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Research Article

In-vitro Anticancer and *In-silico* Assessment of *Oxalis corniculata* against Colorectal Cancer (HCT 116) Cell Line

Saif MS Ansari[1], Veeru Prakash[1], Sushma A[1], KP Rao[2], Danish Ahmed[3], Ibrahim Khan[3], Alok M Lall[1*]

[1]*Department of Biochemistry and Biochemical Engineering, Jacob Institute of Biotechnology and Bio-engineering, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh, India.*
[2]*Department of Biological Science, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh, India.*
[3]*Department of Pharmaceutical Sciences, Shalom Institute of Health and Allied Sciences, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh, India*

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ABSTRACT

Colorectal cancer is the third most frequently diagnosed cancer in both genders and the fourth foremost cause in respect to cancer-associated mortality. The metastasis mechanism of colorectal cancer shows that it gradually develops in the form of polyps under granular cells in the large intestine, which damages blood and lymph vessels. At present, chemotherapy and surgery are the major clinical approaches in treatment. Therefore, developing novel and effective drugs requires the use of natural derivative compounds, a potent and significant effect in restricting the progression of colorectal cancer (CRC). The present research investigates the *in-vitro* anticancer properties of various extracts of plant *Oxalis corniculata* counter cell viability of CRC cell line (HCT116) using an MTT assay. *In-silico* docking studies of hexadecanoic acid were performed with cytochrome P450CYP17A1 protein (3RUK) to predict potential inhibitors and drug-likeness as potential CRC inhibitors using ADME profiling adhered to five rule of Lipinski. The finding indicated the extracts of the *O. corniculata* have anticancer and anti-proliferative activity. The cell cytotoxic observed against chloroform extract exhibited the highest inhibition against the HCT116 cell line, following ethanol and aqueous extract. The determined IC_{50} values for the ethanol, aqueous, and chloroform extracts were 53.94 ± 1.29, 61.85 ± 0.43, and 47.34 ± 1.24 µg/mL, respectively. The *in-silico* molecular docking result shows that n-hexadecanoic acid compound was found to be effective against 3RUK protein associated with CRC. Therefore, the finding suggests *O. corniculata* plants could be used to formulate potential therapeutic drugs for the development of anticancer agents.

Introduction

Cancer is the most threatening disease to humankind globally. Cancer develops by transforming of normal cells into cancerous by rapid uncontrolled growth and spread to other adjacent tissues referred as metastasis. As reported by WHO 2020, cancer disease stands as one of a prominent contributor to global mortality. Nearly ten million deaths were reported in 2020 due to cancer.[1] Prevalence of colorectal cancer (CRC) in recent past and current a major health concern.[2] By the end of 2030, the estimated progression of CRC is anticipated to exceed two million cases, with over one million resulting in mortality.[3] According to ACS, globally CRC is placed third most identified cancer and stands as the fourth major cause of cancer based mortality.[4] Two basic components, genetic and environment, contributing in advancement and inclined around 75 to 80% of CRC cases. Approximately 35% of CRC cases arise due to genetics while 25% cases appeared with no genetic background. About 60 to 70% of CRC patients who exhibit clinical symptoms receive a diagnosis when the illness has already progress. [5] Heavy westernized lifestyle and unhealthy diet plant, smoking and rising incidence of alcohol consumption in low and

***Corresponding Author:** Dr. Alok M Lall

Address: Department of Biochemistry and Biochemical Engineering, Jacob Institute of Biotechnology and Bio-engineering, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh, India.

Email ✉**:** alok.lall@shiats.edu.in

Tel.: +91-9335138408

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middle economical countries are leading to progression of CRC. The prevalence of CRC is attributed to risk factors such as westernized lifestyle and unhealthy diet plan, smoking and rising incidence of alcohol consumption particularly pronounced in low and middle economical countries contributing to its progression**.** These have been reported around 10 to 11% of CRC causing 9 to 9.5% of death in 2018.[6,8] The colorectal cancer is maturing and eventually forming polyps under granular cells in large intestine. As increasing size of polyps leads to cancer. The stationary polyp-cancer process can require more than ten years before polyps in the colorectal to transform into cancerous cells.[5] The colon or rectum are the major site of CRC, damage blood and lymph vessels. The extension of metastasis leads to damage of other vital organs.[9] In emergence of CRC progression, three pathways likely to be involved such as chromosomal instability (CIN) responsible for 70 to 85% of sporadic CRC whereas, CpG island methylator phenotype (CIMP) and microsatellite instability (MSI) contribute in remaining cases.[10]

To counteract the CRC effect, various chemoprotective agents include non-steroidal anti-inflammatory drugs (NSAIDs) and aspirin considered the most potent class of drug against CRC. [11] Celecoxib and metformin also reported as anti-CRC targets. Along with this, several naturally isolated molecules have substantiated to be beneficial in resistant either through inhibiting the proliferation or by promoting apoptosis in cancerous cell presents in humans.[12-14] Various naturally derived compounds such as curcubitacin B, curcumin and resveratrol has been reported as potent pharmacological agents to lower the risk of CRC.[14-17]

Oxalis corniculata belongs to family Oxiladeaceae, natively growing in moist condition in Asia, Africa and Central America region. In India, this plant known as Indian sorrel. It has importance in Ayurveda, Siddha & Unani traditional system, ethnobotanical used to cure various disease including fever, dyspepsia, antifungal, antimicrobial, appetizer, wound healing and inflammation.[18] GCMS screening of methanol fraction of *Oxalis corniculata* identify various compounds includes oleic acid (fatty acid), vitamin F, octadecanoic acid, vitamin E, squalene, stigmast-5-en-3-ol oleate glycosides, phytosterols, n-hexadecanoic acid (palmitic acid). Furthermore, various other compounds such as β-sitesterol, lutolin, flavonoids, betulins and apigenin, ethyl gallate, p-hydroxybenzoic acid, syringic acid, citric acid, oxalic acid, isovitxin isoirientin, trimethoxyflavone and teramethoxyflavone isolation are reported anticancer and antioxidant activity.[19-21] The phyto-pharmacological properties of compound listed in Table 1.

n-hexadecanoic acid or palmitic acid ($CH_3(CH_2)_{14}COOH$) is one of highest obtained saturated fatty acid in plants reported in various therapeutic and protecting activity like antibacterial, antifungal, anti-inflammatory, antitumor and cytotoxic activity against HCT116 cell lines.[22-24]

Molecular docking is one of the computer aided drug design (CADD) that virtually interpret prosperous molecular relationship of targeted protein-ligand binding interaction ensuring fast development of novel potential drug molecules for diseases. For estimation of distinct number of protein-ligand attraction by structure conformation, several commercial software is available like AutoDock, AutoDock Vina v4.2; BIOVIA Discovery studio analyzer and PyMOL are used throughout molecular docking studies.[25,26]

This study aims to investigate cytotoxic activities of *O. corniculata* extracts against CRC cell lines (HCT116) using MTT assay and assessing n-hexadecanoic acid a potential inhibitor against CRC using P450 CYP17A1 (PDB id: 3RUK) through *in-silico* molecular docking study.

Materials And Methods

Plant Collection

O. Corniculata plant was collected in the winter season at herbal garden of Department of Biological Science, SHUATS, Prayagraj and specimen's authentication was done by Prof. Satyanarayan, Department of Botany, Allahabad University. The voucher herbarium under approval no. 01-2020 was deposited at the institutional herbarium.

Extraction Process

O. corniculata plant was placed in air to shade dry after thoroughly cleaning. In order to preparation of extracts, sample was crushed and pulverized into fine powders and were placed in soxhlet against ethanol, aqueous and chloroform solvent separately for 48 hours at 60°C. The extracts were concentrated and removed excess solvent using rotavapour (Buchi). The extract was then stored at 4°C in airtight vessel. To prepare stock solution for MTT activity, extracts were suspended in dimethyl sulphoxide (DMSO) at concentration of 4 mg/mL.

Preliminary Phytochemical Investigation of *O. corniculata*

The preliminary investigation of phytochemicals include alkaloids, flavonoids, saponins, glycosides, saponins, tanins and phenols identify by standard method reported by Kathariya *et al.*, (2010).[27]

Maintenance of HCT116 culture

The cell line with 19 passage number was accessed from NCCS, Pune, India. The cell line was cultured in Dulbecco's modified eagle medium supplemented with 10% of fetal bovine serum, 100 U/mL of 1% of penicillin and streptomycin and then incubated for 72 hours in 37°C with 5% CO_2 to maintained cell line.

Cell Viability or MTT assay

The HCT-116 was accessed to investigate the cytotoxic efficacy against all extracts using the MTT assay reported by Badmus *et al.* (2015).[28] The cell line was grown in DMEM and cultures for 24 hours at a density of 5×10^4 cell/well in 96 well plates. The extracts were diluted to 100 µL of medium and incubated for 48 hours in the wells. The cells in the incubation phase were exposed to varying log concentration of extracts which included 0.1, 10, 20, 50, 100, 200 and 500 µg/mL. After specified extract exposure times, 20 µL of a PBS-based MTT solution with 5 mg/mL was filled to wells, and mixture was preserved for three hours at 37°C with 5% CO_2 in the air. Following extracting the supernatants, 150 µL of isopropanol was applied. The formazan crystals were dispersed after gently shaking the plates for 15 minutes. Cell viability of HCT116 cell was analyzed through absorbance of plates using spectrophotometer at 570 nm against control.[29,30] The Cell viability (%) was determined applying following formula:

$$\text{Cell viability (\%)} = \frac{\text{Absorabnce test sample at 570nm}}{\text{Absorbace of control at 570nm}} \times 100$$

IC_{50} Value Calculation

By constructing a dose dependence curve, the half of the maximum inhibitory value, i.e., IC_{50} was determined to correspond to the dosage of the test sample, resulting in a 50% reduction in the growth of cells. IC_{50} was calculated by establishing a non-linear regression curve. The y-axis displayed cell inhibition percentages, while the x-axis displayed the dose necessary for inhibition.[31]

Table 1: Preliminary phytochemicals present in *O. corniculata* extracts

Phytochemicals	*O. corniculata*
Protein	++
Carbohydrates	++
Fatty acids	++
Lipids and oils	--
Alkaloids	++
Flavonoids	++
Terpenoids	--
Glycosides	++
Phenols	++
Flavonoids	++
Tannins	++
Steroids	--

(++); presence, (--); absent

Molecular Docking for Potential Anticancer Compound

The molecular docking studies is challenging part of bioinformatics to predict the mode of intermolecular binding interaction, active site interpretation and calculation of activation energies of the bioactive compound towards the potential target protein by using docking tools such as AutoDock Vina, SwissADME, Chimera and discovery studio. To explore efficient binding site, the AutoDock tools was used to identify active site of cytochrome P450 CYP17A1 (PDB id: 3RUK) for binding of ligand. Ligand and receptor were prepared by MGL Autodock tool. 3D images were generated by Discovery studio visualize. [25] Initially, ligand and protein were prepared by adjusting charges and removing hydrogen, atoms, and water and saved the result in pdbqt format. Subsequently, the protein-ligand complex was strategically placed within a molecular grid to create a molecular confirmation in the x, y, and z dimensions, specifically 29.538, 3.247, and 32.115. The energy range was set at 4, and exhaustiveness was set to 8 for P450 CYP17A1 (3RUK). The Autodock tools was run under Lamarckian genetic algorithm 4.2 and interpreted the result through binding energy score, participating amino acids in hydrogen bond formation and ligand-receptor complex images. [32] Furthermore docked score of ligand-protein interaction was observed in Discovery studio visualizer.

Preparation of Ligands

The chemical structure of ligands n-hexadecanoic acid (HAD) was drawn using chemSketch, ACD/Labs based free access chemical drawing software and structure was refined using discovery studio tool in pdb format. The ligand was prepared by polar hydrogen bond addition whereas, energy minimization was done by chimera tool. Using Autodock4.2 software to explored substantial affinity between ligand with target protein. The drug likeness attributes that satisfy Lipinski's rule of five of n-hexadecanoic acid compound was evaluate using SwissADME free access tool.

Preparation of Protein

The crystal 3D structures of cytochrome P450 CYP17A1 (PDB ID: 3RUK) were retrieved using RCSB database. The non-polar groups, hydrogen atoms, and water molecules were eliminated from 3RUK before docking process using the discovery studio free available software tools. Employing valence monitor options and adding possible conformations, the protein file was improved. Certain changes addressed the existence of empty valence atoms in the structure of proteins and also crystallographic irregularities. YASARA minimization server (http://www.yasara.org) applying for energy minimization of resultant protein. This free access server conducts energy minimization using YASARA force field. After following

energy minimization, protein file subjected to explored potential binding site in 3RUK protein using tools. [32,33]

Analysis of Docking Outcome

The docking score between ligand-protein interactions was the elementary for the docking outcome. Discovery studio, tools was used to visualize the interaction of amino acid of active site of protein with ligand.

ADME Analysis

The rules set by Lipinski (RO5) serves as a set of guidelines in drug design and development, providing information about drug-like attributes and potential bioavailability of targeted compound achieving medicinal properties. The ligand can be screened for RO5 properties by accessing. Pharmacokinetics characteristics of targeted ligand were evaluated through ADME screening by open access server, SwissADME.[33] The Lipinski's rule consists of multiple parameters including lipophilicity, molecular weight along with quantity of donate and acceptance of hydrogen bonds. If a chemical violates more than one of these guidelines, it may not be a potential oral drug candidate. Lipinski rule of five is a useful guideline in drug development when predicting the possibility of a molecule succeeding as an oral medication. [34,35]

RESULT

Phytochemical Screening

Several phytochemicals screened out from the extracts *O. corniculata* includes alkaloids, phenols, tannins, flavonoids and saponins and results are shown in Table 2.

MTT Assay

The cell proliferating (%) of ethanol, aqueous and chloroform extract of *O. corniculata* was explored on HCT116 cell using MTT assays., IC_{50} cell inhibition (%), dose response curve of every extract estimated. IC_{50} value. All *O. corniculata* extracts showed cell cytotoxic activity in dose dependent manner (Fig. 1). The HCT116 colorectal cancer causing showed highest sensitivity to the chloroform extract as shown by its cell proliferating IC_{50} value with 47.34 ± 1.24 µg/mL compared to ethanol extract with 53.94 ± 1.29 µg/mL and aqueous extract 61.85 ± 0.43 µg/mL respectively is less sensitive to cell lines as reported in Table 3.

Molecular Docking Interpretation

Development of computer based drug designing approached towards designing for target specific novel drugs globally trending. For *in-silico* study of targeted protein specific to source of cancer cytochrome P450 CYP17A1multigene family enzyme was retrieved in 3D structure from PDB source. ADME toxicity (ADME) profiling was carried out for ligand n-hexadecanoic acid to evaluate potential impact of compound or drug on a living system. The application

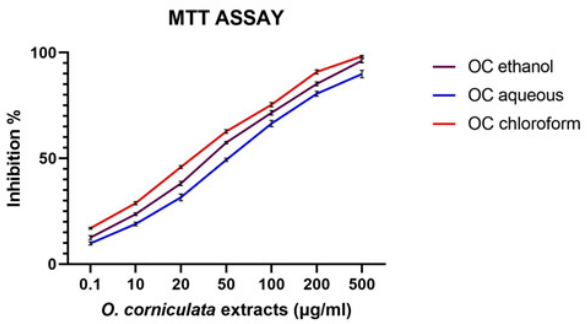

Fig. 1: Effect of *O. corniculata* cell proliferation inhibition (%) of extracts of *O. corniculata* in HCT116 cell lines. y-axis represented cells inhibition (%) against different dose, at x-axis. OC= *Oxalis corniculata*

of Lipinski's (RO5) identifies efficiency of the potential drug candidate that further subjected to ligand–protein docking evaluation. The compound n-hexadecanoic acid was screened against P450 CYP17A1 shown in Fig. 2. The targeted compound was docked against targeted protein responsible for CRC. Throughout this mechanism, binding energy (Kcal/mol) between ligands and receptor, amino acid involved in hydrogen bond formation and other type of bond formed was evaluated. Formation of hydrogen bond and removing of charges facilitates in ligand preparation that access to binding to the target specific protein. Specific bonds interaction between compounds and amino acid are analyzed and visualized using discovery studio. The 3D structure of CytochromeP450 CYP17A1 that regulates apoptosis executed very efficient with amino acid interaction. The target ligand exhibited significant affinity with selected protein showing lowest energy and substantial noncovalent hydrogen binding interaction between ligand and protein. Based on the outcome in Table 4, Cytochrome P450CYP17A1 protein (3RUK) exhibited -6.1 kcal/mol binding energy with n-hexadecanoic acid (HDA). This interaction involved ARG236 and ASP238 amino acid forming conventional hydrogen bond. Additionally, Vander Waals interactions were noted with LEU243, ILE198, ASN202, GLY301, ALA302, VAL483, LEU209, ALA105 and SER106. Alkyl and pi-alkyl bond were observed with TYR201, PHY300, ILE205, PHE114, ILE206 and VAL482. In addition, during docking process, receptor protein associated in donated 1 H-bond and accepted 2

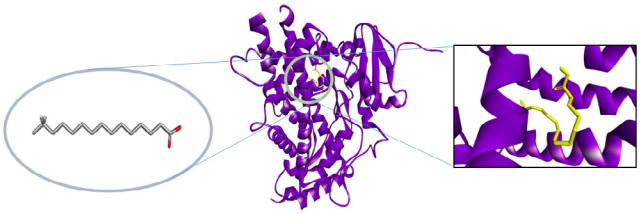

Fig. 2: Chemical structure of n- hexadecanoic acid, interaction of position of compound n-hexadecanoic acid (yellow) with 3RUK and close view of active side

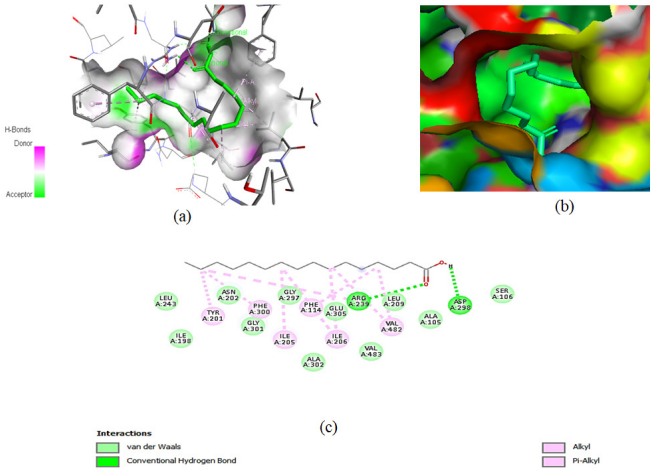

Fig. 3: Cytochrome P450 CYP17A1 (3RUK)-n-hexadecanoic acid docking complex: (a) interaction of n-hexadecanoic acid with catalytic site of 3RUK protein, (b) pocket view of n-hexadecanoic acid with 3RUK (c) pocket view and (d) 2D representation hydrophobic bond interactions between amino acid residue of 3RUK with n-hexadecanoic

Table 2: Phyto-pharmacological properties of compounds reported in *O. corniculata*

Compound	*Class*	*Pharmacological properties*
Oleic acid (Monosaturated omega-9-fatty acid)	Fatty acid	Antiinflammatory, cardiovascular, skin health, nutrient absorption
linoleic acid polyunsaturated omega-6-fatty acid)	Fatty acid	Essential fatty acid, dietary source, cardiovascular protection, improve pancreatic beta cells
stearic acid	Fatty acid	Antiinflammatory, decrease cholesterol level, anticancer, skin health,
vitamin E acetate (tocopheryl acetate)	Vitamin E	Antioxidant, anticancer
Squalene	Polyunsaturated hydrocarbons	Antioxidant, cardiovascular, anticancer and antiinflammatory, immune support, detoxifier
Octadecatereonic acid	Fatty acid	Anticancer, antiinflammatory, hepatoprotective, free radical scavenger
Stigmasterol (Stigmast 5 en 3 ol oleate)	Phytosterol	Anticancer, antiinflammatory, lowering cholesterol
n-hexadecanoic acid (palmitic acid)	Fatty acid	Anticancer, antiinflammatory,
β-Sitosterol	Phytosterol	Anticancer, reduce cholesterol level, antiinflammatory, prostate health
luteolin	Natural flavonoid	Antioxidant, antiinflamatory, neuroprotective, cardiovascular
Betulins	Triterpenoids	Anticancer, antiviral, antinflammatroy
apigenin	Flavonoid	Antioxidant, antiinflammatory, anticancer, neuroprotective, cardiovasuclar
Ethyl gallate	Ester derivative gallic acid	Antioxidant, antiinflammatroy, antimicrobial,
p-Hydroxybenzoic acid	Derivative of benzoic acid	Antioxidant
Syringic acid	Phenolic	Antioxidant, antimicrobial, antiinflammatory
Citric acid	Organic acid	Improve blood circulation, nephroprotective, skin health
Oxalic acid	Organic acid	Antiinflammatory, anticancer, immune health
Isovitxin	c-glycosyl flavones	Antioxidant, antidiabetic antiinflammatory, anticancer, cardiovasucular
Isoorientin	Glycoside	Hepatoprotective, antioxidant, anticancer, antinociceptive
Trimethoxyflavone	Flavone	Anticancer, antiinflammatory, antitumor
Teramethoxyflavone		

Table 3: *O. corniculata* of ethanol, aqueous and chloroform extract IC_{50} value against HCT116 cell proliferation (%)

Concentration (µg/mL)	*Cell viability (%) (ethanol)*	*Cell viability (%) (aqueous extract)*	*Cell viability (%) (chloroform extract)*
Control	0.00	0.00	0.00
500	12.52 ± 0.97	9.76 ± 0.76	16.95 ± 0.42
200	23.65 ± 0.70	18.98 ± 0.87	28.78 ± 0.76
100	38.12 ± 1.00	31.55 ± 1.60	45.86 ± 0.68
50	57.49 ± 0.61	49.32 ± 0.74	62.74 ± 0.91
20	71.52 ± 1.10	66.46 ± 1.49	75.35571 ± 1.02
10	85.17 ± 0.97	80.50 ± 1.22	90.86 ± 1.01
0.1	96.16667 ± 1.00	89.87 ± 1.74	98.28 ± 0.44
IC_{50} (µg/mL)	53.94 ± 1.29**	61.85 ± 0.43***	47.34 ± 1.24**

Data are presented in table as the means ± standard error (SEM). The Data were analyzed using One way Anova. Significance levels ($p < 0.005$) and ($p < 0.001$) are indicated by descriptive characters

Table 4: Docking status of n-hexadecanoic acid with Cytochrome P450CYP17A1(3RUK) based on binding energy, hydrophobic bond interaction and Lop P (≤ 5)

Docking details	
Molecular formula	$C_{16}H_{32}O_2$
mw (g/mol)	256.42
docking score (kcal/mol)	-6.1
H-Bond interaction	ARG236, ASP238
Other bonds (Van der Waals, pi-sigma, pi-alkyl and C-H bond) interaction	TYR201, PHY300, ILE205, PHE114, ILE 206, VAL482, LEU 243, ILE198, ASN 202, GLY 301, ALA 302, VAL 483, SER 106
Lipinski's (RO5)	Yes
H-bond donors	1
H-bond acceptor	2
Molar refractivity	80.80
TPSA	37.30 Å2
Log P (≤ 5)	4.15

Table 5: ADME profiling based on lipophilicity and water solubility of n-hexadecanoic acid

Lipophilicity	
iLOGP	3.85
XLOGP3	7.17
WLOGP	5.55
MLOGP	4.19
Silicos-IT Log P	5.25
Consensus Log P	5.2
Water solubility	
ESOL Log S	-5.02
ESOL Class	Moderately soluble
Ali Log S	-7.77
Ali Class	Poorly soluble

h-bond when docked. The docking interaction between the 3RUK protein and n-hexadecanoic acid is depicted in Fig. 3. Table 5 presents a range of values associated with the lipophilicity (logP) of n-hexadecanoic acid, indicating its solubility in lipids or nonpolar solvents. The iLOGP (Calculated LogP) is a numerical representation of octanol/water partial coefficient (Lipiphilicity). Specifically, for n-hexadecanoic acid, the calculated logP is 3.85. Another calculated logP, XLOGP3, is reported as 7.17. The WLOGP (Wildman and Crippen LogP) value is 5.55, and the MLOGP (Moriguchi LogP) is determined to be 4.19. Silicos-IT Log P is registered at 5.25, and the Consensus Log P, an averaged or calculated value from multiple sources, is recorded as 5.2. The filters of water solubility in ADME profiling of n-hexadecanoic acid is expressed in Table 5. The logarithm of the aqueous solubility (ESOL LogS) of n-hexadecanoic acid is -5.02 indicated low solubility. Solubility based on the ESOL is suggesting a moderate level of solubility in water. The alternative solubility is calculated as -7.77, indicated low solubility and Ali Class similar to ESOL class of n-hexadecanoic acid classified as poorly soluble.

Table 6 provided information pertains n-hexadecanoic acid to various pharmacokinetic attributes. Notably, n-hexadecanoic acid has been predicted to have high gastrointestinal absorption, and the ability to permeate blood brain barrier (BBB) and enter to central nervous system. The compound n-hexadecanoic acid is potential inhibitor for CYP1A2 and CYP2C9 and but support for CYP2C19, CYP2D6 and CYP3A4 protein belongs to cytochromeP450 family. The result of drug-likeness and medicinal chemistry properties indicated that n-hexadecanoic acid having one violation for Lipinski's, 0 voilation for Ghose violation, 1 voilation for Veber and 0 voilance for Muegge and achieved 0.85 bioavailability score suggested potential drug candidate for oral availability and drug-likeness. In addition, n-hexadecanoic acid count 0 for PAINS alerts and Brenk alerts suggested no alerts for compound that could make biological assays inaccurately.

Table 6: ADME screening of n-hexadecanoic acid

Pharmacokinetics	
Gastrointestinal (GI) absorption	High
Blood brain barrier (BBB) penetration	Yes
P-glycoprotein (P-gp) substrate	No
Cytochrome CYP1A2 inhibitor	Yes
Cytochrome CYP2C19 inhibitor	No
Cytochrome CYP2C9 inhibitor	Yes
Cytochrome CYP2D6 inhibitor	No
Cytochrome CYP3A4 inhibitor	No
Permeability coefficient (log Kp) (cm/s)	-2.77
Drug-like attribute	
Lipinski violations	1
Ghose violations	0
Veber violations	1
Egan violations	0
Muegge violations	1
Bioavailability score	0.85
Medicinal/Pharmaceutical chemistry	
PAINS alerts	0
Brenk alerts	0
Leadlikeness violations	2
Synthetic accessibility	2.31

Discussion

The application of herbal medicine as anticancer drawn attention due to their substantial biological activity and constituent presents. Phytochemicals are considerably viable and have potential role as inhibitor against various type of cancer. The advantages of phytochemicals include their wide pharmacological information, low noxiousness, ubiquity and high curative potential in nature. Many cutting-edge analytical techniques have emerged to support the novel breakthrough of phytochemicals in pharmacological stats.[36,37] *O. corniculata*, plant used in conventional herbal therapy to treat a variety of diseases including anti-inflammatory, liver-protective, antimicrobial, anticancer and anithyperglycemic activity.[38] This plant contain diverse group of phytochemicals includes fatty acid, phenol, glycosides, triterpenoids and flavons identified through preliminary investigation. Some phytochemicals play a major role in anticancer, antioxidant and antiinflammatory activity (Table 1).[21,38] Ethanol, aqueous, and chloroform extracts were used to prepare a variety of solvent extracts. Each extract was subjected to high pressure concentration before being utilized to examine its potential cytotoxicity on cell lines.

Evaluating the anticancer potential of plants extract is important to identify the plant's inherent toxicity along with potential adverse effects of an acute overdose. The MTT cell cytotoxic assay was performed to analyze the potential of *O. corniculata's* crude extracts and reveal any cytotoxic activity against cell lines.[39] MTT is mono-tetrazolium salt comprised with tetrazole ring consisted thiazolyl ring and phenol groups. The tetrazole ring formed violet blue molecules called formazan generated by mitochondrial dehydrogenase. The MTT reagent undergone reduced when bound to cancer cells and produced formazan, which is chromogenic substance. It serves to analysis of cell viability, metabolic activity and drug cytotoxicity by spectrophotometric method (Fig.1). Notably, the outcome suggested that the ethanol and chloroform extracts were almost equally efficient against HCT116 cell at lower concentration (0.1 µg/mL), however, aqueous extract was not as effective. Additionally, the cell viability of the chloroform extract consistently increased (90.86 ± 1.01, 75.35571 ± 1.02, 62.74 ± 0.91, 45.86 ± 0.68, 28.78 ± 0.76, 16.95 ± 0.42) as concentrations increasing between 10 to 500 µg/mL, indicating superior efficacy when compared to ethanol (96.16 ± 1.00, 85.17 ± 0.97, 71.52 ± 1.10, 57.49 ± 0.61, 38.12 ± 1.00, 23.65 ± 0.70, and 12.52 ± 0.97) and aqueous extracts ((96.16 ± 1.00, 85.17± 0.97, 71.52 ± 1.10, 57.49 ± 0.61, 38.12±1.00, 23.65±0.70, and 12.52 ± 0.97)). Notably, the extract prepared in ethanol showed effectiveness than aqueous extract. The determined IC_{50} values for the ethanolic, aqueous, and chloroform extracts were 53.94 ± 1.29, 61.85 ± 0.43, and 47.34 ± 1.24 µg/mL, respectively. The IC_{50} stand for half maximal inhibitory concentration at 50%, which is applicable to determining the amount of substance required to kill 50% of cells. The IC_{50} values of ethanol, aqueous and chloroform extracts indicate the concentration at which they inhibit the specified biological activity. The IC_{50} results for all three extracts at different concentration showed that chloroform exhibited more effective against HCT116 cell line. Therefore, the cytotoxic activity of plant *O. corniculata* showed ability to suppress cancer cell. Recent investigation suggested for that hydroethanolic extract of *O. corniculata* extract is also an effective cytotoxic treatment against hepatocarcinoma (Hep-G2) cell lines.[39]

n-hexadecanoic acid (palmitic acid) belongs to the class of sterol and bioactive compounds found in plants. Supported by docking investigation, it inhibits DNA topoisomerase-I action, and prevent human fibroblast cells propagation. [24,25,40] The ligand displayed affinity with -6.1 kcal/mol with 3RUK and were evaluated for druglikeness by using a guideline of Lipinski's rule of 5 (RO5). In order to evaluate a compound's potential for use as a medication, Lipinski's rule of five established the maximum limits for lipophilicity (logP ≤ 5), molecular weight (MW ≤ 500), and the total count of hydrogen bonds donor (HD ≤ 5) along with acceptor sites (HA ≤ 10). Compounds with more than two Ro5 breaches are prone to cause complications with gastrointestinal absorption.[41] The n-hexadecanoic acid acquired molecular weight (≤ 500 g/mol) and LogP (<5) favor oral bioavailability. Any drug

higher than that are unable to cross membrane barrier, therefore does not consider for oral bioavailability.[41] Furthermore, the Log S values for n-hexadecanoic acid are between −5.02 and −7.77 indicated moderate solubility in water,[42] and skin permeation coefficient i. e., logKp is -2.77 indicates a limited skins permeate and reduced toxicity.[43] In the process of developing new drugs, solubility play crucial role in determining compound's bioavailability and possible therapeutic efficacy. A drug required to must able to penetrate the BBB in order to have beneficial effects on the neurological system. The blood brain barrier functions as filter, preventing polar chemicals away from the brain.[44]

Molecular docking results revealed that compound n-hexadecanoic acid interpreted has high rate of absorption through the GI and demonstrates the capability to penetrate the BBB and glycoprotein (Pg-p), which is a cell membrane based efflux carrier pump. This pump is accountable for transported drugs out of the cell membrane consequently, caused therapeutic ineffectiveness when the drug's concentration is decreased. With a bioactive score of 0.85, n-hexadecanoic acid exhibit remarkable pharmacokinetics characteristics. [45] *In-silico* virtual docking study and drug likeness studies predicted that n-hexadecanoic acid are potential against the cytochrome P450 CYP17A1 target receptor protein and satisfy Lipinski's rule of five

In summary, extracts from *O. corniculata* plants demonstrated a possible cytotoxic impact and decreased colorectal cancer cell proliferation (HCT 116 cell). The n-hexodecanoic acid potential inhibitor for the cytochrome P450 CYP17A1 (3RUK) protein that causes colorectal cancer was supported by the molecular analysis.

Conclusion

Based on that study we concluded that *O. corniculata* plant containing phytochemicals with the potential inhibitor for the growth cancer cells. Moreover, n-hexadecanoic acid was discovered to be a possible inhibitor for the protein cytochrome P450 CYP17A1 that causes colorectal cancer by molecular docking research. Furthermore, these investigations offer new scientific understandings that allow for the investigation of pathways that could eventually result in CRC through *in-vivo* studies and the development of prospective anticancer medications that could eventually be prescribed to treat CRC-related diseases.

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