

EVALUATION OF ANTICANCER POTENTIAL OF *EPIPREMNUM AUREUM*: AN IN-VITRO AND IN-SILICO APPROACH

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Abstract

The use of phytochemicals in cancer therapeutics is an approach that has gained significant attention in recent years. Several plants commonly found in the environment have been observed to possess secondary metabolites with medicinal properties. This study aims to examine the anticancer activity of the methanolic extract of *Epipremnum aureum* (MEEA), a well-known ornamental vine. The constituents of the extract have been identified by the use of biochemical tests, as well as Gas Chromatography Mass Spectrometry. The extract has been shown to exhibit a cytotoxic effect against the HCT-116 cell line, with an IC₅₀ value of 35.7 µg/mL. Docking and Molecular Dynamics Simulation (MDS) conducted against the Yes-associated protein (YAP) using the constituents of *E. aureum* as ligands showed that Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (HE) and Octadecanoic acid, 2,3-dihydroxypropyl ester (OE) may be able to inhibit the interaction of YAP with TEA DNA-binding domain protein (TEAD) of the Hippo pathway, and hence prevent cell proliferation.

Keywords: Anticancer; *Epipremnum aureum*; Hexadecanoic acid; 2-hydroxy-1-(hydroxymethyl)ethyl ester; Octadecanoic acid; 2, 3-dihydroxypropyl ester; Yes-Associated Protein

Introduction

Cancer is a condition characterized by the abnormal growth and division of cells in the body, wherein they lose their identity and regular function. When gone unchecked and uncontrolled, this condition can result in organ failure and eventual system failure, leading to eventual death of the person. It is

a leading cause of death worldwide, due to the absence of prophylactic and treatment methods that are completely effective. Current treatment options such as chemotherapy and radiotherapy are known to have side effects (Albano *et al.*, 2021). Historically, plant extracts have been effective in

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treating disorders through the interactions between their constituents and cellular biochemicals (Ashraf, 2020). This encouraged us to focus our research on the use of phytochemicals as an auxiliary treatment method, by using naturally occurring compounds and their derivatives to select tumor cells for targeted cancer therapy.

Phytochemicals are secondary metabolites produced by plants, often as a mechanism of defense, or a result of their metabolic pathways. These are associated with many medicinal properties, including but not limited to anti-diabetic, antimicrobial, anthelmintic, antioxidant as well as anticancer activities (Bansal and Priyadarsini, 2021). Terpenes and their derivatives, alkaloids, steroids and sterols, flavonoids, as well as fatty acid-derived compounds are some common examples of phytochemicals (Bansal and Priyadarsini, 2021).

Several commonly found household plants, as well as invasive species have often been observed to contain anticancer compounds (Desai *et al.*, 2008). Additionally, the fact that they grow easily and quickly in various environments indicates that these are reliable candidates for reproducible research as well as feasibility of drug development. This study focuses on one such plant, namely *Epipremnum aureum*.

E. aureum, better known as money plant and Devil's ivy, is an ornamental climber vine commonly grown in houses. Compounds such as alkaloids, tannins, saponins, terpenoids and flavonoids are present in this plant, and are known to exhibit antimicrobial, antioxidant, diuretic, anti-inflammatory, pesticidal, anti-diabetic, and antiulcer activities (Meshram *et al.*, 2016).

Cancer is often associated with the upregulation of pro-survival pathways, and downregulation of mechanisms detrimental to cell growth and proliferation (Hernández-Lemus *et al.*, 2019). The Hippo pathway is necessary for ensuring survival, as well as the proliferation of cells. The Yes-Associated Protein (YAP) is an important transcription factor involved in the Hippo pathway. This pathway is crucial in regulating tissue homeostasis and its deregulation causes increased angiogenesis, thus supporting tumor growth (Boopathy and Hong 2019). Along with its co-activator called transcriptional coactivator with PDZ-binding motif (TAZ), YAP binds to the TEA DNA-binding domain (TEAD) in the nucleus. This complex activates the transcription of genes responsible for survival and cell proliferation (Boopathy and Hong, 2019). Thus, the inhibition of YAP-TEAD interaction prevents the transcription of downstream genes, thereby keeping the proliferation of cells in check (Boopathy and Hong,

2019). In the current study, the constituents present in the methanolic extract of *Epipremnum aureum* were identified and checked for their overall anticancer activity. Additionally, an *in-silico* approach was used to examine the ability of these phytoconstituents to inhibit YAP.

Materials and methods

Preparation of methanolic extract of *Epipremnum aureum* (MEEA)

The leaves of *E. aureum* were collected from Bangalore, India in the month of December, 2021. The sample herbarium was authenticated by Dr. TG Umesh, Department of Botany, Bangalore University, India as *Epipremnum aureum* (Linden & Andre) G.S. Bunting of the Araceae family. The plant material was then shade dried at room temperature for three weeks and then powdered by coarse grinding. The extract was prepared by macerating the sample at room temperature for 24 h., using 100% methanol (HPLC grade, obtained from Fisher scientific). The ratio used for preparation was 0.1 g of powdered dry plant material per 1 mL methanol. The obtained extract was subjected to filtration twice to remove any impurities. The extract was placed in a hot air oven at around 70°C until all the methanol was evaporated, after which it was weighed and stored at 4°C for reconstitution and use as was required.

Phytochemical characterization of MEEA

MEEA was subjected to some specific biochemical tests to examine the presence of common bioactive secondary metabolites (Table 1). To obtain a more detailed understanding of the chemical nature of these constituents, Gas Chromatography Mass Spectrometry (GCMS) of the extracts was carried out. The GCMS constituents were identified by comparing their retention time to those of the compounds recorded in the NIST libraries.

Evaluation of cytotoxicity of MEEA

MTT assay was conducted to assess the cytotoxicity of MEEA on HCT-116 (human colon cancer) cells. The cell line was obtained from NCCS, Pune. The assay was performed in triplicates for a time point of 24 h., using concentrations ranging from 23.4 µg/mL to 750 µg/mL. Untreated cells were used as negative control.

In-silico studies

In-silico studies were conducted to understand the anticancer potential of MEEA in a more detail-

oriented manner. While the MTT assay provides an overall outlook on cytotoxicity of the extracts, docking and molecular dynamics simulation (MDS) studies focus on interactions at the molecular level of the mechanism.

Selection of target protein and ligands

The yes-associated protein (YAP) was chosen as the target for *in-silico* studies. YAP is an important transcription factor involved in the Hippo pathway. YAP binds to TEA DNA-binding domain (TEAD) in the nucleus, which is necessary to activate the transcription of genes responsible for survival and cell proliferation (Boopathy and Hong 2019). Thus, the inhibition of YAP prevents the activation of TEAD in turn, thereby keeping the proliferation of cells in check. The structure of YAP was selected from RCSB PDB (PDB ID: 3KYS). This is an X-ray diffraction structure (of the resolution 2.80 Å) of the YAP-TEAD complex. Only the B-chain of YAP that contains interface 3 of the protein was retained and all other constituents were removed.

For this *in-silico* study, the GCMS constituents of MEEA were chosen as ligands, to check if they could bind YAP at the site of its interaction with TEAD, and possibly inhibit this interaction. The chemical structures of the 22 constituents of MEEA were obtained using the PubChem database. As a positive control, Narciclasine, an inhibitor of YAP was used (Kawamoto *et al.*, 2021). The structure of this compound was obtained from PubChem (PubChem ID: 72376).

Docking studies

Docking studies were performed using PyRX (version 0.9.8). The structures of all the ligands were energy minimized using the Open Babel feature. Using the AutoDock Vina program, protein-ligand docking was carried out in triplicates. The grid parameters were set to enclose the whole protein as the selected region for binding

(blind docking). The results were obtained in the pdbqt file format and the corresponding binding energies were noted. The docked complexes were visualized in PyMOL (version 2.3.2) and their specific interactions were determined with Ligplot (version v.2.2.5).

Molecular dynamics simulation (MDS)

The docked complexes of all the constituents of MEEA with YAP were examined and the best potential ligands were chosen for molecular dynamics simulation using Maestro Desmond (version 11.9.011). Protein preparation wizard was used to pre-process the complexes, and an orthorhombic box was drawn to enclose the complex. Since the inhibition of YAP is a less explored area from an *in-silico* approach, the simulation environment was set up using a study on the binding of TEAD as a reference (Pobbati *et al.*, 2019), with the assumption that there is no significant variation in the cellular environment wherein these two proteins interact with each other. The simulation environment was set up using the system builder. Additionally, Na⁺ and Cl⁻ ions were added at a concentration of 0.15M, and the pH was set to 7.4. The NPT ensemble was used, and the water model chosen for the simulation was TIP3P. The temperature was set to 300K using the Nose-Hoover thermostat and the pressure was maintained at 1.01325 bar using the Martina-Tobias-Klein barostat. The MDS was performed for 50ns in triplicates.

Results

Preparation of methanolic extract of *Epipremnum aureum* (MEEA)

MEEA was prepared and subjected to complete evaporation of methanol, after which the weight of the extract was obtained. After this, the concentration of plant extract was found to be 2.725 mg/mL using the following formula:

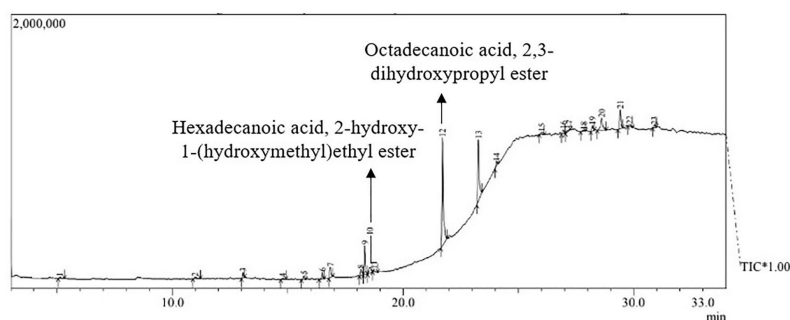


Figure 1. GCMS peaks of MEEA constituents

Concentration = Weight of extract obtained / Total volume of methanol used

Phytochemical characterization of MEEA

Biochemical analysis of MEEA (Table 1) showed that the extract contains compounds which

can exhibit anticancer activity. A detailed GCMS analysis provided a clear scheme of the specific components present in the extracts. Twenty-two compounds (Figure 1) were identified in MEEA with 11 of these constituents having exhibited anticancer activity in previous studies (Table 2).

Table 1. Biochemical characterization of MEEA

Compound	Assay	Procedure	Indication of positive results	Results
Alkaloids	Wagner's test (Pandey and Tripathi, 2014)	Add 1mL KI solution to 1mL extract and shake	Reddish-brown precipitate	Negative
Steroids	Liebermann Burchard's test (Abubakar and Haque, 2020)	Few drops of acetic acid was added to 1mL chloroform extract. Conc. sulfuric acid added along sides of the test tube	Violet-blue ring formation	Positive
Diterpenes / Terpenoids	Copper acetate test (Pandey and Tripathi, 2014)	3-4 drops of copper acetate added to 1 mL extract and mixed	Emerald green coloration	Positive
Flavonoids	Lead acetate test (Pandey and Tripathi, 2014)	Few drops of lead acetate added to 1mL extract and shaken	Yellow precipitate	Negative
Phenols	Ferric chloride test (Pandey and Tripathi, 2014)	3-4 drops of 2% Ferric chloride added to 1 mL extract	Bluish black coloration	Negative
Triterpenes	Salkowski's test (Abubakar and Haque, 2020)	Add 2 mL chloroform to 1 mL solution and then a few drops of conc. sulfuric acid. Mix and allow to stand.	Golden yellow precipitate	Positive
Saponins	Froth test (Pandey and Tripathi, 2014)	2 mL distilled water added to 1 mL extract and shaken vigorously	Presence of foam layer and persistence for 10 minutes	Negative
Anthocyanin	Sodium hydroxide test (Rajesh et al., 2014)	Add 1 mL 2 M NaOH to 1 mL extract and heat at 100 deg. Celsius for 5 minutes	Bluish green coloration	Positive

Table 2. Constituents of MEEA characterized by GCMS and their history of anticancer properties

Sl no	Compound	Pub Chem ID	Molecular formula	Molecular weight	Peak area %	Known to have anticancer activity
1	17-(1,5-Dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol	304	C ₂₇ H ₄₆ O	386	1.16	No
2	2-Pentadecanone, 6,10,14-trimethyl-	10408	C ₁₈ H ₃₆ O	268	1.12	Yes (Esghaei, 2018)
3	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	638072	C ₃₀ H ₅₀	410	1.39	No
4	3,5,3',5'-Tetramethyl-N4-propyl-biphenyl-4,4'-diamine	624765	C ₁₉ H ₂₆ N ₂	282	0.59	No
5	9,12-Octadecadienoic acid, methyl ester	5284421	C ₁₉ H ₃₄ O ₂	294	2.35	No
6	Cholest-5-en-3-ol (3.beta.)-	5997	C ₂₇ H ₄₆ O	386	1.16	No
7	Diethyl Phthalate	6781	C ₁₂ H ₁₄ O ₄	222	1.48	Yes (Mohansrinivasan et al., 2015)
8	Ergost-5-en-3-ol, (3.beta.)-	6428659	C ₂₈ H ₄₈ O	400	1.84	Yes (Bae et al., 2021)
9	gamma.-Tocopherol	927729	C ₂₈ H ₄₈ O ₂	416	0.67	Yes (Jiang, 2017)
10	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	123409	C ₁₉ H ₃₈ O ₄	330	34.97	Yes (Sivakumaran, 2019)
11	Octadecanoic acid	5281	C ₂₁ H ₄₂ O ₄	284	0.63	Yes (Manivannan, 2017)
12	Octadecanoic acid, 2,3-dihydroxypropyl ester	24699	C ₂₁ H ₄₂ O ₄	358	19.98	Yes (Rongpan, 2017)
13	Pentadecanoic acid	13849	C ₁₈ H ₃₆ O ₄	270	3.13	Yes (To, 2020)
14	Pentadecanoic acid, 14-methyl-, methyl ester	21205	C ₁₇ H ₃₄ O ₂	270	1.69	No
15	Phenylacetic acid, 2-(1-Adamantyl)ethyl ester	610091	C ₂₀ H ₂₆ O ₂	294	0.84	No
16	Phytol	5280435	C ₂₀ H ₄₀ O	296	7.7	Yes (Sakthivel, 2018)
17	Propanenitrile, 3,3'-iminobis-	8149	C ₆ H ₉ N ₃	123	0.61	No
18	Silicic acid, diethyl bis(trimethylsilyl) ester	77092	C ₁₀ H ₂₈ O ₄ S ₃	296	0.85	No
19	Stigmasterol	5280794	C ₂₉ H ₄₈ O	412	6.67	Yes (Bae et al., 2020)
20	Sucrose	5988	C ₁₂ H ₂₂ O ₁₁	342	1	No
21	Tetradecanoic acid	11005	C ₁₄ H ₂₈ O ₂	228	0.58	Yes (Zheng et al., 2019)
22	Z,Z-8,10-Hexadecadien-1-ol	5364617	C ₁₆ H ₃₀ O	238	2.88	No

Evaluation of cytotoxicity of MEEA

The results of MTT assay have been graphically represented in Figure 2. It was observed that MEEA has an IC_{50} value of 35.7 $\mu\text{g/mL}$, indicating that the extract used at a concentration of 35.7 $\mu\text{g/mL}$ can inhibit the growth of HCT-116 cells by 50%. This indicates that the extract exhibits anticancer behavior, which may be owed to its constituents.

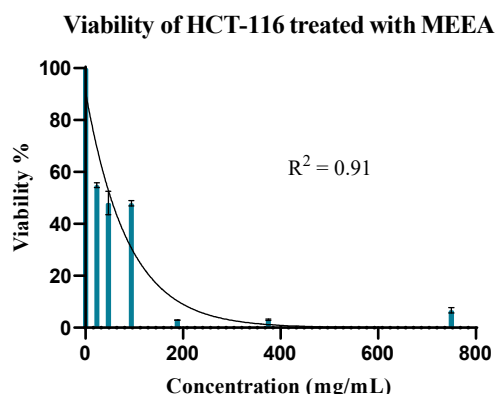


Figure 2. Evaluation of percentage viability of HCT-116 cell line treated with MEEA

In-silico studies

The docked complexes of all ligands obtained as a result of docking studies done in triplicates were visualized with Ligplot. Each trial of docking gave nine conformations of protein-ligand binding. The best binding ligands were chosen based on the following criteria - specificity of interaction with the aforementioned amino acids, higher number of hydrogen bonds formed with these residues, as well as more negative binding energy. Thus, the best three conformations obtained for hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (HE) and octadecanoic acid, 2,3-dihydroxypropyl ester (OE) were selected for further MDS studies. The docking results of these conformations, along with those of the control molecule, have been displayed in Table 3. Figures 3, 4, 5 represent the protein-ligand interactions for one of these conformations, for each ligand.

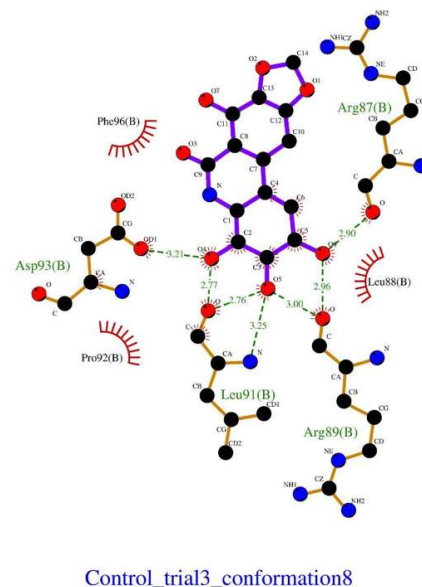


Figure 3. Narciclasine (control) docked with YAP - The visualization of bond types, number and strength on Ligplot

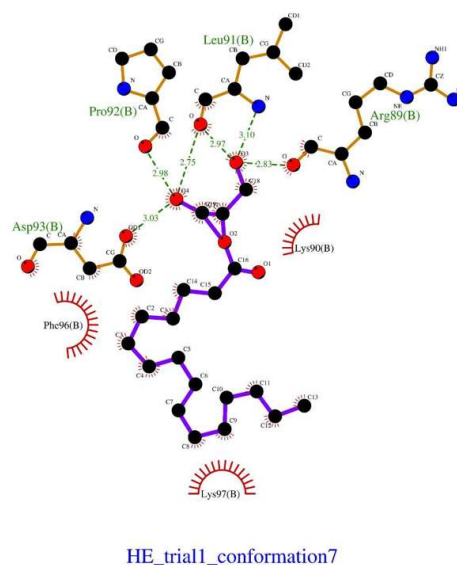
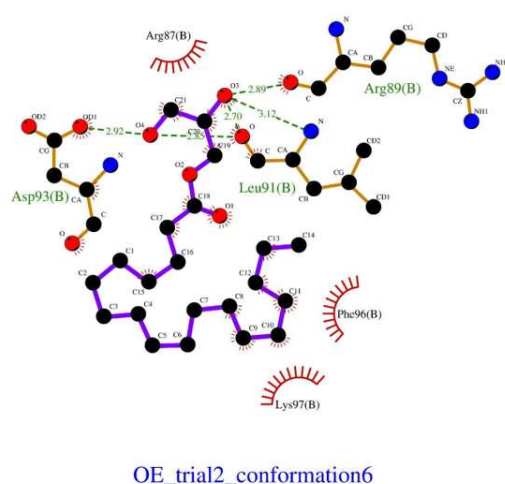


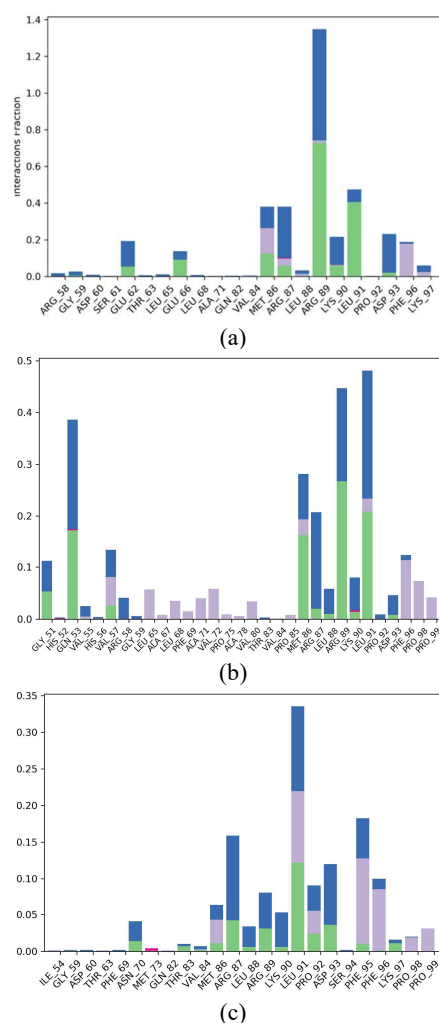
Figure 4. Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester docked with YAP - The visualization of bond types, number and strength on Ligplot

Table 3. Docking results of selected compounds from MEEA with YAP

Ligand	Conformation number	Binding energy (kcal/mol)	Interactions with desired residue numbers (HB = hydrogen bond, HP = hydrophobic interactions; the number in brackets indicates number of bonds)
Control	Trial3_conformation8	-5.2	HB - 89 (2), 91 (3); HP - 96
	Trial2_conformation2	-5.7	HB - 89 (1), 91 (1); HP - 96
	Trial3_conformation2	-5.7	HB - 89 (1), 91 (1); HP - 96
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	Trial2_conformation1	-3.5	HB - 89 (2), 91 (2); HP - 96
	Trial1_conformation7	-3.5	HB - 89 (1), 91 (3); HP - 96
	Trial1_conformation4	-3.5	HB - 89 (1), 91 (2); HP - 96
Octadecanoic acid, 2,3-dihydroxypropyl ester	Trial1_conformation3	-4.1	HB - 89 (2), 91 (2); HP - 96
	Trial2_conformation8	-3.6	HB - 89 (2), 91 (2); HP - 96
	Trial2_conformation6	-3.7	HB - 89 (1), 91 (3); HP - 96

**Figure 5. Octadecanoic acid, 2,3-dihydroxypropyl ester docked with YAP - The visualization of bond types, number and strength on Ligplot**

MDS was performed to check the effect of ligand binding on YAP in simulated cell conditions. YAP in its native state was simulated to study its default behavior in the specified environment. This information was used to differentiate the changes in the protein structure caused as an effect of ligand binding, from those caused by the interaction of the native protein with its environment. On performing MDS for 50 ns, it was observed that HE and OE displayed specific and strong hydrogen bonding with residues Met 86, Arg 89 and Leu 91 of YAP. OE also showed strong hydrophobic interactions with Met 86, Leu 91 and Phe 95, while HE had moderate hydrophobic interactions with Met 86 and Leu 91. Both ligands also interacted with Phe 96 hydrophobically. These interactions were visualized through the interaction graphs (Figure 6), protein RMSD (root mean square deviation) graph (Figure 7) as well as the protein RMSF (root mean square fluctuation) graph (Figure 8).

**Figure 6. Type and strength of ligand interactions with YAP. (a) Interactions of control with YAP. (b) Interactions of hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester with YAP. (c) Interactions of octadecanoic acid, 2,3-dihydroxypropyl ester with YAP. Here, green, gray, pink and blue represent hydrogen bonds, hydrophobic interactions, ionic bonds and water bridges, respectively**

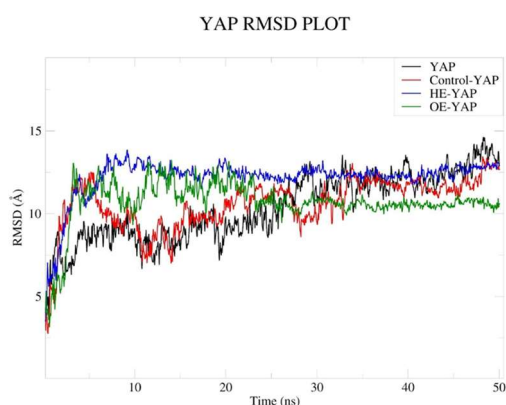


Figure 7. YAP RMSD plot upon interaction with Narciclasine (control), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (HE) and Octadecanoic acid, 2,3-dihydroxypropyl ester (OE) overlapped using QTgrace software (version: 5.6.0)

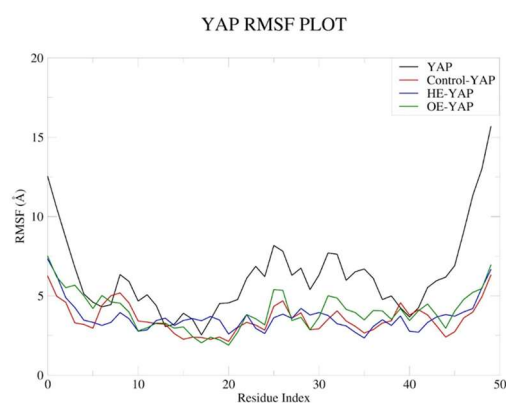


Figure 8. YAP RMSF plot upon interaction with Narciclasine (control), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (HE) and Octadecanoic acid, 2,3-dihydroxypropyl ester (OE) overlapped using QTgrace software (version: 5.6.0)

RMSD gives a measure of how much the structure of the protein complex has fluctuated over time. This can be useful to compare various structures (protein, protein with ligand, etc.) to check for similarity and differences in stability. In this study, average RMSD values have been calculated from the period of 10ns to 50ns, since the protein ligand complexes took approximately 10 ns to attain stability in the simulation environment. It has been observed that HE-YAP and OE-YAP had average RMSD values of 13.2 Å and 12.2 Å respectively, while control-YAP had an average RMSD of 11.9 Å. OE-YAP and control-YAP

displayed similar RMSD values to that of the native protein (11.9 Å). Although HE-YAP displayed a relatively higher RMSD, there seemed to be minimal fluctuation in its RMSD values over time.

Additionally, RMSF is a measure of the total fluctuations of individual residues of the protein during the entire period of molecular simulation. This can be useful to estimate behavior of individual residues of the complex in its environment. It has been noted that the average protein RMSF for the region consisting of the amino acid residues 85-100 is 7.23 Å. Average RMSF value for the Control-YAP complex was found to be 3.71 Å. HE-YAP and OE-YAP displayed values of 3.74 Å and 4.30 Å respectively. The effect of the interaction of HE with YAP seems to be comparable to that of our control, while OE has a higher RMSF value than the control.

Discussions

The medicinal properties of plant extracts are generally attributed to the chemical constituents present in them. To prepare the extract, methanol as the solvent was selected, because previous studies have reported that its small molecular size and high polarity allows for greater permeation into plant tissues and dissolution of required compounds within (Valakatte and Kavitha, 2022). MEEA was first subjected to phytochemical characterization to check if it contains any components with known, or possible anticancer activity. On performing GCMS analysis, it was observed that 50% of the total number of compounds present in MEEA have been identified to have anticancer properties, from prior studies (Table 2). Several of these substances have been previously shown to inhibit the growth of HCT-116 cells, in separate studies (Campbell *et al.*, 2006; Frassini *et al.*, 2019; Abdel-Mageed *et al.*, 2021). Since different chemical compounds have different mechanisms of interfering with biochemical pathways, it was hypothesized that the constituents of MEEA may be capable of working synergistically to inhibit cancer cell growth and proliferation. This was supported by the results of the MTT assay carried out against the HCT-116 cell line. MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) is a yellow-colored tetrazolium salt which can be reduced to purple colored formazan by the NAD(P)H dependent oxidoreductase enzymes present in the mitochondria of viable cells (Liu *et al.*, 1997). The amount of formazan formed is proportional to the number of cells, thus quantifying the viability of cells when subjected to our extracts. From Table 2

and Figure 2, it was observed that MEEA, with an IC_{50} of 35.745 $\mu\text{g/mL}$, has significant cytotoxicity against cancer cells. This agrees with a previous study (Ali, 2018) wherein the acetone extract of *E. aureum* roots could inhibit the growth of HEPG-2 cells with an IC_{50} of 36.7 $\mu\text{g/mL}$. This is also in accordance with commercially used anticancer drugs which can have IC_{50} values over a broad range, such as 0.368 $\mu\text{g/mL}$ - 35.5 $\mu\text{g/mL}$ as seen in the work done by Cai *et al.* (2019).

While the MTT assay provided insight on the activity of MEEA at a cellular level, it was also necessary to understand the significance of this phenomenon at the molecular level. This was done using *in-silico* studies. The constituents of MEEA were docked to YAP, to see if they can inhibit its activity by inhibiting its interaction with TEAD. YAP contains a TEAD binding domain with three interfaces, through which it binds to TEAD in the nucleus to facilitate its activation. The interaction between YAP and TEAD is mediated through these interfaces, out of which interface 3 is necessary for TEAD binding (Zhang *et al.*, 2014). Interface 3 of YAP is the twisted coil region (also called Ω -loop) which ranges between 86-100 residues. Arg 89, Ser 94, Met 86, and Leu 91 are the most crucial residues in interface 3, necessary for strong and efficient hydrogen binding to TEAD (Zhang *et al.*, 2014). Additionally, Met 86, Leu 91 and Phe 95 form a part of the hydrophobic core of YAP, and are necessary for hydrophobic interactions with TEAD (Zhang *et al.*, 2014). It has been seen that inhibition or loss of any of these residues, along with Phe 96, can lead to a loss of YAP activity (Zhang *et al.*, 2014). It was observed that our ligands display these specific hydrogen and hydrophobic interactions, which indicates that they are theoretically capable of binding YAP in the place of TEAD. These findings were supported by the trends seen in the RMSD (Figure 7) and RMSF (Figure 8) values of the protein-ligand complexes.

RMSD is the overall deviation caused in the structure of protein over time, as a result of its interaction with the ligand, and the surrounding environment. The RMSD value of protein-ligand complexes has a dependency on the molecules present in the environment, such as water and the salts. Since these conditions were maintained to be constant across all cases, it is possible that they did not have a significant contribution to the variability across different protein-ligand complexes, which explains the similarity amongst the RMSD values obtained for these complexes. On the other hand, the RMSF value is a direct representation of the effect of ligand binding on the protein stability. It provides an idea about the local fluctuations caused in the protein spatially, due to its interaction with

the ligand. It was observed that the RMSF value of the protein-ligand complexes used in the study are roughly half the RMSF of the nascent protein. This implies that the hydrogen bonds and hydrophobic interactions formed by this region of the protein with the ligands of interest can reduce the fluctuation in the structure of the protein, and increase its compactness. The information obtained by examining the RMSD and RMSF plots suggests that the changes in the nature of the protein are more likely to be caused due to the binding of the ligands, rather than any environmental fluctuations. Thus, it can be concluded that HE and OE interact with the selected region of YAP in a favorable manner *in-silico*, and may possibly inhibit its activity *in-vitro*.

Conclusions

This study was focused on examining the anticancer potential of the methanolic extract of *Epipremnum aureum* (MEEA). The plant extract was preliminarily subjected to phytochemical analysis, to determine the constituents that may be responsible for their therapeutic action. It was found that the extract contains compounds with previously established anticancer behavior. Next, cytotoxicity assay showed that MEEA can inhibit the growth and proliferation of HCT-116 cells, with an IC_{50} value of 35.7 $\mu\text{g/mL}$. *In-silico* studies were conducted to understand this inhibition at a molecular level. Docking results of MEEA indicated that two compounds, hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester and octadecanoic acid, 2,3-dihydroxypropyl ester are capable of disrupting YAP-TEAD interactions and may also inhibit its activity in the cellular environment. The fact that these two compounds constituted a large fraction of the extract (HE = 34.97%, OE = 19.98%) indicates that MEEA may prevent cancer cell proliferation by regulating the Hippo pathway, and explains the high potency (IC_{50} = 35.7 $\mu\text{g/mL}$) of the extract.

It is known that the constituents of plant extracts can often interact with each other to produce synergy, which can have a beneficial effect on therapeutics (Valakatte and Kavitha, 2022). Since cancer involves an aberration in the functioning of several pathways, it is possible that the various constituents of MEEA may be working together synergistically to inhibit or activate many of these pathways at the same time, thus giving an overall inhibition of cancer growth. For instance, a previous study conducted in our laboratory showed that stigmasterol present in *Justicia wynaadensis* can bind thymidylate synthase effectively, with a binding energy of -8.3 kcal/mol when compared to the reference drug capecitabine

(binding energy -6.6 kcal/mol) (Vandana *et al.*, 2020). This indicates that this compound can possibly inhibit the activity of this enzyme. Stigmasterol constitutes about 6.67% of MEEA, which indicates that the extract may employ this mechanism of cancer inhibition also. Additionally, *J. wynaadensis* extract containing this compound was also seen to inhibit the growth of HCT-116 cells with an IC₅₀ of 28.5 µg/ml (Vandana and Shanti 2017), which further supports our findings on the same cell line.

Venkatesh *et al.* (2021) have shown that treatment with ethanolic extract of *E. aureum* can reduce tumor volume in mice. This can be validated further by using the *in-vitro* and *in-silico* findings of our study in mouse tumor models. We performed a preliminary *in-silico* analysis of MEEA constituents against YAP, but there is scope to perform more detailed studies on the same, as well as explore the effect of these ligands on other proteins involved in cancer pathways. Further, this study has only examined the cytotoxic activity of the entire extract *in-vitro*, but it would be useful to assess the effect of our lead molecules HE and OE individually and in combination with other MEEA constituents on HCT-116 cells as well.

Thus, it can be concluded that the methanolic extract of *Epipremnum aureum* has significant anticancer potential, and its phytochemical constituents have the potential in becoming efficient therapeutic molecules for cancer therapy by interfering with the biochemical pathways involved in the survival, growth and proliferation of cancer cells.

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