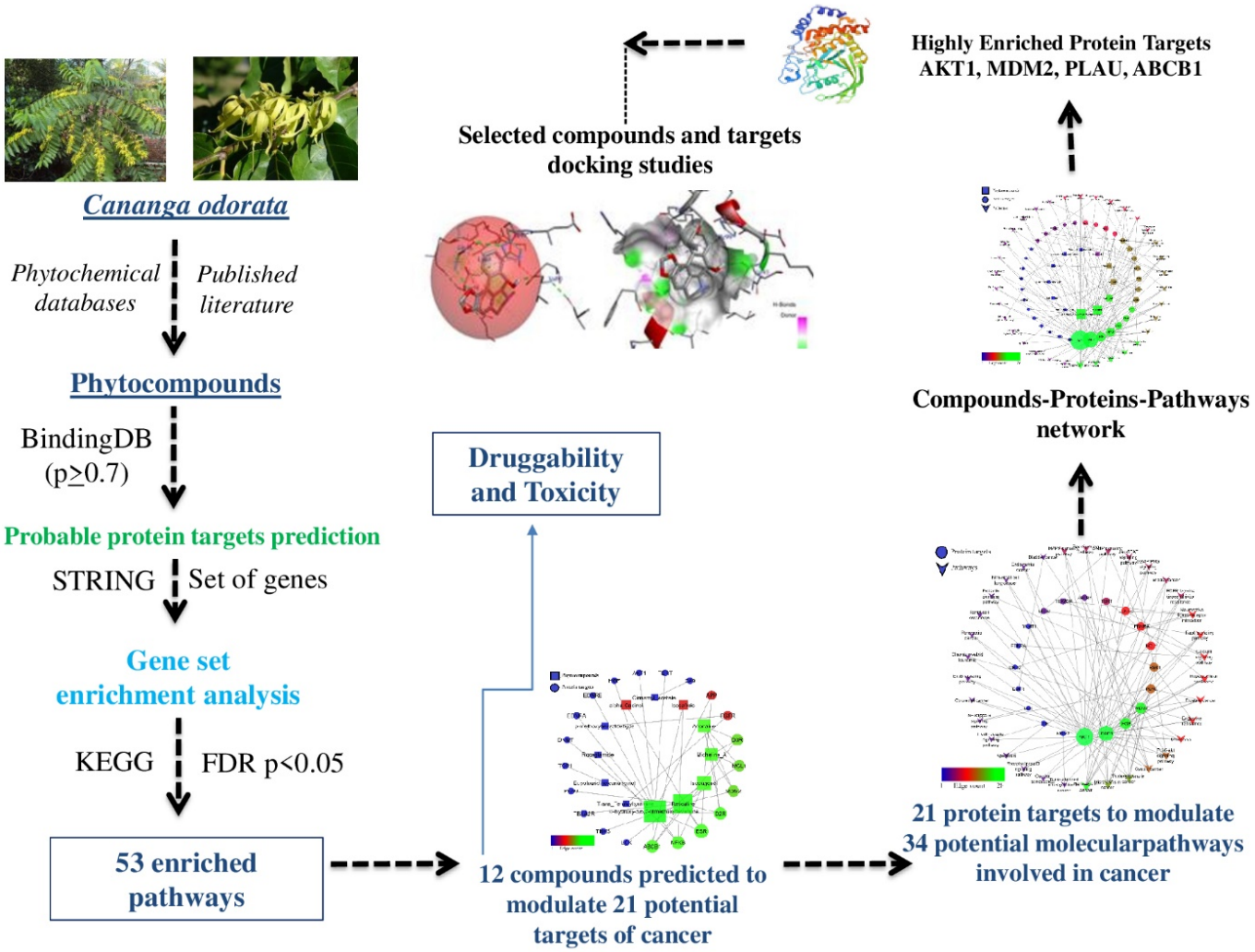


Figure 2. Probable protein targets protein-protein interaction network

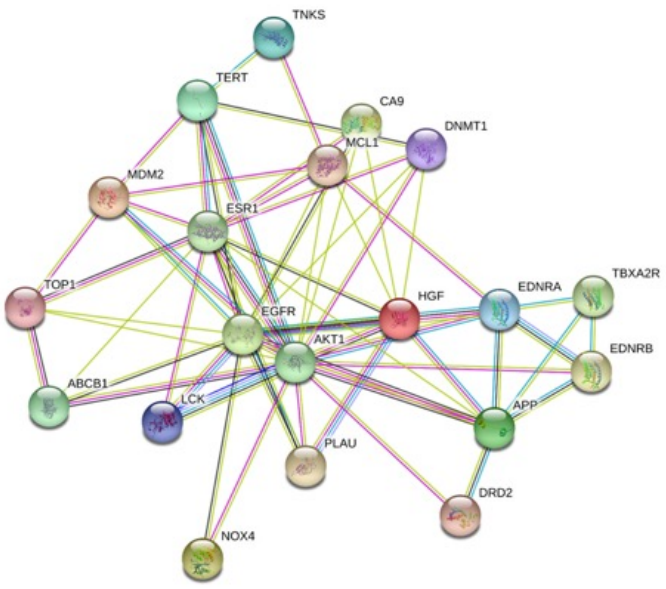


Figure 3. Network representation of compounds and proteins interactions

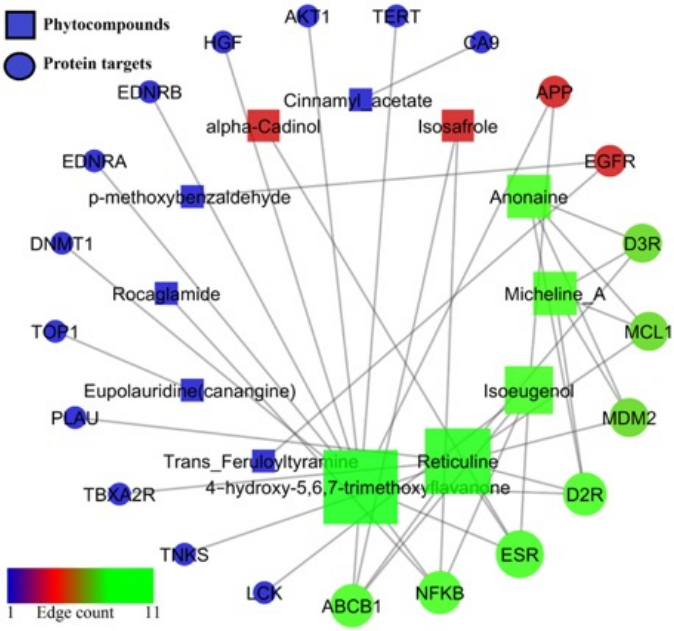


Figure 4. Network representation of protein and pathway interactions

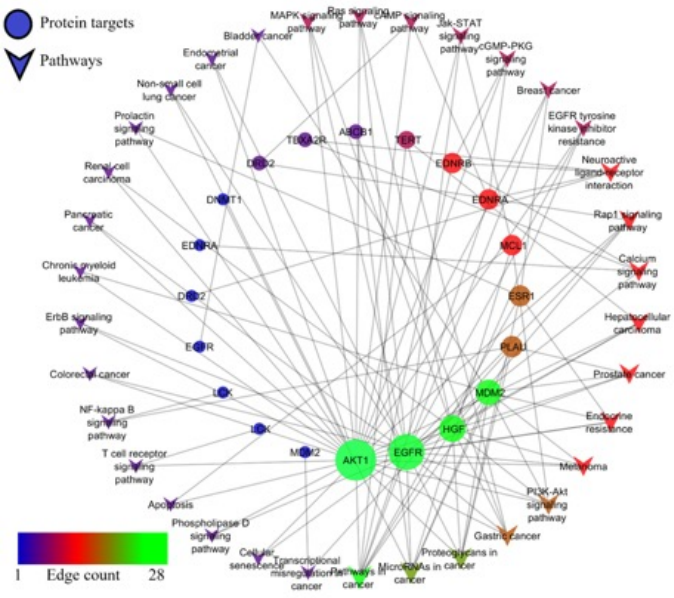


Figure 5. Network representation of compounds, proteins, and pathway interactions

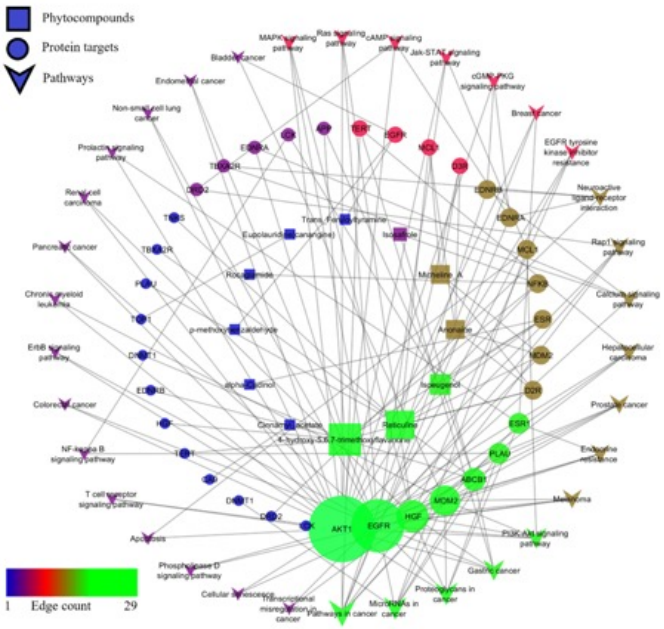
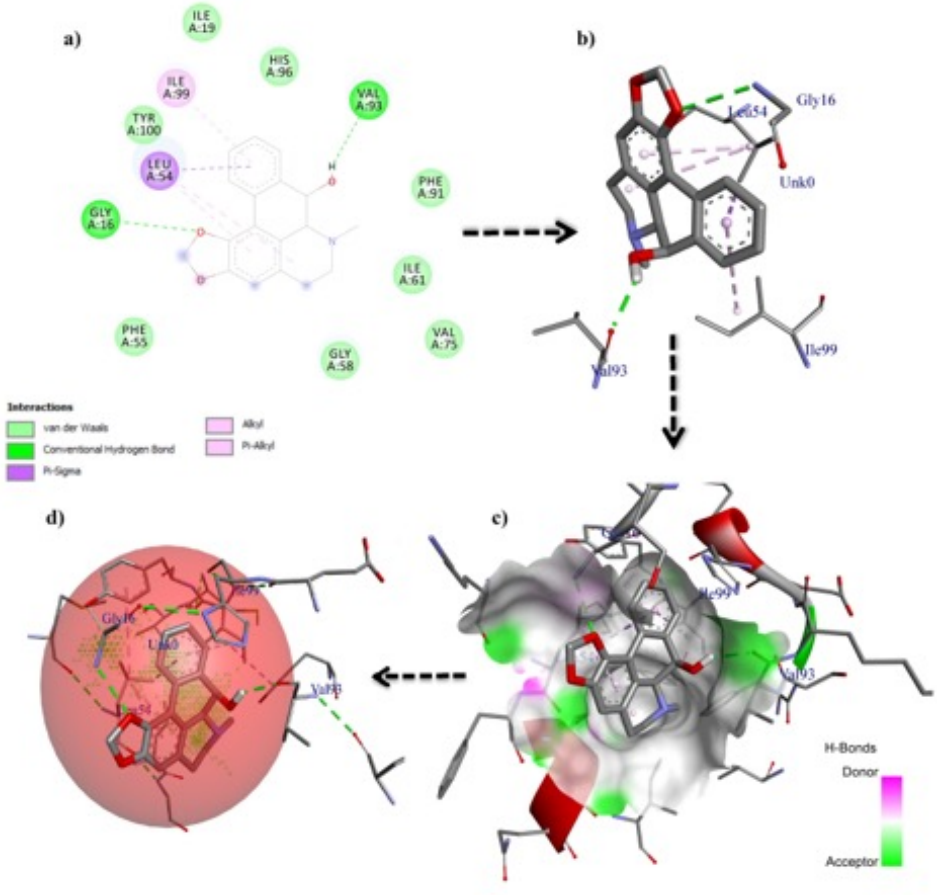


Figure 6. Interaction of Micheline A with MDM2 a) 2D representation b) 3D representation c) of Micheline A binding at MDM2 pocket d) Micheline A binding at MDM2 active site 1 region.



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