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Original article

Biochemical characterization, cytotoxic, antimutagenic, anticancer and molecular docking studies on *Tecomella undulata*

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ABSTRACT

In this study bioassay-guided screening of Tecomella undulate was performed for its cytotoxic, antimutagenic and anticancer potential. The ariel parts were extracted on a polarity basis (methanol, dichloromethane and hexane). The in vivo toxicity was assessed on Caenorhabditis elegans, and its locomotion was affected by Tecomella undulata hexane (TUAH) the most. Ames test for antimutagenicity showed Tecomella undulata methanol (TUAM) exhibited against mutagen 2AA showed inhibition of 71.03% and 26.32% 2AA in TA98 while in in vitro MTT assay on carcinoma cell lines TUAM showed 68.1% cytotoxicity. Moreover, In resazurin assay on fibroblast cells African green monkey kidney VERO and on the panel of carcinoma cell lines, the most effective extract was TUAM on liver HepG-2 with CC_{50} value 117. 37 ± 4.73 μg/ml followed by on lungs A549 with 142.01 ± 5.3. Furthermore, for the bioassay-guided screening, the selectivity index was calculated for TUAM CC_{50} ratio on HepG-2 and VERO which showed a decent 2.77 score. After column chromatography, the fraction TU-63 should remarkable cytotoxic effect in dose–response manner assay as (Hep-G2) CC_{50} value 11. 67 ± 1.37 μg/ml followed by (A549) CC_{50} value 17.23 ± 0.58 μg/ml. For qualitative analysis of anticancer potential LC-ESI-MS/MS the potential phytochemicals were identified. In silico molecular modelling against selected carcinogenic proteins. The results suggest Tecomella undulate the substantial anticancer potential which supports potential natural anticancer therapeutic drug candidate development for combating cancer.

1. Introduction

Cancer is caused by abnormal cell proliferation, which leads apoptosis suppression and uncontrolled proliferation of malignant cells due to the defective regulatory pathway, mutation, UV radiation or cellular stresses defects in the regulatory pathways (Burger, 2016). Over the years, 40% of anticancer drugs developed including novel compounds, are either phytochemical based on their derivatives. Folk herbal medicinal system in various part of the world is utilized successfully against different ailments. Phytochemicals have the massive potential the regular screening of secondary metabolites of folk herbs leads to the discovery of effective and novel antimicrobial, antiviral and anticancer phyto-compounds. The scientists have a great interest in secondary metabolites of plants because of their accessibility, least adverse effects and superior biodegradability and enhanced bioavailability as compared to other available synthetic agents. Higher green plants are producing some structurally unique secondary metabolites belongs to phytochemical classes like alkaloids, flavonoids saponin, steroids, carbohydrates and terpenoids exhibit antimicrobial, contraceptive, antitumor, anti-hypertensive, laxatives, cardiotonic agent and antivirals. Medicinal Plants have been used in the traditional drug system and explored for their pharmacological importance for some the remarkable present modern drugs (Alamgir, 2017).

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Pakistan is geographically located in South East Asia, with rich agriculture and diverse flora (Salma et al., 2012; Grimmett et al., 2016). Worldwide only 8% of the plants are pharmacologically investigated and this percentage is even much lower in the case of Pakistan (Younis et al., 2018).

Traditionally *Tecomella undulata* is used as herbal medicine in South Asian countries like India, Bangladesh, and Pakistan (Saggoo et al., 2017). Pakistan has a sound basis for successful traditional medicine as part of cultural heritage which has played a significant role in health care provision to a large number of populations mostly residing in rural areas (Zehri et al., 2020). The World Health Organization also appreciates the role of traditional medicines in the prevention and promotion of health care facilities in developing countries and encourages the appropriate policy formulation for the practice of traditional health care services. Several, anticancer medicinal plants from the traditional medicinal system are reported by researchers after evaluations on cell lines and animal models to comprehend the pharmacological, mechanistic and physiological alterations associated with the anticancer potential of phytochemicals. *Figonia creatica* aqueous extract has shown promising anticancer potential on MCF-7 breast cancer cell line via up-regulation of p53 gene activation. In Pakistan, *Tecomella* undulata is recommended by traditional healers of South Punjab and Sindh for leucorrhoea, urinary tract infection, wound healing, piles, and microbial infections and predominantly for liver complications. The present study was designed to identify the bioactive potential of *Tecomella* undulata along with cytotoxicity, antimutagenecity and *in vitro* anticancer potential in bioassay-guided manner validated with *in silico* molecular docking simulations.

2. Materials and methods

2.1. Field trips for the collection of plant material

The field trip was managed (2016–2017) for the *Tecomella undulata* plant collection, Bio Park BZU, Multan in Punjab province of Pakistan. The geographic coordinates were recorded by global positioning system (GPS) 30°16′05.5″N 71°30′05.6″E were maintained in decimal degrees as latitude and longitude were identified. The Satellite view of locations was also generated from Google map and labelled with the plant's botanical name.

2.2. Extraction of plant material

The percolation extraction was employed for small-scale extraction with three solvents with variable polarity parts that were shade dried under pressurized air. Plant extracts labelled with four capital letters refer to botanical name initials, part used and solvent. *Tecomella undualata* ariel parts in solvents methanol (M), hexane (H) and dichloromethane (D), solvents were recovered on the rotary evaporator and crude extracts of *Tecomella undualata* ariel parts (TUAM) (TUAD) (TUAH) were obtained and stored at 4 °C. After percolation extraction was sonication assisted for 15 min to increase the yield and kept at room temperature for 24 h before filtration followed by liquid–liquid extraction and evaporated over rotary evaporator.

2.3. Culture and maintenance of Caenorhabditis elegans strains

The *Caenorhabditis elegans* wild-type was maintained on Nematode Growth Medium (NMG) and *Escherichia coli* strain OP50 for anthelmintic screening (Gontijo et al., 2019). Briefly, the L1s were transferred to an incubator on Nematode Growth Medium (NMG) plate with *E. coli* OP50 at 20 °C; the culture plates were prepared by washing worms thrice with M9 buffer.

2.3.1. Caenorhabditis elegans toxicity assay

The assay was carried out in a 96-well microplate (flat-bottomed, TPP Techno Plastic Products AG, Switzerland). Adult worms were placed in M9 buffer and managed around 3000 larvae/ml. Almost 40 larvae in 12 μl of buffer were seeded per well in 96well microplate with 182 μl of *E. coli* (OP50 culture) at an Optical density of 600 = 0.5. The 96 well microplate was then properly placed for 16 h in WMicrotracker (Phylumtech, Argentina) at 20 °C. The movement of the worm was analysed and recorded the global locomotion/ movement index (De Silva and Alcorn, 2019; Risi et al., 2019).

2.4. Anti-mutagenic activity

This assay determines the potential mutagenic capability of tested extracts by reverse mutation induction in his operon. Bacterial strain including *Salmonella typhimurium* (TA98 and TA100) cultured in Nutrient broth with a volume of 100 μl and 1×10^9 cell/mL were prepared along with labelled minimal glucose agar plates. The mutagenic agents used here were sodium azide 2-aminoanthracene (2AA) 5 μg of each mutagenic agents was dissolved in 100 μl of DMSO to make a solution (Kumar et al., 2020). To a (sterilized) tube 100 μl from mutagenic agent solution and 100 μl of plant extracts were mixed properly. Correspondingly, 100 μl of *Salmonella typhimurium* were added to tubes and thoroughly mixed or vertexes and incubated at 37 °C for 30 min. The 2 ml of top agar 45 °C accompanied with small histidine was mixed in a sterile tube; this addition of histidine will allow the bacterial to first time grow and can have the ability to mutate. The whole tube material was transferred and applied on the surface of the minimal glucose agar plate after that incubated for 48 h at 37 °C. Subsequently, after the incubation period, the revenant colonies were counted by employing a formula.

% Antimutagenic Activity = A-B/A X 100

Where A = Number of revertant sin [media + mutagen] Control; B = Number of revertanats in [media = mutagen = test compounds] Sample

2.5. MTT (3-[4, 5-dimethylthiazol-2-y1]-2, 5- diphenyltetrazolium bromide) cytotoxicity assay

MTT assay is a colourimetric cell proliferation and chemosensitivity analysis assay. MTT (3-[4, 5-dimethylthiazol-2-y1]-2, 5- diphenyltetrazolium bromide) is a dye that can reduce viable cells the formazan crystals are insoluble in the aqueous environment. The cells attached to the bottom are viable cells with more adhesion (Salam and Quave, 2019; Loizzo et al., 2020). In 4×10^4 cells were seeded per in 96 well cell culture grade plates. The medicinal plant extracts were added to the plates with a conventional cell culture environment of 5% CO_2 at 37 °C for 24 h. After the incubation period, the fresh 100 μl culture media and 30 μl of MTT solution were also dispensed. The plate was further wrapped in aluminium foil and placed in the incubator for 4 h at 37 °C incubators. The media was removed and formazan crystals were dissolved in 200 μl of DMSO (dimethyl sulfo-oxide) in each well and absorbance was taken at 570 nm and 620 nm reference wavelength as reference.

Cell Viability % = (Test 570 nm-620 nm)/ (Control 570 nm-620 nm) × 100

2.6. Resazurin (7-hydroxy-3H-phenoxazin-3-one 10-oxide) chemosensitivity assay

Resazurin or Almar blue dye monitors the reduced oxidative environment of cells any change can be observed by quantitative

Table 1
In vivo Caenorhabditis elegans toxicity assay.

Plant Extracts	*Caenorhabditis elegans* mobility index (% reduction in locomotion)			
	Time (hours)			
	0–12	12–24	24–36	36–48
Negative Control	97.78761	92.73973	91.30841	90.18018
Levamisole	22.62578	13.37543	8.52083	3.70833
TUAM	57.76712	41.0885	30.18692	29.18919
TUAD	70.17699	65.75342	64.95327	58.64865
TUAH	34.41441	27.67512	20.46729	6.28386

measurement in the form of fluorometric analysis. Resazurin (7-hydroxy-3H-phenoxazin-3-one 10-oxide) blue, is a highly fluorescent redox dye it can be reduced by the metabolically active live cell to resorufin (7-hydroxy-3H-phenoxazin-3-one) pink. The cultured cells were counted and a cell suspension containing 15000–20000 cells/well was prepared in the complete culture medium. Then 200 µl of cell suspension was brought into each well of a 96-well plate containing the pre-diluted sample, except in the negative control wells. The plates were then incubated for 3 days at 37 °C with 5% CO_2. The plates were taken out of the incubator and 20 µl of resazurin working solution was added to each well of the microtiter plate. The plate was then further incubated for 4 h at 37 °C. Each experiment included a set of negative controls (untreated cells), a DMSO control group and positive control gossypol. The fluorescence of the plate was read using the wavelength of excitation 550 nm and emission 590 nm and finally, the %reduction of the cell viability was compared with the positive control. The viable cells reduce the resazurin to pink-coloured fluorescent resorufin. Subsequently, fluorescence is measured by cell plates in a FLEXstation II microplate reader (Molecular Devices) (Suksiriworapong et al., 2018).

2.7. Chemical characterization

2.7.1. Silica gel column chromatography

It is the type of preparative chromatography which deals with the separation of constituents using different elution techniques like adsorption.

2.7.2. Column packing and chromatography

A glass column (8 cm X 30 cm) was packed with silica gel 200 g (mesh size: 63–200 µm) mixed in a screw-capped bottle with 500 ml of 100% hexane to form a slurry and transferred to glass column. Once the silica gel slurry has settled, the dried plant extract-silica mixture was evenly placed on the silica gel bed. Stepwise elution gradient was developed in four solvents on increasing polarity order; Fractions of 40 ml each were collected in 50 ml falcon tube labelled before and fractions were air-dried. All the fractions obtained were tested *in vitro* chemotherapeutic and pharmacological assays.

2.8. HPLC DAD and LC-ESI MS/MS analysis

The most effective fraction was subjected to LC ESI MS/MS Analysis in this system HPLC (Shimadzu Corp., Japan) was coupled with Electrospray Ionisation linear Trap(LTQ-XL) along with liquid chromatography equipped with a spectrometer (LC-MS) (Thermo Fisher Scientific, USA). Through auto-sampler, the sample was injected into HPLC. An HPLC system with a diode array detector (Nwachukwu et al., 2017) was used for HPLC purification. Aliquot of 0.5 mg dried active fraction obtained after assessment on chemotherapeutic assay was dissolved in 1.0 ml of methanol and chromatographed. The flow rate was 1.0 ml/minute. A small aliquot of the dried fraction was dissolved in 2.0 ml of 20% acetonitrile (ACN) in a water mixture in the beginning and purified by C18 column (Sunfire®, 10x250 mm, 5 µm) for 60 min. The photodiode array and mass spectrometer connected detected the prominent peaks and corresponding natural compounds were ionized employing Atmospheric Pressure Electrospray Ionization (Modarresi et al., 2019) probe at negative ion mode with a flow rate of 9 L/min and the auxiliary gas flow rate of 2 L/min. The temperature of 300 °C, with (voltage 4.5 kV. The fragment pattern obtained was confirmed with compound library and mass spectra simultaneously; the qualitative evaluation was performed concerning the time of retention and quantitative with measurement of liquid chromatogram peaks in mass spectra.

2.9. Molecular docking analysis

The preparation of five selected proteins that were involved in overexpression, or uncontrolled proliferation of abnormal cells. These proteins play their part in various types of cancers including hepatocellular carcinoma, colorectal carcinoma, pulmonary, breast, and ovarian cancers. The 3D structures of 5 different proteins were retrieved from Protein Data Bank (PDB) (http://www.rcsb.org), autodock vina uses the same PDBQT molecular structure file format for input the autodock tool 1.5.6. The structures of the molecules being docked were subjected to auto grid parameter files (GPF, DPF) and grid map files. Ligands with a value greater than −7 kcal/mol were selected for further *in vitro* assessment. PyMOL can produce high-quality 3D images of small molecules and biological macromolecules, such as proteins (Tahara et al., 2020) for analysis, the docked PDB files were uploaded in the PyMOL and the results were analysed. The phytochemicals selected for *in silico* evaluation were also subjected to assessment of classical drug likeliness properties (ADME) absorption, distribution, metabolism and elimination or excretion and Lipinski's rule of five a conventional virtual evaluation method to assess the bioavailability.

2.10. Statistical analysis

The results were analysed statistically and IC_{50} and CC_{50} values were carried out using Graph Pad Prism 8.0 (Graph Pad Software Inc., San Diego, CA).

3. Results

3.1. Collection

The plant material was collected from an identified site in Multan South Punjab province of Pakistan, the geographic coordinates were recorded by global positioning system (GPS) record was maintained in decimal degrees as latitude and longitude were identified and satellite view of location was also generated from Google map. After Identification, the specimen was submitted to the herbarium at University of the Punjab Lahore voucher submission number LAH #28120B.

Table 2
AMES test for anti-mutagenic potential.

Plant Extracts	Concentration	No. of Revertant Colonies			
		TA98 (AZS)	TA98 (2AA)	TA100 (AZS)	TA100 (2AA)
Control	(μg/plate)	216.31 ± 4.5	187.4 ± 9.3	263.3 ± 14.1	305.4 ± 8.1
TUAM	125	126.3 ± 1.7	131.4 ± 3.2	45.4 ± 3.2	173.3 ± 5.1
	250	93.2 ± 0.9	104.1 ± 1.6	84.0 ± 3.2	122.6 ± 9.6
	500	78.2 ± 2.6	93.14.2	63.3 ± 3.6	113.1 ± 8.1
	1000	73.5 ± 4.8	73.8 ± 6.4	56.6 ± 1.6	84.01 ± 0.7
TUAD	125	923 ± 1.8	876 ± 1.2	1378 ± 5.3	1124 ± 5.7
	250	864 ± 0.3	765 ± 0.5	1276 ± 0.3	947 ± 0.5
	500	654 ± 0.6	643 ± 1.5	946 ± 0.7	732 ± 0.2
	1000	586 ± 0.7	483 ± 4.5	876 ± 0.6	538 ± 0.5
TUAH	125	674 ± 3.7	562 ± 2.6	628 ± 1.2	539 ± 4.6
	250	537 ± 4.6	483 ± 4.6	528 ± 5.5	414 ± 1.9
	500	384 ± 4.3	384 ± 2.1	432 ± 2.0	383 ± 2.6
	1000	294 ± 3.3	304 ± 2.4	413 ± 1,6	326 ± 0.5

3.2. Extraction and % yield

The percolation extraction was employed with three solvents with variable polarity methanol, chloroform and hexane then dried under pressurized air. Plants extracts labelled with four capital letters refer to botanical name initials, partly used and solvent. *Tecomella undualata* ariel parts % yield recorded as 5.2 ± 0.2 (TUAM), 3.6 ± 0.3 (TUAD), and 2.1 ± 0.2 (TUAH) for plant extract solvents were represented by methanol (M), dichloromethane (D) and hexane (H) in liquid–liquid extraction.

3.3. Caenorhabditis elegans WMicroTracker based in vivo toxicity assay

Here *Caenorhabditis elegans* were selected as laboratory model experimental organisms for *in vivo* biotoxicity assessment of *Tecomella undulata*. The WMicroTracker offers the competency to quantity the locomotive activity and *practicality* of *Caenorhabditis elegans* and parasitic nematodes cultured in liquid media and 96well plates (Doyle and Parker, 2018). To measure the motility index in the infrared detection system WMicroTracker ™ Phylumtech, reading was recorded in microtiter plate after every 30 min excluding the first-hour test samples were compared to DMSO control. The quantitative data obtained was to evaluate the toxic potency on *Caenorhabditis elegans* for *Tecomella undualata* ariel parts (TUAM) (TUAD) (TUAH). The Global mobility index, depicting the effect of plant extracts measured by WMicroTracker after every 12 h on the mobility of *Caenorhabditis elegans* concerning the time for 48 h, the results showed lethality by the decrease in mobility *Caenorhabditis elegans* lethality was observed by *Tecomella undulata* (TUAH) as shown in Table 1.

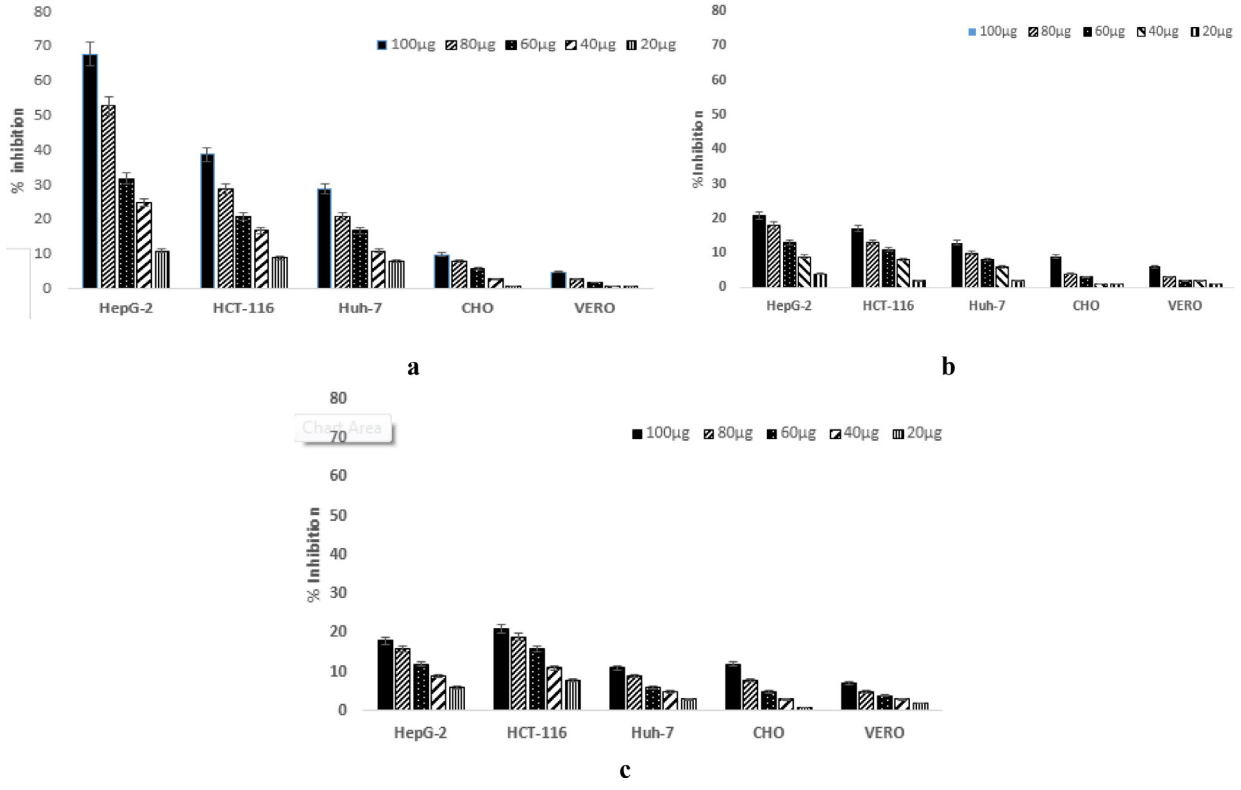

Fig. 1. % cytotoxicity by MTT 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) for *Tecomella undualata* ariel parts (a) TUAM (b) TUAD (c) TUAH on Chinese Hamster (CHO) normal fibroblast followed by homo sapiens carcinoma cell lines, liver (HepG-2) colorectal (HCT-116) and liver (Huh-7).

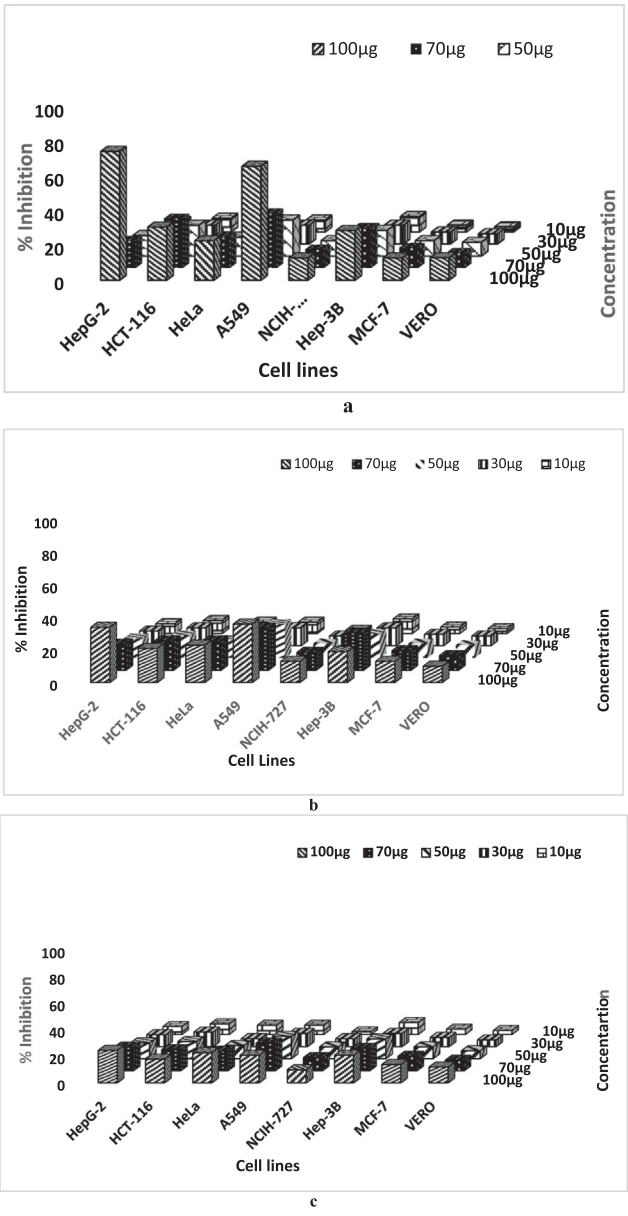

Fig. 2. % cytotoxicity by resazurin fluorescence assay for *Tecomella undualata* ariel parts (a) TUAM (b) TUAC (c) TUAH African green monkey kidney (VERO) normal fibroblast followed by homo sapiens carcinoma cell lines, liver (Hep-G2), cervix adenocarcinoma (HeLa), epithelial tissue of lung (A549), lung bronchial (NCIH-727), liver integrated HBV (Hep-3B) and breast (MCF-7).

3.4. AMES test for antimutagenicity

The mutagen sodium azide (AZS) acts with direct-acting mechanism mutagenicity is intervened by L-acid adenine production which interacts with DNA and point mutation is originated by induction of G: C → A: T transition. The other mutagen 2-aminoanthracene (2AA) acts indirect DNA adduct and its electrophilic reactive metabolites reported for their potential to mutate the strain and restore the ability to grow even in histidine deficient culture media. The anti-mutagenic substance makes the mutagen vulnerable and hampers or blocks this alteration in the genetic code of DNA. Here, *Tecomella undualata* ariel parts methanol (TUAM) were tested for their antimutagenic potential with negative control 0.2% DMSO and sterility control was with bacterial inoculum tested substance as given in Table 2. The antimutagenic potential of *Tecomella undualata* ariel parts methanol (TUAM) activity was moderate for strain TA100 AZS against mutagen at a concentration of 1500 µg/ml and 125 µg/ml inhibition recorded was 73.15% and 42.85% activity respectively but for strain TA98 at same concentrations inhibition was 83.13% and 64.12%. For *Tecomella undualata* ariel parts, methanol (TUAM) against mutagen 2AA showed inhibition of 71.03% and 26.32% 2AA in TA98 while strain TA 100 was 76.55% and 44.05% at the concentrations fore mentioned in Table 2.

3.5. MTT 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) in vitro cytotoxicity assay

MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) *in vitro* cytotoxicity assay is a rapid, profound sensitive *in vitro* assay for the evaluation of cytotoxic effect by plant extracts this spectrophotometer based assay absorbance of MTT dye is measure in viable cells. MTT assay was performed separately on Chinese Hamster (CHO) normal fibroblast cell lines and carcinoma cell lines HepG-2, HCT116 and Huh-7. An incubation of 24 h and five different concentrations from 10 µg/ml to 100 µg/ml were tested *Tecomella undualata* ariel parts (TUAM) (TUAD) (TUAH). During this test the mitochondrial metabolic reduction of 3-(4, 5-dimethyl-2-thiazolyl)-2, 5- diphenyl-2H-tetrazolium bromide to form formazan (insoluble). As three extracts (TUAM) (TUAD) (TUAH) showed limited % inhibition cytotoxicity on fibroblast CHO and Vero cell lines as at highest concentration were TUAM 10% and 5.4% TUAD 12.1%, and 7.2%, TUAH 9%0.0 and 6.0%, respectively. Whereas, cytotoxicity on carcinoma cells HepG-2 cell line for TUAM 68.0%, TUAD 18.6%,TUAH 21.9 while on HCT-116, TUAM 39.5%, TUAD 21.2%, and TUAH 17.5%, on Huh-7, TUAM 29.5%, TUAD 11.3% and TUAH 13.0% respectively at highest concentrations were documented (Fig. 1).

3.6. Resazurin fluorescence chemosensitivity assay

Resazurin based cytotoxicity assessment is a rapid, sensitive, cost-effective fluorescence quantitative assay (Helm et al., 2017). The non-toxic resazurin compound can pass the cell-permeable membranes the reducing potential of viable cells converts blue resazurin (7-hydroxy-3H-phenoxazin-3-one 10-oxide) non-fluorescent dye to resorufin (7-hydroxy-3H-phenoxazin-3-one) red fluorescent dye (Koutsoni et al., 2019). The viable cells proliferate and create a reduced environment in cytosol although by acceptance an electron from the respiratory chain of mitochondrion of viable cells, non-viable cells maintained an oxidized environment. The amount of resorufin produces is directly proportional to the number of viable cells the fluorescent signal of the resorufin was observed by a wavelength of excitation 550 nm and an emission wavelength of 590 nm. *Tecomella undulata* (TUAM) (TUAD) (TUAH) extracts were carried out step by step to isolate bioassay-guided fractionation containing chemotherapeutic constituents. The resazurin cell viability fluorescence assay is a sensitive and colourimetric assay that measures the viability of cell cytotoxicity it provides homogeneity for fluorimetric detection of viable cells (Metta et al., 2017). The extracts were tested against the panel of seven tumour cell lines, homo sapiens colon colorectal carcinoma cell (HCT-116) ATCC CCL-247, homo sapiens liver carcinoma cell (Hep-G2) ATCC HB8065, homo sapiens cervix adenocarcinoma (HeLa) ATCC CCL-2, homo sapiens epithelial cell from lung tissue (A549) ATCC, CCL-185, homo sapiens lung bronchial carcinoma (NCIH-727) ATCC, RL-5815, homo sapiens liver cancer cell, integrated HBV (Hep-3B) ATCC HB8064 and homo sapiens breast carcinoma cell (MCF-7) ATCC HTB-22 and African green monkey kidney (VERO) normal fibroblast cell line ATCC CCL-81-VHG (Fig. 2). The most effective extract was TUAM on HepG-2 with 74.7% followed by on A549 with 66.1% and on VERO normal cell

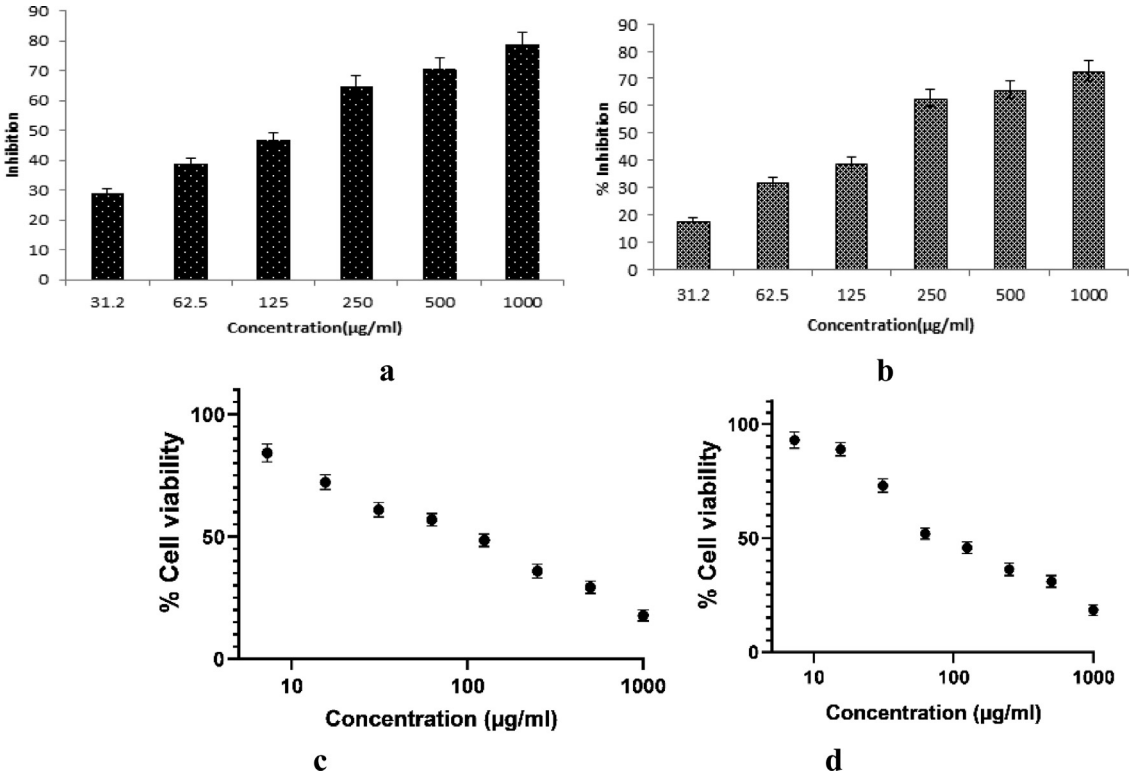

Fig. 3. Dose-response assay *Tecomella undulata* (TUAM) results are expressed in mean ± SEM on Homo sapiens carcinoma cell lines (a) liver (Hep-G2) CC_{50} value 117.37 ± 4.73 µg/ml, (b) lung A549) CC_{50} value 142.01 ± 5.3 µg/ml; Dose-response assay for active fraction TU-63 from *Tecumella undulata* (TUAM) results are expressed in mean ± SEM, on Homo sapiens carcinoma cell lines (c) liver (Hep-G2) (d) lung (A549).

line 13.2% the % inhibition cytotoxicity for TUAD and TUAH were also recorded Fig. 2.

3.6.1. Selectivity index (SI)

As *Tecomella undulata* (TUAM) remained cytotoxic to homo sapiens liver (Hep-G2) carcinoma cells and the selectivity behaviour of extracts provides information about being selectively cytotoxic on cancer cells and less cytotoxic on normal. The extract with such behaviour is considered suitable for further bioassay-guided high throughput screening and anticancer effect of its phytocompounds. The Selectivity index (SI) was calculated for *Tecomella undulata* (TUAM) with 50% cytotoxic concentration (CC_{50}) of the extract on normal cells *cerocepithecus aethiops* kidney (VERO) cells $CC_{50\ or}$ TD_{50} = 377.2 ± 2.01 to its ratio (SI = TD_{50} /CC_{50}) on chomo sapiens liver carcinoma cells (HpG-2) $CC_{50=}$135.9 ± 1.71 and selectivity Index recorded was SI = 2.77 which depicts TUAM possess a decent selectivity and can be proceeded further for analysis.

3.7. Bioassay-guided evaluation

The result shows *Tecomella undulata* (TUAM) showed promising cytotoxicity 74.7% against Homo sapiens liver carcinoma cell (Hep-G2) and 66.2% against Homo sapiens epithelial cells from lung tissue (A549). The fluorescence intensity for each extract on an individual cell line was quantified in 9-well plates by FlexStation II microplate reader with advanced quality and enhanced throughput efficiency in an automated system. Further, tested for concentrations from serial dilution up to 1000 µg/ml and cytotoxic concentration CC_{50} (µg/ml) value was calculated in a dose response assay *Tecomella undulata* (TUAM) on homo sapiens liver carcinoma cell (Hep-G2) CC_{50} value 117.37 ± 4.73 µg/ml and on homo sapiens epithelial cell from lung tissue (A549) CC_{50} value 142.01 ± 5.3 µg/ml (Fig. 3).

The positive control doxorubicin on Homo sapiens colon colorectal Homo sapiens liver carcinoma cell (Hep-G2) CC_{50} value 3.81 ± 3.7 µg/ml and on homo sapiens epithelial cell from lung tissue (A549) CC_{50} value 3.57 ± 6.1 µg/ml. After column chromatography bioassay-guided fractionation analysis, for *Tecumella undulata* showed the most effective fraction F-63 of all fractions, on homo sapiens liver carcinoma cell (Hep-G2) and homo sapiens epithelial cell from lung tissue (A549) cell line the fraction was eluted by Ethylacetate: Methanol (7:3) with % inhibition of 87.67% and 83.13% respectively. For, *Tecomella undulata* fraction F-63 on Homo sapiens liver carcinoma cell (Hep-G2) CC_{50} value 11.67 ± 1.37 µg/ml and on Homo sapiens epithelial cell from lung tissue (A549) CC_{50} value 17.23 ± 0.58 µg/ml (Fig. 4 c-d).

3.8. High-performance liquid chromatography (HPLC-DAD) and LC-ESI MS/MS analysis

The High-Performance Liquid Chromatography (HPLC) analysis was performed on a Shimadzu, LC-20AT system, model DGU 20A3 equipped with LC-20AT quaternary pump coupled with LC/MS. The *Tecomella undulata* fraction TU-63 resulted in 60 subtractions for fraction each subtraction was dried on SpeedVac Concentrator, subtractions were subjected to resazurin assay for identification of active peak which corresponds to active constitute (Zhao et al., 2020; Fernández-Poyatos et al., 2021). The most effective peak was collected at retention times 21.84, 28.37and 28.84 min as it corresponds to the maximum inhibition in resazurin assay (Fig. 5 a-d). The peak was collected and subfraction was subjected to Electrospray ionization for active phytochemicals mass spectrometry analysis showed the presence of Apigenin-7-O-gulucronoide, Isoquercitrin and Quercitrin with molecular weight 446.4, 286.21, 464.0955 and 448.38 g/mol (Fig. 6 a-d).

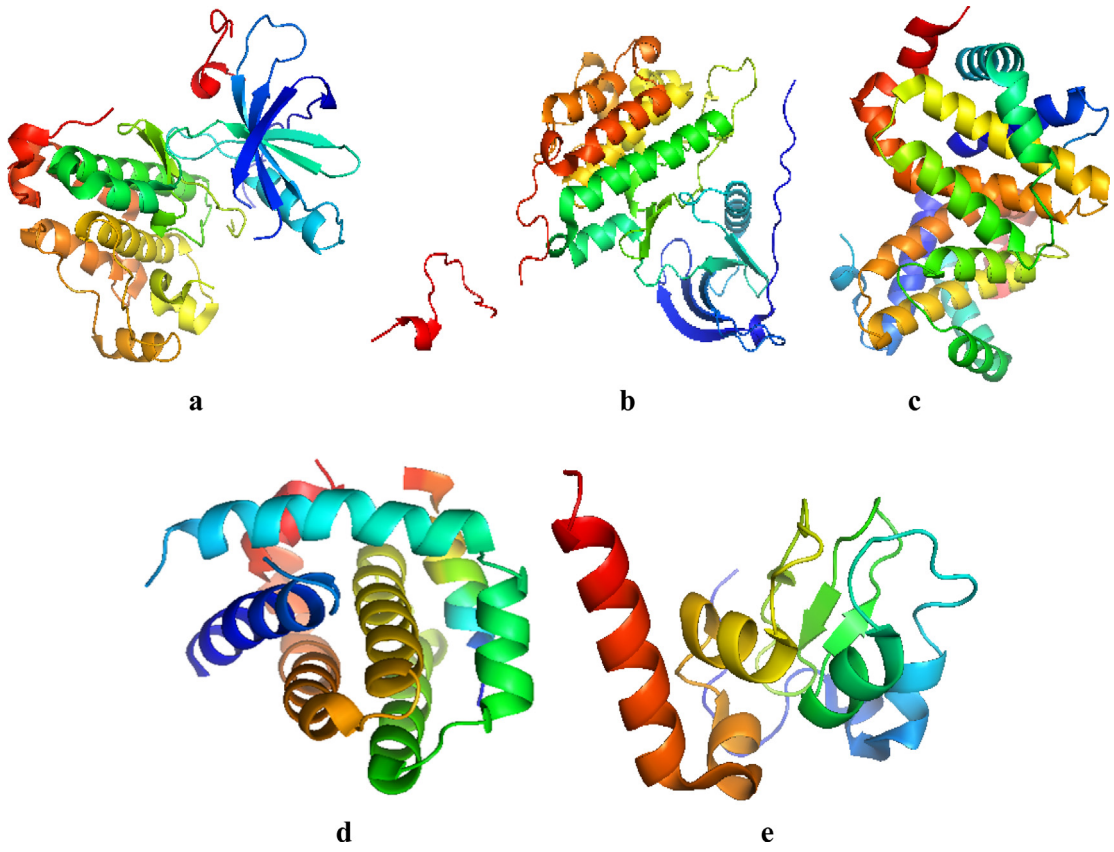

Fig. 4. Selected Proteins for Molecular Docking Analysis (a) Crystal structure of mutated EGFR kinase (EGFR) (ID 2eb3) (b) Epidermal growth factor receptor tyrosine kinase domain (EGFRK) (ID 1 m17) (c) Crystal structure of BCL-XL peroxisome proliferators-activated receptor proteins PPARs gamma (ID 2xyj) (d) The MCL-1 BH3 members of the BCL-2 family (ID 3mk8) (e) Apoptosis proteins fragment-drug target (ID 5c3h).

3.9. Molecular docking analysis

In modern *in silico* drug designing and development approaches were utilized to develop a novel effective drug range with target specificity (Husain et al., 2017). For protein modelling after identification and validation of targeted proteins and their role in cancer enhancement five different proteins and their 3D crystal structures were retrieved. The phytochemicals selected for *in silico* evaluation were subjected to assessment of classical drug likeliness properties (ADME) absorption, distribution, metabolism and elimination or excretion (Zhao and Zhou, 2015). Lipinski's rule of five is a conventional virtual evaluation method to assess the bioavailability by five critical properties includes molecular mass, high lipophilicity C LogP, H-bond donors, H-bond acceptors and molecular using virtual tools. Lipinski filter provided information about the effectiveness of the possible drug candidate ligands which were further selected for ligand–protein molecular docking (Fig. S1). *Tecomella undulata* ligand structures, ursolic acid, betulinic acid, Quercitrin, xyloidone, 5-iodopentyl acetate, skrofulein, sitosterol, stigmasterol, rutin, 4-[(4-Bromophenyl)-Hydroxymethylidene]-1-(5-methyl-1,2-oxazol-3-Yl)-5methylphenyl)pyrrolidine-2,3-dione. Formation of hydrogen bond facilities the ligand binding to the target protein and inhibit its biological activity; all such bonds are analyzed individually by using pymol 1.3 and their specific interaction to amino acid residues the minimum bond length of < 4.5 Å were considered significant (Table 3). Here, the crystal structure of B cell lymphoma a member of the Bcl-2 regulator protein family which regulates the cell death and apoptosis protein showed maximum amino acid interactions by all the compounds in the study. The lead compounds selected bind efficiently to the target molecule, poses the lowest energy and is expected to be a better drug candidate *in vitro*. The non-covalent hydrogen bonding predicts the robust binding interactions between ligand and protein residues; almost all compounds showed 2–4 hydrogen bonds in different more conformations (Fig. S1). The set of five proteins involved in cancer enhancement or caused due to mutation were selected for *in silico*. These proteins were epidermal growth factor protein PDB ID; 1 M17, the crystal structure of mutated EGFR kinase PDB ID; 2 EB3, the crystal structure of Bcl-xl PDB ID; 2YXJ, MCL-1 BH3 PDB ID; 3MK8 and apoptosis proteins PDB ID; 5C3H (Fig. 4).

4. Discussion

The traditional medicinal plants have been scientifically proven for their centuries-old therapeutic properties against various diseases, including cancer. Moreover, the interest of researchers in natural products has enhanced due to resistance to cancer therapy (Panda et al., 2017); (Saroj et al., 2020). Some plant compounds or their derivatives have unique structures and specific orientation with the least toxic effect on normal healthy cells and sometimes their synergist effect enhances their efficacy, efficiency and specificity. Furthermore, *in vivo* screening, *Tecomella udulata* plant extracts were selected being cytotoxic in initial experiments. *Caenorhabditis elegans* are small nematodes or that can easily maintain at a low cost for standard *in vivo* toxicity assessment. The advantage of *Caenorhabditis elegans* assay is it provides information regarding toxicity on the animal as a whole (Hunt, 2017). Therefore, different strategies can be developed using *Caenorhabditis elegans* for initial drug safety testing these organisms are easy to

handle results can be analyzed rapidly due to their short life cycle (Shen et al., 2018). Caenorhabditis elegans were used as a model organism to screen plant extracts with most pharmacological activities. Here, the activity or locomotion was the ability to record the consequence of extract treatment within 48 h tracked by WMicroTracker ™ Phylumtech. The Incase of Tecomella undulata (TUAH) and (TUAM) mobility was reduced to 6.2% and 29.1% respectively. The bacterial DNA in genetically modified strains of *Salmonella typhimurium* strains TA98 for frame-shift mutation and TA100 for base-pair substitutions. These strains have a definite mutation in histidine operon and thus cannot grow without amino acid histidine, to these mutants, it is mandatory to add histidine in culture media. The scientist's investigation of phytochemicals affecting genetic material was the subject of the antimutagenic assay. Antimutagenic activity by plants like *Linum usitatissimum*, *Ipomoea batatas* and *Poincianella bracteosa* increases the evidence of chemotherapeutic potential as mutation-related pathogenic process can be prevented or inhibited mechanistically the carcinogenic effect of mutagens (De Silva and Alcorn, 2019). These mutagens can cause environmental factors or chemical exposure which causes a mutation in genetic codes of DNA. *Salmonella typhimurium* strains T98 frame-shift mutations and T100 base-pair substitutions, mutagens Sodium azide (AZS) and 2-aminoanthracene (2AA) were taken as control induces mutation in bacterial DNA and enables them to grow in histidine lacking media (Figat et al., 2020).

In the present experiment, *Tecomella undulata* TA100 (AZS) 74. 7 ± 1.6 μg/ml previous reports show flavonoids have the potential to inhibit mutation caused by several mutagens and also predict the relationship between high antimutagenic activity and antioxidant potential of herbal extracts. Some other secondary metabolites have been reported to constrain the mutation triggered by a range of mutagens (Ramadan et al., 2019; Sarwari et al., 2019).

The MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) the assay is colourimetric assay for cytotoxicity assessment (Bahuguna et al., 2017) was performed homo sapiens liver carcinoma cell (Huh-7), homo sapiens colon colorectal carcinoma cell (HCT-116) and homo sapiens liver carcinoma cell (Hep-G2). The promising cytotoxic effect was observed for *Tecomella undulata* (TUAM) on Homo sapiens liver carcinoma cell (Hep-G2) tumour cell lines with HepG-2, 68.17%.

Selectivity index (SI) is the degree of selectivity of the compounds, which is expressed as SI value as suggested by Badisa (Fabre et al., 1984; Demirgan et al., 2016). The selectivity of extracts was calculated by fibroblast normal *cerocepithecus aethiops* kidney (VERO) cell line and Homo sapiens liver carcinoma cell (Hep-G2). The Selectivity index (SI) provides the information for the performance of bioassay-guided fractionation of extracts with more selectivity and least cytotoxic effect on normal cells here *Tecomella undulata* (TUAM) were selected for resazurin fluorescence assay for bioassay based on their selective index (SI) 2.77 respectively. The bioassay guided fractionation of most effective constituents the cytotoxicity was tested on a panel of seven tumour cell lines homo sapiens colon colorectal carcinoma cell (HCT-116), homo sapiens liver carcinoma cell (Hep-G2), homo sapiens cervix adenocarcinoma (HeLa), homo sapiens epithelial cell from lung tissue (A549), homo sapiens lung bronchial carcinoma (NCIH-727), homo sapiens liver cancer cell, integrated HBV (Hep-3B) and homo sapiens breast carcinoma cell (MCF-7). The results were in concordance with MTT 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay as *Tecomella undulata* (TUAM) showed cytotoxicity as on resazurin assay fluorescence assay performed on FlexStation II. The resazurin fluorescence assay for the assessment of cell viability is a potential reduction assay for the *in vitro* screening of anticancer drugs significant linearity of fluorescence intensity of resazurin reduction product resofurin

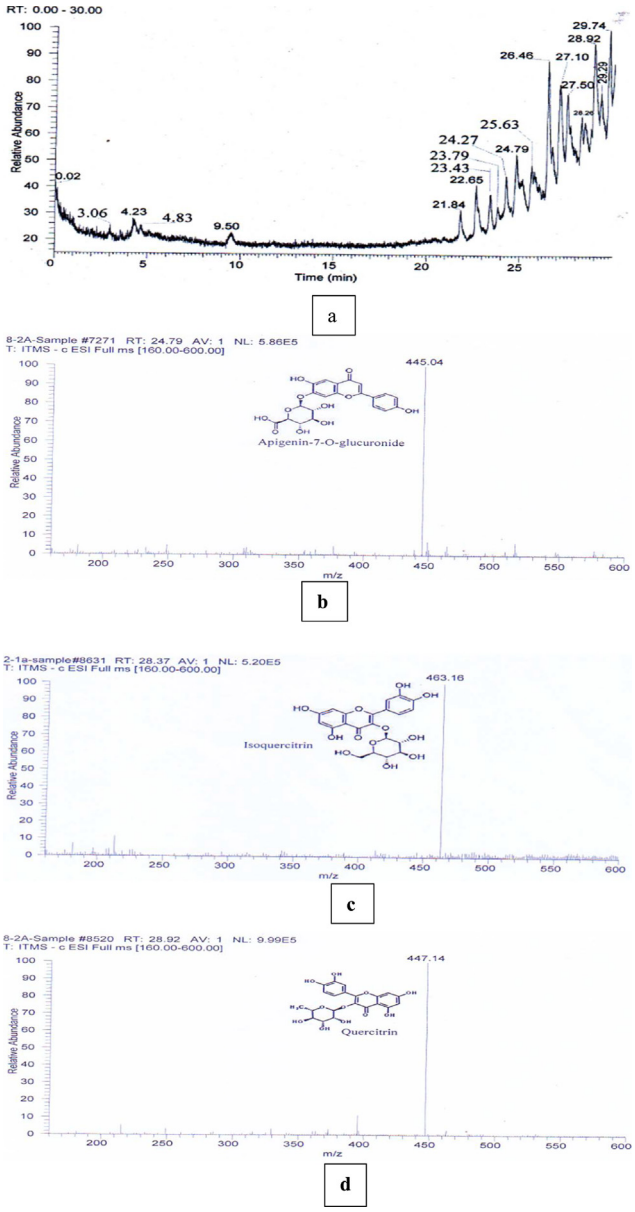

Fig. 5. (a) TUAM Chromatogram: Mass spectrum, (b) Apigenin-7-O-gulucronoide (Retention time = 24.79), (c) Isoquercitrin (Retention time = 28.37), (d) Quercitrin (Retention time = 28.92).

(Rodríguez-Corrales and Josan, 2017) was observed for *Tecomella undulata* (TUAM) on Homo sapiens epithelial cell from lung tissue (A549) cell line. The most promising decrease in viability of cells was also reported in Homo sapiens liver carcinoma cell (Hep-G2) by *Tecomella undulata* (TUAM). The 50% of the cytotoxic concentration CC_{50} (μg/ml) value was calculated *Tecomella undulata* (TUAM) on homo sapiens liver carcinoma cell (Hep-G2) CC_{50} value 117.37 ± 4.73 μg/ml and on homo sapiens epithelial cell from lung tissue (A549) CC_{50} value 142.01 ± 5.3 μg/ml. The silica gel column of methanol extract in methanol was an interest of investigation after stepwise column chromatography, total (111) fractions for *Tecomella undulata* (TUAM) were collected and tested in 96-well plates by same resazurin fluorescence assay the fluorescence was measured by Flaxstatio II at the fluorescence of emission determined by 530 nm and excitation as 590 nm. The most efficient *Tecomella undulata* (TUAM) fraction was F-63 which has diminished significant cancer cells in homo sapiens epithelial cell from lung tissue (A549) cell line both active fractions have remained effective.

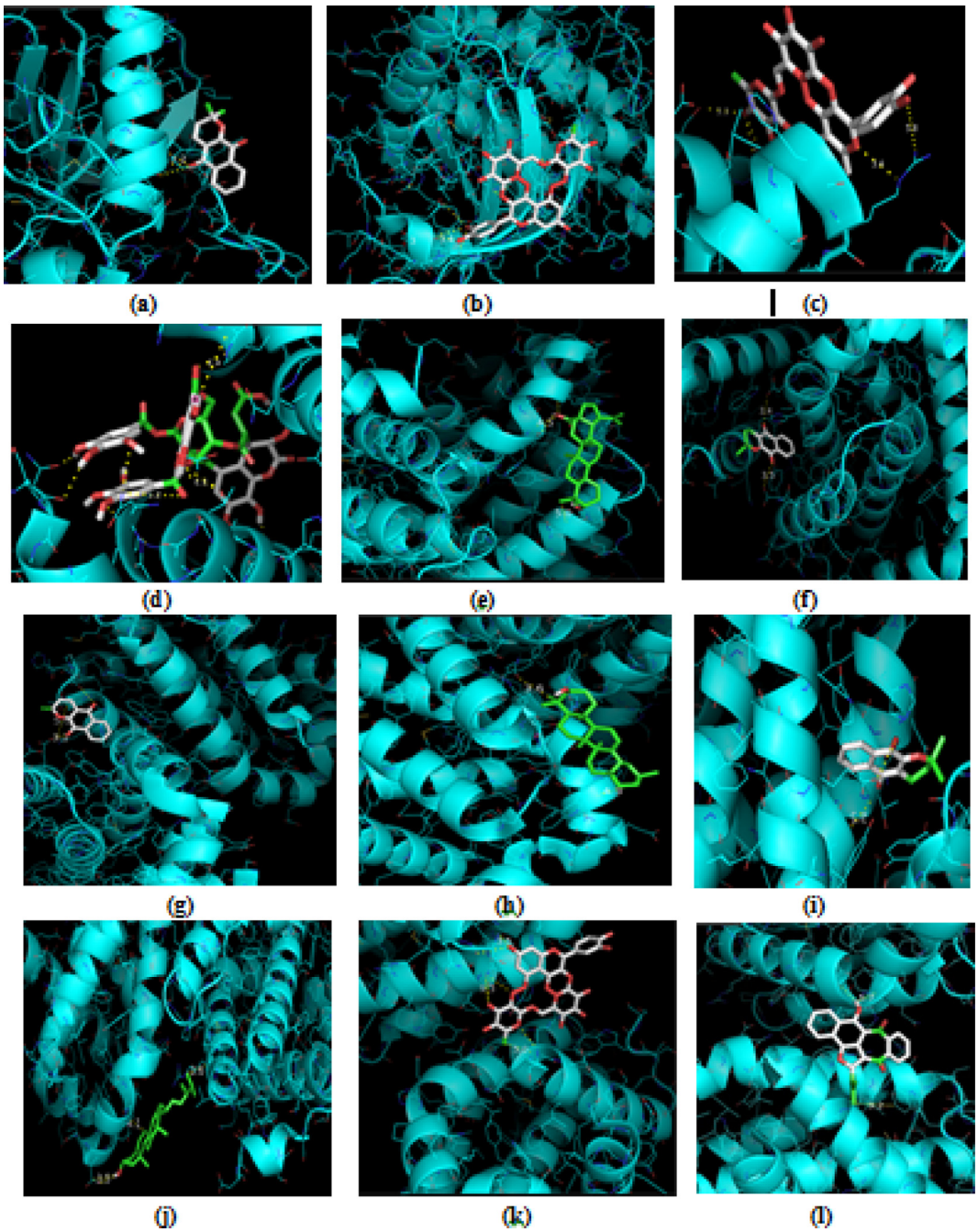

Fig. 6. *Tecomella undulata* 3D ligand structures and Protein docking complex (a) ursolic acid-5c3h (b) Betulinic acid-5c3h(c)α-Lapachone-2xyj(d) xyloidone-2xyj(e) Patamostat-2xyj(f) ursolic acid-2xyj(g) Sitosterol −2xyj(h) Betulinic acid-2xyj (i) Stigmasterol-2eb (j) Quercetin-2eb3(k) Quercetinl-3mk8(l) Sitosterol-1 m17.

For, *Tecomella undulata* fraction F-63 on Homo sapiens liver carcinoma cell (Hep-G2) CC_{50} value, 11. 67 ± 1.37 μg/ml and on Homo sapiens epithelial cell from lung tissue (A549) CC_{50} value, 17.23 ± 0.58 μg/ml. The active fractions were subjected to structural information by Liquid chromatography coupled with spectrometer and High-Performance Liquid Chromatography (HPLC) and Electrospray ionization mass spectrometer analysis (Loizzo et al., 2020; Lu et al., 2021). The results for *Tecomella undulate* fraction F-63 for of its every subtraction tested on resazurin fluorescence fractional and % inhibition on the cell-based assay. In the case of *Tecomella undualata* the most effective peak was collected at retention times 21.84, 28.37and 28.84 min as it corresponds to the Apigenin-7-O-gulucronoide, Isoquercitrin and Quercitrin with molecular weight 446.4, 286.21, 464.0955 and 448.38 g/mol. Quercitin from medicinal plants is reported to be antioxidant and anticancer by some researchers (Baghel et al., 2012) and identified from *Tecomella undulata* recently (Ali et al., 2017; Zehri et al., 2020). The top hits scoring phytochemicals in molecular docking were selected the scoring function provides information about *in silico* efficiency highest binding affinities, polar bonds and interactions with active residues (Wang *et al.,* 2015). The set of selected proteins included were epidermal growth factor protein (PDB ID; 1 M17), the crystal structure of mutated EGFR kinase (PDB ID; 2 EB3), the crystal structure of Bcl-xl (PDB ID; 2YXJ), apoptosis regulator protein MCL-1 BH3 (PDB ID; 3MK8) and apoptosis proteins (PDB ID; 5C3H). Ligand protein interactions showed some phyto-

Table 3
Binding affinities and residual interactions *Tecomella undulata* leads with activated proteins.

PubChem ID	IUPAC names	Mol. Formula	PDB ID	Residues Interact via H-bonding	Residues in contact To ligand
Tecomella undulata					
58472	Ursolic acid	$C_{30}H_{48}O_3$	5c3h	Val245, Phe324	Phe301, Lys311, Pro316, Ala324, Leu331
58496	Betulinic acid	$C_{30}H_{48}O_3$	5c3h	Leu317, Thr253	Leu256, Ser261, Thr271, Ala322,
65571	Lapachone	$C_{15}H_{14}O_3$	2xyj	Leu275, Ser428	Trp181, Gly186, Phe191, Gly196
65573	Xyloidone	$C_{15}H_{12}O_3$	2xyj	Asp176, Tyr173	Ala118, Gln121, Ser122, Val127, Asn128
65921	Patamostat	$C_{20}H_{20}N_4O_4S$	2xyj	Met 365, Phe 274	Gln121 Val127Thr134, leu142
58472	Ursolic acid	$C_{30}H_{48}$	2xyj	His177, Ile6166	Thr115, Pro116, Thr119, Glu123
192962	Sitosterol	$C_{29}H_{50}O$	2xyj	Cys251, Asn 276	Asp176, Leu180, Gly186, Gly196
58496	Betulinic acid	$C_{30}H_{48}O_3$	2xyj	Ile113, Gly118	Thr115, Pro116, Thr119, Glu123, Phe122
4444352	Stigmasterol	$C_{29}H_{48}O$	2eb3	Met793, Leu792	His851, Val876, Ala883
5280343	Quercitrin	$C_{21}H_{20}O_{11}$	2eb3	Val 726, Gln 791	Met881, Gln885, Gln901, Trp951, Asp956
5280343	Quercitrin	$C_{21}H_{20}O_{11}$	3mk8	Leu 194, Asp 131	Asp172, Ile181, Leu186, Thr191
192962	Sitosterol	$C_{29}H_{50}O$	1 m17	Thr766, Leu 820	Ile756, Thr761, Leu763, Thr766
58472	Ursolic acid	$C_{30}H_{48}O_3$	5c3h	Val245, Phe324	Phe301, Lys311, Pro316, Ala324, Leu331

compounds possess promising potential during docking in *Tecomella undulata* showed ursolic acid-5c3h, betulinic acid-5c3h, xyloidone-2xyj, patamostat-2xyj, ursolic acid-2xyj, sitosterol-2xyj, betulinic acid-2xyj, stigmasterol-2eb, Quercitrin-2eb3, Quercitrin-3mk8 and sitosterol-1 m17. Molecular Docking studies enhanced the drug (ligand) receptor (protein) interactions with virtual high throughput screening before biochemical experimentalization, and this has increased the chances of successful drug development with reduced false-positive results, low cost and within the limited time (Iwaloye et al., 2020; Wani et al., 2021). Our results reveal the presence of *Tecomella undulata* anticancer natural compounds *in vitro* in bioassay-guided manner evaluation the results condors with *in silico* molecular docking findings.

5. Conclusion

These natural compounds possess promising potential *in silico*, *in vivo, and in vitro* experiments were identified by bioassay-guided purification. The selectivity efficiency and efficacy on carcinoma cell lines as compare to normal fibroblast cells prove their potential for mechanistic studies which can help to enhance the efficiency of anticancer phytocompounds.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors' contributions

MAJ, IN and TJ concept and design of the study, data acquisition, and literature search, SR supervision of the study manuscript preparation, TJ critical review and TJ, SR final revision of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2021.12.015.

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