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Antioxidant properties and cytotoxic effects of *Oxalis corniculata* on human Hepatocarcinoma (Hep-G2) cell line: an in vitro and in silico evaluation

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Abstract

Background *Oxalis corniculata* is a well-known medicinal plant used in folk medicine for the management of many diseases. The aim is to determine the physico-chemical properties, microscopic study, phytochemical properties, in-silico, in-vitro antioxidant and anticancer activity on human Hepatocarcinoma (Hep-G2) cell line of *O. corniculata* plant extract.

Results Microscopical study reaveals that presence of pericyclic fibres, starch gains, trichomes etc, and phytochemical screening is carried to find out secondary metabolites. The molecular docking study concluded that some of the phytocompounds showed inhibition of epidermal growth factor receptor tyrosine kinase domain (PDB ID: 1M17) inhibitor. Furthermore, ADMET and drug likeness study hints some of phytocompounds may act as lead for anticancer drug discovery and development. Among selected phytocompounds, compound apigenin possesses − 7.90 kcal/mol as compared to standard drug doxorubicin possesses − 7.63 kcal/mol against the epidermal growth factor receptor tyrosine kinase. The plant extract shown antioxidant activities based on the different tests were performed. The hydroalcoholic plant extracts were found to be selectively cytotoxic in vitro to human Hepatocarcinoma (Hep-G2) cell line with IC_{50}values 34.494 ± 0.42 µg/ml and EAF showed at IC_{50} value 30.245 ± 0.58 µg/ml of the cells were inhibited at the concentration of 50 µg/ml as compared with standard doxorubicin at IC_{50} value 24.8939 ± 0.25 µg/ml, respectively.

Conclusion The present study concluded that *O. corniculata* possesses potential antioxidant and cytotoxic properties based upon the computer aided drug design models and in-vitro activity.

Keywords *Oxalis corniculata*, Antioxidant, Hep-G2 cell-line, Molecular docking, Cytotoxic effect

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Background

Previous studies were concluded that the about ayurvedic and unani medicines have lot of benefits and information regarding herbal remedies The Charaka Samhita (1000 B.C.), one of the first texts on Indian medicine, discusses the usage of more than 2000 plants for medical purposes [1, 2].

One of the most versatile medicinal herbs, *Oxalis corniculata Linn* (Fig. 1). (Family: Oxalidaceae), is popular in India and exhibits a broad range of biological activity [3, 4]. It is frequently referred to as wood sorrel that creeps and is a superb natural plant that contains all the necessary elements for human health and wellbeing. Astringent herbs treat skin conditions, quartan fevers, dysentery and diarrhoea. They also remove kapha, vata, and piles. Warts and corneal opacities can be treated externally with an infusion of the tiny leaves [5, 6].

Reactive oxygen species (ROS) which are generated in the body during metabolism causes protein and DNA damage along with lipid peroxidation. These changes contribute to cancer, atherosclerosis, cardiovascular and inflammatory diseases. ROS have been accountable for hundreds of diseases states ranging from arthritis and connective tissues disorders to carcinogenesis, aging, physical injury and infection [7]. Plants have been used as a source of medicine throughout human history. They are the source of secondary metabolites. Phenolics compounds and flavonoids are widely distributed in plants which have been reported to exert their biological effects such as antioxidant, anti-inflammatory, and anti-carcinogenic. Antioxidants of naturally generated origin reduce the oxidative stress that many hepatotoxins cause. Over the past few decades, the research for such naturally occurring antioxidants has grown significantly as a scientific challenge [8].

Fig. 1 Representation of *Oxalis corniculata*. Drug profile of *Oxalis Corniculata* [12]. Kingdom: Plantae. Division: Magnoliophyta. Class: Magnoliopsida. Order: Oxalidales. Family: Oxalidaceae. Genus: Oxalis. Species: O. corniculata

Cancer is the biggest collection of illnesses responsible for 10 million deaths globally. Females are more likely to get breast, cervical, colorectal, lung, and thyroid cancers than any other cancer type. Among them, cervical cancer is the fourth most common reason for women's deaths and accounts for 270,000 fatalities each year. It is well known that % of anticancer medications come from different plant species [9, 10].

Free radical regulation in hepatic cells involves a complex interplay between enzymatic and non-enzymatic antioxidants such as superoxide dismutase, catalase and glutathione. These antioxidants scavenge and neutralize free radicals preventing oxidative damage to cellular components. Additionally, transcription factors such as nuclear factor erythroid 2- related factor (Nrf2) play a crucial role in regulating the expression of these antioxidants and maintaining redox homeostasis [11].

In this study, the hydroalcoholic extract was prepared from the plant *Oxalis corniculata* and further study assessed for their physico-chemical properties, microscopic characteristics, phyto-chemical properties, fluorescent properties of the extract and antioxidant activity was studied using DPPH-free radical scavenging, Ferrous ion chelating assay, ferric reducing antioxidant power (FRAP), Nitric oxide scavenging activity, ABTS radical scavenging activity, Superoxide radical scavenging activity (NBT) and Antilipid peroxidation activity. The current study also reports a method for evaluating anticancer activity against on human carcinoma hepatoma HepG2 cell line and in-silico studies, in order to predict the hypothetical binding mode of phyto compounds.

Therapeutic uses

Anti-cancer, antidiabetic, hepatoprotective, hypolipidemic, abortifacient, antimicrobial, wound healing, anti-inflammatory, anxiolytic, anticonvulsant, antifungal, antiulcer, antinociceptive and antifungal [13].

Phytochemistry

Oxalis corniculata Linn plant contains number of phyto-constituents based on the literature such as tannins, palmitic acid, a combination of stearic 8-oleic and linolenic acids. This plant also contains carbohydrates, glycosides, phytosterols, phenolic compounds, flavonoids, proteins, amino acids, and volatile oil [4]. Palmitic acid, 8-oleic acid, Linoleic acid, linolenic, stearic acids, tartaric acid, citric acid, acacetin, 7,4′- diOMe apigenin, P-hydroxybenzoic acid, Vanillic acid, syringic acid, isoorientin, isovitexin, swertisin, β-sitosterol, betulin, ethyl gallate, 5-hydroxy-7,8-dimethoxyflavone, 5-hydroxy-3′, 4′, 6, 7, 8-pentamethoxyflavone, 5-hydroxy-3, 6, 7, 4′-tetra methoxyflavone, apigenin these are the responsible for

anticancer activity. Phytochemistry of common phytocompounds were described (Table 1) [14].

Methods

Chemicals

All the chemicals used in this research were of analytical grade and including 2,2-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), butylated hydroxy anisole (BHA), ABTS, sodium nitroprusside, sodium carbonate, FRAP (ferric reducing antioxidant power), NBT (nitro blue tetrazolium), TBA (thiobarbituric acid assay) chloroform and ethanol were purchased from sigma (Sigma Aldrich GmbH, Steinheim, Germany) while Merck supplied the penicillin G (Quality level 200), streptomycin (Quality level 100) and doxorubicin.

Cell culture technique

The Human tumour cell line HePG2 was purchased from the National Centre for Cell Sciences (NCCS), Pune, India. Cells were maintained in DMEM supplemented with 2 mM l-glutamine and balanced salt solution (BSS) adjusted to contain 1.5 g/l Na_2CO_3, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 2 mM l-glutamine, 1.5 g/l glucose, 10 mM HEPES (4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid) and 10% fatal bovine serum (GIBCO, USA). Penicillin and streptomycin (100 IU/100 µg) were adjusted to 1 mL/l. The cells were maintained at 37 °C with 5% CO_2 in a humidified CO_2 incubator.

Microscopic studies

For microscopic examination, several parts of *Oxalis corniculata* were examined under magnifying lens by Labomed Lx-300 trinocular halogen microscope in KLE College of Pharmacy, Belagavi.

Physicochemical evaluation

Physicochemical parameters like percentage of loss on drying (LOD), total ash, acid insoluble ash and water-soluble ash were examined as per the guidance of Indian Pharmacopeia.

Fluorescence study

On a grease-free, clean microscopic slide, a totally powdered plant material was placed and 1–2 drops of reagent solutions (ethanol, 1 N HCL, 1 N H_2SO_4, 1 N NaOH, iodine and $FeCl_3$) were added. After mixing well for 1–2 min, the slide was seen in the UV chamber using long (365 nm) and short (254 nm) ultraviolet radiations. The colours observed by application of different reagents in different radiations were reported [15].

Preparation of extract

The plant of *Oxalis corniculata* was collected from Kurani village of Hukkeri Tq (Dist. Belgaum, Karnataka). Authentication of plant is done from Shri B.M.K. Ayurveda Mahavidyalaya, Central Research Facility, (Shahpur, Belgaum). The samples were collected, cleaned, drained, cut and dried before being used. A crude extract was prepared by adding 750 g of powder in 2.5 l mixture of ethanol and water (70:30) and leaving the mixture for 7 days while stirring often. The extract was then filtered, added to a soxhlet apparatus with the same solvent system and left for 3 days for the extraction of a lipid from a solid material and extraction of compound has a limited solubility in solvent.

Preliminary phytochemical analysis

Preliminary phytochemical screening of the *Oxalis corniculata* plant extract for the determination of the presence of secondary metabolites like Flavonoids, Alkaloids, Saponins, Triterpenoids, Steroids, Tannins, Glycosides and Phenolics by standard methods [16].

Preparation of flavonoid fraction

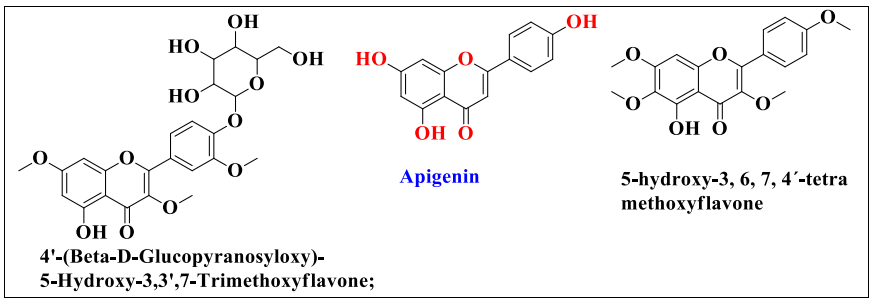
Ethyl acetate was used for sequential liquid–liquid extraction on the hydroalcoholic extracts. The ethyl acetate fraction (Flavonoid Fraction/EAF) was then obtained by collecting and drying each fraction under reduced pressure.

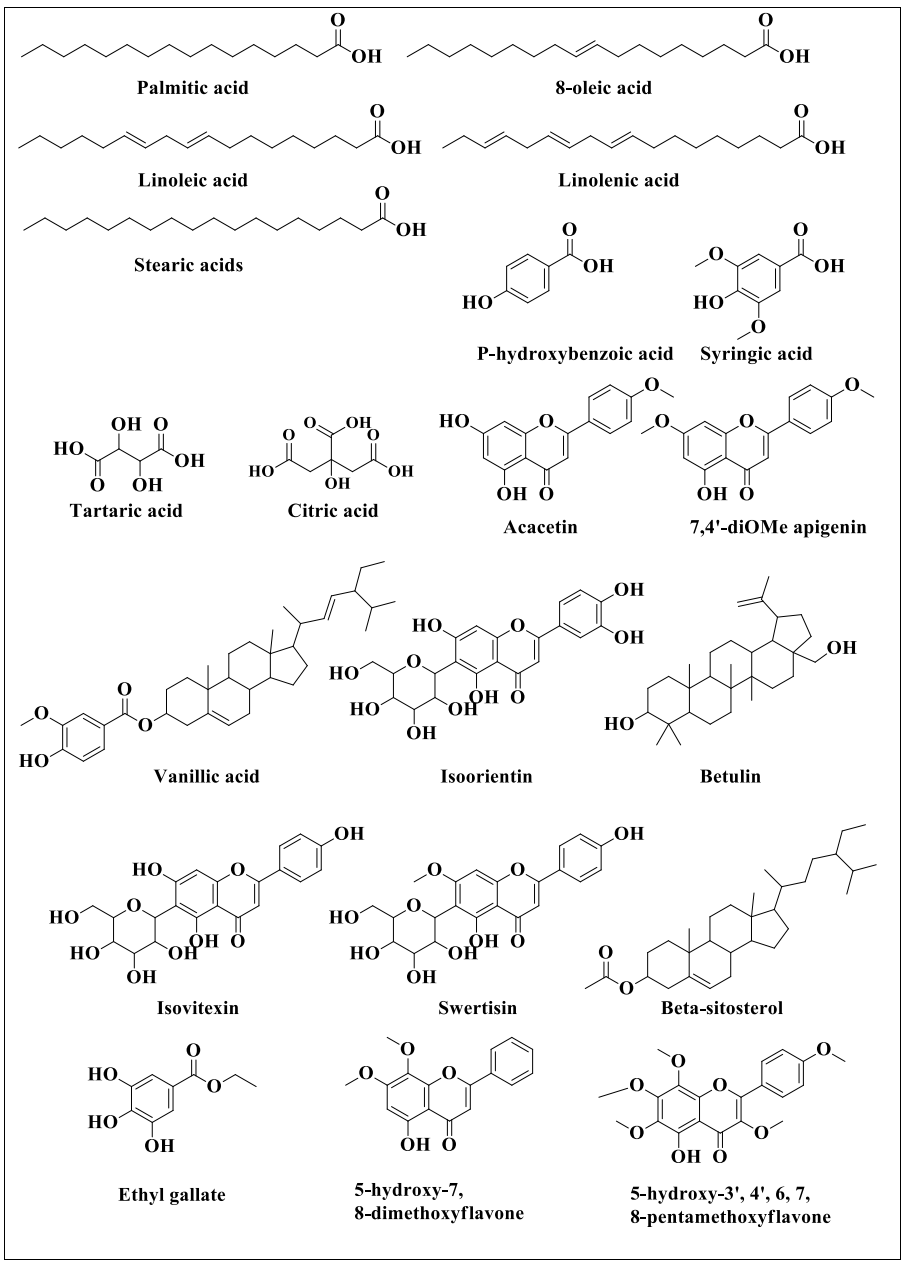
In-silico anticancer test

The protein data bank-obtained molecule combines the native ligand and the binding pocket molecule. Native ligand molecule 4-anilinoquinazoline inhibitor and binding pocket molecule epidermal growth factor receptor tyrosine kinase domain with protein code 1M17. The coordinates for the epidermal growth factor receptor tyrosine kinase were downloaded from the RCSB protein data bank (PDB ID:1M17) [17, 18]. The protein preparation wizard (PPW) was used to process the protein structure, and the prime module of the Schrödinger suite was used to verify and alter its integrity by adding the missing residues. The protein was reduced by using the OPLS 2005 force field, and the pH range was set to 7.0. Finally, restricted minimization was followed until the non-hydrogen atoms average root mean square deviation (RMSD) converged to 0.30 Å [19].

A total of 23 molecules were created using Schrödinger's maestro molecule builder and then optimized using the OPLS 2005 force field in the LigPrep module at a pH of 7.4, ionizer was used to identify all the protomers and ionisation states for ligands.

Table 1 Phytochemistry of selected phytocompounds of *Oxalis corniculata Linn*





The GLIDE (Grid-based Ligand Docking with Energetics) docking module of the Schrödinger suite was used to carry out molecular docking studies. The prepared ligands were docked into the generated receptor grid using glide XP docking precision. It utilizes an energy-based grid-based ligand docking approach to investigate for advantageous interactions between a ligand and a protein-like receptor molecule. Using the Schrödinger suite, the interactions of each complex were analyzed, and the 3D poses indicative of the molecular recognition interactions were obtained.

The pharmacokinetic properties like absorption, distribution, metabolism and excretion of *Oxalis corniculata* play an important role in drug development process. So, we have used physico-chemical and ADME properties, which were estimated using the Qikprop module of Schrödinger, assist in predicting both physico-chemical significant descriptors and pharmacokinetically important properties of the molecules.

In- vitro determination of antioxidant activity and free radical scavenging activities

The radical scavenging activity of the extracts was studied using DPPH free radical assay, ferrous ion chelating, nitric oxide scavenging activity, ABTS radical scavenging activity, and ferric-reducing antioxidant power (FRAP). Deoxyribose assay was carried out according to the method proposed by Halliwell et al. [20]. Superoxide radical scavenging activity of the extracts (NBT method) was determined and Antilipid peroxidation activity (thiobarbituric acid [TBA] method) was carried out as described by Gupta et al. [21].

In-vitro determination of anticancer activity

Evaluation of cytotoxicity

Determination of the inhibitory concentration (IC_{50}) value using an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) assay. Cultured cells (1×10^5) were seeded in a 96-well plate and incubated for 48 h at 37 °C at 5% CO_2 incubator. After 48 h, the monolayer was washed with medium and 100 µL of different test concentrations (10, 20, 30, 40 and 50 µg/ml) of samples were added on to the monolayer and the cells were further incubated at the same conditions. Removed the cultured medium and 100 µl of the MTT solution was added to each well and incubated at 37 °C for 4 h. After the supernatant has been removed, 100 µL of DMSO was added to each of the wells and incubated for 10 min to dissolve the crystals of formazan Measurement of the optical density at 590 nm. The percentage growth inhibition was calculated and results were expressed as IC_{50} values using dose response curve. [22].

Statistical analysis

All the estimation was carried out in triplicates. The data obtained in this study were analyzed using relative standard deviation method and the results were expressed as mean ± SD.

Results

Total yield of crude extract

The total yield of crude extracts from *Oxalis corniculata* by using the solvents, namely, 750 g of powder were added completely in ethanol: water (70:30) ratio and found 8.64 g (weight/weight), respectively, in relation to the plant material that has been air dried.

Microscopic studies

The results of powder microscopy showed pericyclic fibers, starch grains, trichomes, calcium oxalates, cork cells, fibers and microscopical images are mentioned in Fig. 2.

Physico-chemical evaluations

Total ash usually contains silicate, phosphate, carbonate, and silica. Ash value is a standard to confirm the identity and clarity of crude plant pharmaceutical material. Total ash value was found to be 13 ± 0.63. The results were expressed as mean ± SD and values are represented in Table 2.

Fluorescence investigation

Any powdered herbal drug's fluorescence investigation feature is very unique and useful in detectable features for the determination of fluorescence properties of the extract and the results are mentioned in Table 3.

Phytochemical screening

Phytochemical investigation of hydroalcoholic extract: by carrying out the different chemical test for the particular class of compounds it shows the presence of secondary metabolites like Steroids, Alkaloids, Flavonoids, Tannins, Triterpenoids, and Carbohydrates and the results are mentioned in Table 4.

In-silico anticancer test

As a result of the molecular docking test, the binding energies of the reference and test molecules are shown in Table 5. The selected compounds were docked against the selected targeted protein in order to predict the optimum conformational position within the active region of the target protein. On the premise of minimum energy values (Kcal/mol) and bonding interaction patterns, all the produced docked complexes were assessed (hydrogen, hydrophobic, and electrostatic). The apigenin possesses − 7.90 kcal/mol (Fig. 3a) whereas other, 7,4′- diome apigenin, acacetin and 5-hydroxy-3,

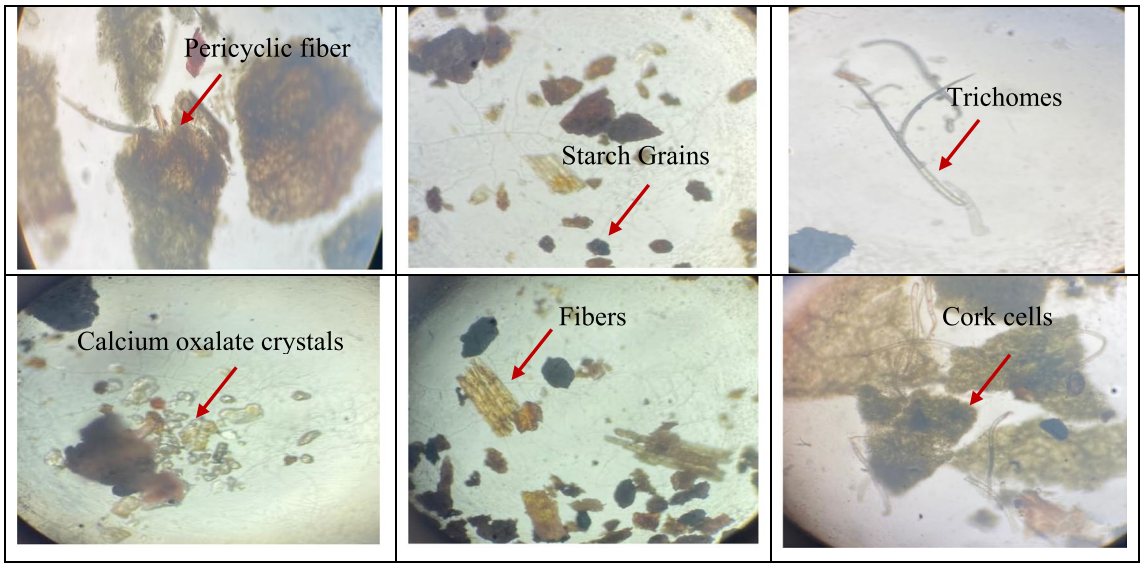


Fig. 2 Microscopic properties of *Oxalis corniculata*

Table 2 Physico-chemical evaluations of *Oxalis corniculata* powder

Parameters	Values (%w/w)
Total ash	13 ± 0.63
Acid insoluble ash	1.5 ± 0.33
Water soluble ash	1.6 ± 0.25
Alcohol soluble extractive	22 ± 0.42
Water soluble extractive	18 ± 0.53

Values are mean ± SD ($n = 3$)

6, 7, 4′-tetra methoxyflavone, exhibited − 7.33, − 7.297, and − 7.61 kcal/mol, respectively. Docking results demonstrated that compounds have high values for docking energy as compared to standard drug doxorubicin possesses − 7.63 kcal/mol (Fig. 3b) against the Epidermal growth factor receptor tyrosine kinase, respectively. In the Qikprop module of Schrodinger's, they were predicted for the ADMET prediction. The development of the pharmacokinetic profile of bioactive depends heavily on adsorption, distribution, metabolism, and excretion. Table 6 includes all of the descriptors.

In-vitro antioxidant activity

Determining antioxidant activity of the hydroalcoholic extract of *Oxalis corniculata. Plant* extracts' antioxidant activity is significantly influenced by the nature of the extracts and the evaluation procedure. The antioxidant capabilities are also influenced by numerous aspects that are difficult to fully describe using a single method. As a result, it is required to conduct multiple antioxidant capacity assays to account for the varied mechanisms of antioxidant action.

Hydroalcoholic extract showed remarkable free radical scavenging activity. The % inhibition values of both plant hydroalcoholic extracts and the standards increase with increases in concentration and Table 7 includes all IC_{50}

Table 3 Fluorescence characteristics of *Oxalis corniculata* powder

Treatment	Day light	Short UV (254 nm)	Long UV (365 nm)
Powder	Greenish brown	Brown	Dark brown
Powder + Water	Brown	Yellowish brown	Greenish brown
Powder + Ethanol	Yellowish brown	Brown	Dark brown
Powder + 1 N H_2SO_4	Dark brown	Greenish brown	Dark brown
Powder + 1 N NaOH	Yellowish brown	Yellowish brown	Bluish brown
Powder + 1 N HCL	Brown	Yellowish brown	Dark brown
Powder + Iodine	Light yellow	Yellowish brown	Bluish brown
Powder + $FeCl_3$	Greenish brown	Yellowish brown	Dark brown

Table 4 Preliminary phytochemical screening results of *Oxalis Corniculata* via chemical reaction

Metabolites	O C extract	Metabolites	O C extract
Carbohydrates	+	Flavonoids	+
Gums	–	Alkaloids	+
Mucilage	–	Phenols	+
Amino acids	+	Tannins	+
Fats and fixed oils	–	Terpenoids	–
Steroids	–	Quinones	–
Glycosides	+	Resins	–
Saponins	–	Volatile oils	+
Proteins	+	Furans	–

(+): the presence; (–): the absence

Table 5 Binding affinity of phytocompounds

Compounds	Docking scores in Kcal/mol PDB ID: 1M17
Palmitic acid	− 0.849
8-oleic acid	− 0.956
Linoleic acid	− 1.37
Linolenic acid	− 1.583
Stearic acid	− 0.689
Tartaric acid	− 4.411
Citric acid	− 4.847
Acacetin	− 7.297
7,4′- diome apigenin	− 7.33
P-hydroxybenzoic	− 4.368
Vanillic acid	− 5.114
Syringic acid	− 5.369
Isoorientin	− 6.916
Isovitexin	− 5.855
Swertisin	− 5.848
B-sitosterol	− 2.252
Betulin	− 3.699
Ethyl gallate	− 5.883
5-hydroxy-7,8-dimethoxyflavone	− 6.82
5-hydroxy-3′, 4′, 6, 7, 8-pentamethoxyflavone	− 6.06
4′-(beta-d-glucopyranosyloxy)-5-hydroxy-3,3′,7-trimethoxyflavone	− 5.86
Apigenin	− 7.90
5-hydroxy-3, 6, 7, 4′-tetra methoxyflavone	− 7.61
Doxorubicin	− 7.653

values of *Oxalis corniculata* in different antioxidant tests and the results were expressed as mean ± SD.

DPPH- *α*, *α* -diphenyl-*β*-picrylhydrazyl, NO-Nitric oxide scavenging activity, ABTS- 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), NBT- Superoxide radical scavenging activity, FRAP- Ferric reducing antioxidant power assay, LPO- Anti-lipid peroxidation activity.

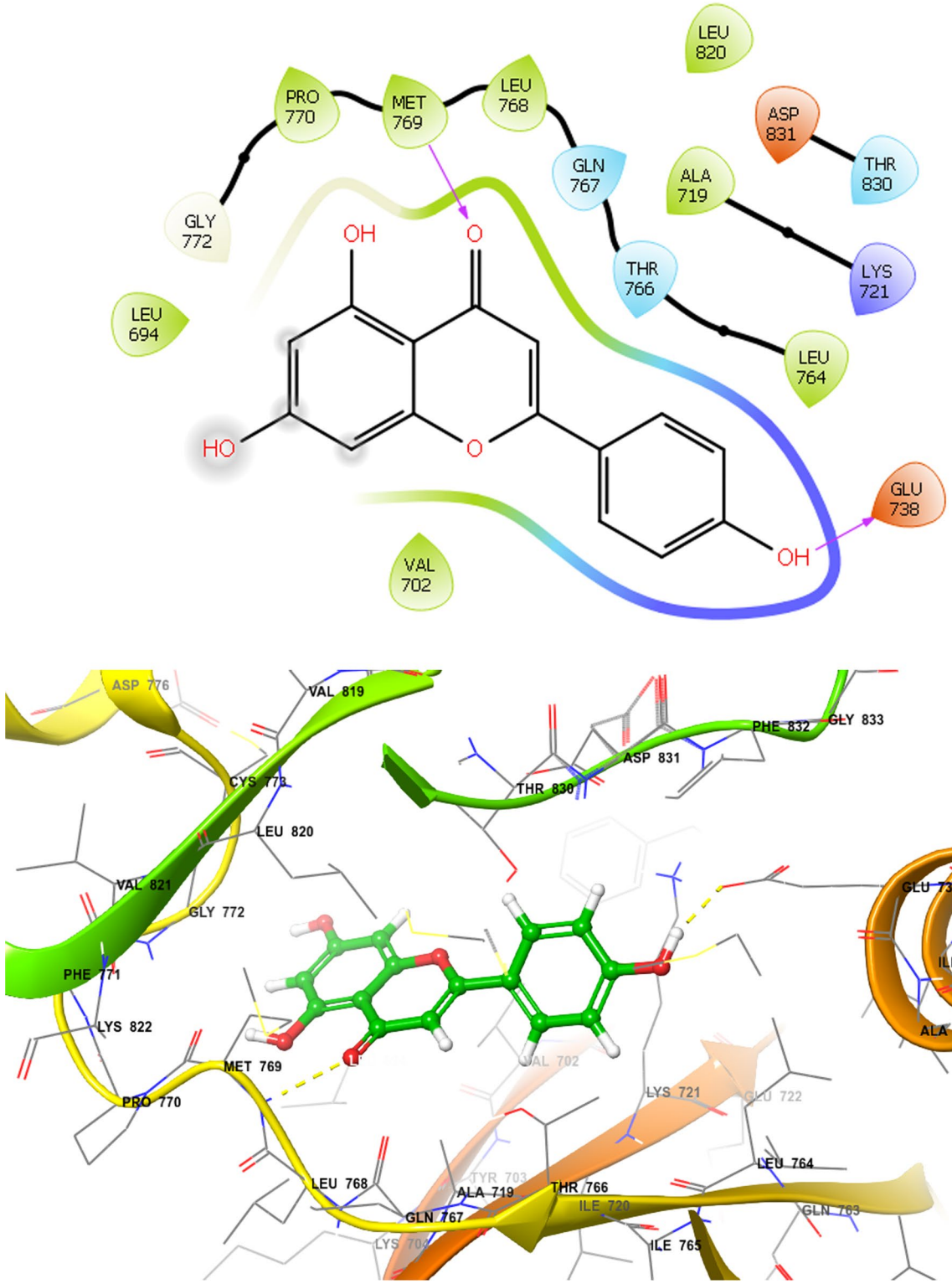
Evaluation of cytotoxicity

Cell viability in terms of growth inhibition of the extract and fraction was found to be dose dependent (Fig. 4). Laboratory testing indicate the % inhibition (Fig. 5) of the hydroalcoholic extracts and ethyl acetate fraction on human Hepatocarcinoma (Hep-G2) cell line of *Oxalis corniculata* at different concentrations and showed cytotoxicity against Hep-G2 cell line, EAF showed at IC_{50} value 30.245 ± 0.58 µg/ml of the cells were inhibited at the concentration of 50 µg/ml and hydroalcoholic extract showed IC_{50} value at 34.494 ± 0.42 (mean ± SD) µg/ml as compared to standard doxorubicin at IC_{50} value 24.8939 ± 0.25 (mean ± SD) µg/ml.

Discussion

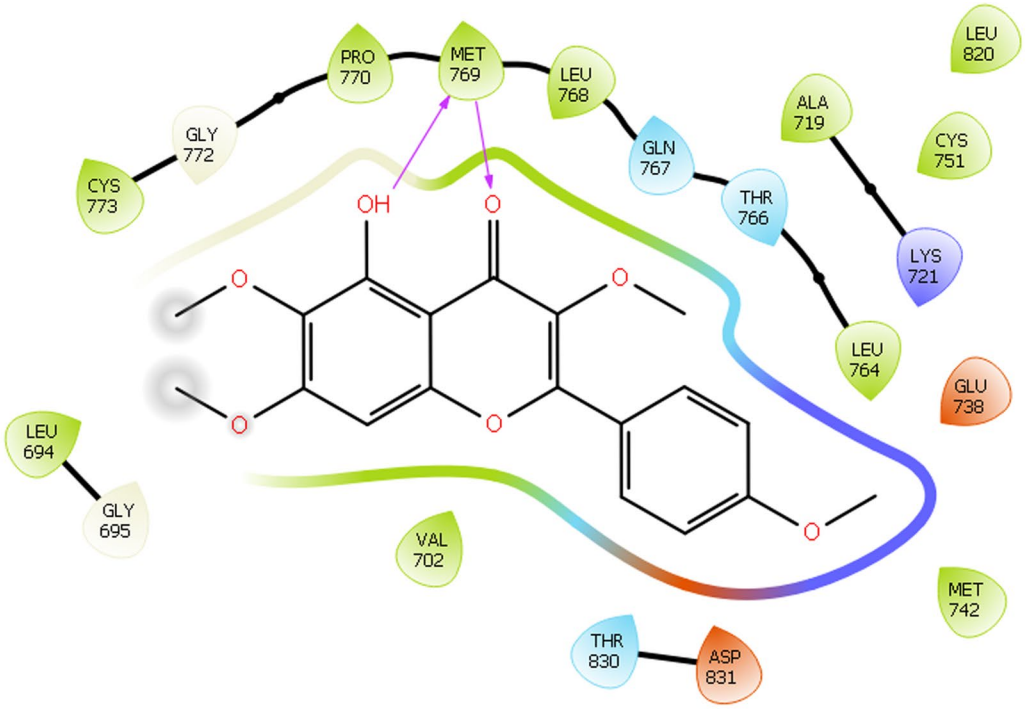
In recent years, due to their diverse phyto metabolic contents and multiple biological activities, the use of herbal medicines in the treatment of cancer has attracted increasing attention. The Western Ghats plants were identified using the taxonomical characteristics of *Oxalis corniculata* and the presence of phytochemicals were analysed with hydroalcoholic extracts and phytochemistry of common phytocompounds were described (Table 1). Microscopic studies confirmed the presence of pericyclic fibers, starch grains, trichomes, calcium oxalates, cork cells, fibers (Fig. 2), physico-chemical evaluation describes percentage of loss on drying (LOD), total ash, acid insoluble ash and water soluble ash (Table 2), the fluorescence study determines the fluorescence properties of extract summarized in (Table 3), secondary metabolites were identified in the selected plant extract via preliminary phytochemical investigation (Table 4). There are numerous biological and therapeutic features associated with these secondary metabolites. Various biochemical assays were used to measure phytoconstituents and ethyl acetate was used for successive liquid extraction of hydroalcoholic extracts. To obtain the ethyl acetate fraction (EAF), each fraction was then collected and concentrated to dryness under reduced pressure and these investigations showed good results than existing some literatures.

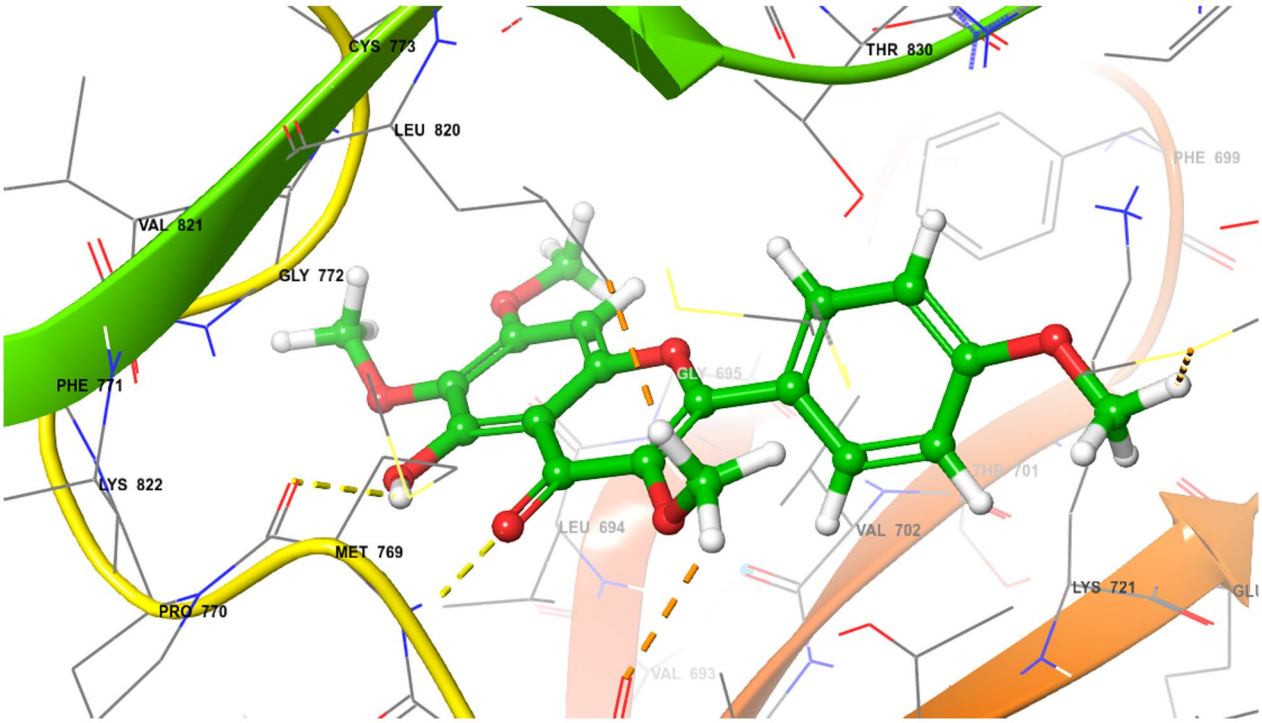
In silico study reveals that (Table 5) the selected compounds were docked against the selected targeted protein in order to predict the optimum conformational position within the active region of the target protein. Among that the apigenin possesses − 7.90 kcal/mol (Fig. 3a) as compared to standard drug doxorubicin possesses − 7.63 kcal/mol (Fig. 3b) against the epidermal growth factor receptor tyrosine kinase and the



(a)

Fig. 3 **a** Pose of Apigenin in Epidermal growth factor receptor tyrosine kinase pocket (PDB ID:1M17): the oxygen atom of apigenin forms 2.14 Å with MET 769 and 1.98 Å. hydrogen bond with alcohol atom of GLU 738. **b** Pose of doxorubicin in epidermal growth factor receptor tyrosine kinase pocket (PDB ID:1M17): the oxygen atom and alcohol of Doxorubicin forms 2.12 Å and 1.14 Å with MET 769





(b)

Fig. 3 continued

Table 6 Pharmacokinetic parameters

QikProp Parameters	QPlog Po/W	QP logS	PSA	QPlog HERG	QPP Caco	QPP MDCK	QP log Khsa	QPlog BBB	HOA	CNS	RoF	RoT
Palmitic acid	5.194	−5.353	50.521	−3.22	241.0	135.1	0.507	−1.344	3	−2	1	0
8-oleic acid	5.934	−6.396	50.56	−3.66	240.5	134.8	0.78	−1.576	1	−2	1	1
Linoleic acid	5.824	−6.367	50.55	−3.651	240.5	134.9	0.77	−1.50	1	−2	1	1
Linolenic acid	5.785	−6.457	50.56	−3.744	240.3	134.7	0.785	−1.446	1	−2	1	1
Stearic acids	5.98	−6.266	50.44	−3.55	241.3	135.3	0.765	−1.628	1	−2	1	1
Tartaric acid	−0.39	−0.469	138.084	1.08	1.63	0.007	−1.315	−1.807	1	−2	0	1
Citric acid	0.06	−0.599	158.14	2.67	0.27	0.143	−1.358	−2.07	1	−2	0	1
Acacetin	2.483	−3.841	85.39	−5.11	385.3	176.4	0.155	−0.96	3	−1	0	0
7,4′- diome apigenin	3.163	−3.979	71.128	−5.15	1272.2	641.7	0.175	−0.48	3	0	0	0
P hydroxybenzoic	0.585	−1.534	72.006	−1.62	76.6	39.1	−0.80	−0.78	3	−1	0	0
Vanillic acid	8.236	−7.626	58.975	−3.78	2057.9	1079.3	2.25	−0.58	1	0	2	1
Syringic acid	0.963	−1.591	86.5	−1.59	82.72	42.5	−0.71	−0.96	3	−1	0	0
Isoorientin	−1.168	−2.88	202.9	−5.32	4.96	1.46	−0.78	−3.41	1	−1	2	2
Isovitexin	−0.535	−3.065	181.4	−5.44	12.50	4.34	−0.61	−2.87	2	−2	1	2
Swertisin	0.053	−3.459	167.86	−5.53	22.39	8.15	−0.57	−2.78	2	−2	1	2
B-sitosterol	8.474	−9.628	35.85	−4.83	3845.1	2121.1	2.425	−0.30	1	−2	1	1
Betulin	5.92	−6.856	40.01	−3.815	1862.3	968.6	1.507	−0.358	1	0	1	1
Ethyl gallate	0.174	−1.822	100.8	−4.09	114.8	47.6	−0.583	−1.55	3	0	0	0
5-hydroxy-7,8-dimethoxyflavone	3.074	−3.656	69.65	−4.86	1274.8	643.1	0.134	−0.44	3	−2	0	0
5-hydroxy-3′, 4′, 6, 7, 8pentamethoxyflavone	3.414	−3.863	87.76	−4.61	1732.2	865.5	0.06	−0.53	3	0	0	0
4′-(beta-d-glucopyranosyloxy)-5-hydroxy-3,3′,7-trimethoxyflavone	0.694	−3.673	174.8	−5.53	46.11	17.7	−0.60	−2.67	2	0	2	1
Apigenin SP	1.636	−3.35	99.6	−5.08	116.76	48.5	−0.02	−1.42	3	−2	0	0
5-hydroxy-3, 6, 7, 4′-tetra methoxyflavone SP	3.409	−4.027	83.65	−4.93	1859.7	967.3	0.11	−0.456	3	0	0	0

PSA Prostate-specific antigen, *HERG* Human ether-a-go-go-related gene, *MDCK* Madin-Darby canine kidney, *BBB* blood brain barrier, *CNS* Central nervous system

Table 7 Antioxidant properties of extract by using different methods

Samples	DPPH (µg/mL)	Ferrous Ion (µg/mL)	NO (µg/mL)	ABTS (µg/mL)	NBT (µg/mL)	FRAP (µg/mL)	LPO (µg/mL)
OC extract	26.159 ± 2.3	74.330 ± 0.39	75.408 ± 7.3	59.884 ± 5.23	118.18 ± 2.3	152.11 ± 9.47	1.810 ± 0.38
Std	14.844 ± 5.2 (AA)	47.20 ± 2.9 (EDTA)	62.958 ± 0.89 (AA)	23.269 ± 2.5 (BHA)	107.113 ± 5.7 (BHT)	163.77 ± 5.09 (AA)	1.55 ± 0.30 (BHA)

Values are mean ± SD ($n = 3$)

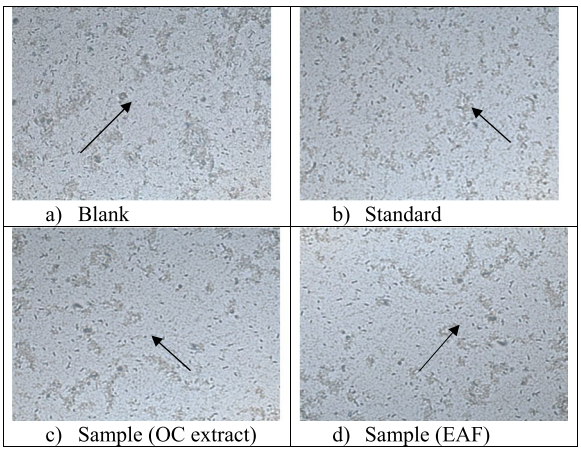


Fig. 4 Cell viability of Hep-G2 cell line treated with hydroalcoholic extract and EAF. Standard- Doxorubicin, OC-oxalis corniculata, EAF-Ethyl acetate fraction

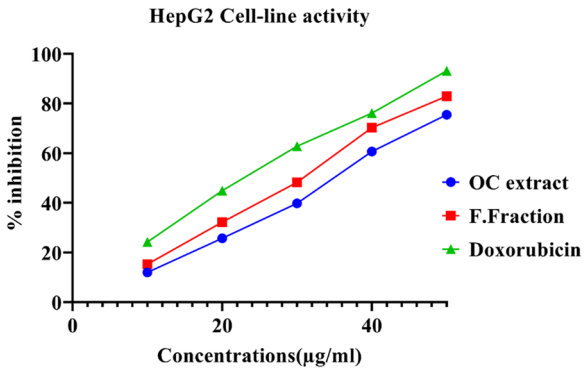


Fig. 5 Determination of % inhibition of (Hep-G2) cell line

pharmacokinetic profile of bioactive were predicted by using Qikprop module (Table 6).

Many human diseases are believed to be influenced by reactive oxygen species. Because free radicals have a harmful role in biological systems, radical scavenging activities are essential. Numerous secondary metabolites, including phenols, polyphenols, and flavonoids, act as scavengers and antioxidant sources. Reactive oxygen species easily combine and oxidise biomolecules including proteins, lipids, and carbohydrates, making them inert and causing subsequent harm to cells, tissues, and organs that promotes the spread of cancer. The total antioxidant capacity of the hydroalcoholic extract was evaluated in the current study using a variety of methodologies, and the findings were superior to those of previous studies that used these 7 assays (DPPH, ferrous ion chelating, nitric oxide scavenging activity, ABTS, FRAP, NBT and TBA). Antioxidant effect is imparted by the presence of antioxidants, which have been demonstrated to break the free radical chain by donating a hydrogen molecule (Table 7).

DPPH, a stable nitrogen-centered free radical, is typically used to assess the capacity of antioxidants found in plant extracts or synthesised compounds to scavenge free radicals. The reduction in absorbance at 517 nm brought on by the antioxidant provides to measure the DPPH radical's capacity for reduction. In the present study, the antioxidant activity of *Oxalis corniculata* was evaluated using hydroalcoholic extract of the plant and was compared with standard ascorbic acid. The experimental data revealed that the extracts are likely to have the properties of scavenging free radicals with extract showing higher antioxidant capacity.

The ferrous-ion chelating effect of samples determined by the percentage inhibition of Ferro zine Fe^{2+} complex formation; the absorbance of the colour produced was measured by spectrophotometer at 562 nm against the blank. In the present study, the antioxidant activity of *Oxalis corniculata* was evaluated using hydroalcoholic extract of the plant and was compared with standard EDTA. The results from the experiment indicated that the extracts are probably capable of scavenging free radicals, with extracts having a higher antioxidant capability.

The nitric oxide scavenging activity of samples determined by the percentage inhibition of NO complex formation, the absorbance of the formed chromophore is measured at 546 nm. In the present study, the antioxidant activity of *Oxalis corniculata* was evaluated using hydroalcoholic extract of the plant and was compared to standard ascorbic acid. The results of the experiments showed that the extracts are probably capable of scavenging free radicals, with extracts having a higher antioxidant capability.

The ABTS (2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) cation radical scavenging activity was performed based on quenching the ABTS by antioxidant compounds at 734 nm to get a blue green colour. The decrease in colour was calculated as percentage reduction of hydroalcoholic extract when to Gallic acid as standard.

Superoxide radicals scavenging capacity of samples was determined and the absorbance was read at 560 nm against blank. The decrease in colour was calculated as a percent reduction of superoxide anion scavenging capacity of hydroalcoholic extract when compared to BHT as standard.

The reduction of ferricyanide Fe^{3+} to ferro cyanide Fe^{2+} caused by the presence of FRAP antioxidants in the extract was measured spectrophotometrically at 700 nm. Depending on the reducing power of the plant extract, the test solution's yellow colour changes in this assay to various shades of green and blue. With an increase in extract concentration, the reducing power also increased. Its potential antioxidant action may be significantly indicated by this. The hydroalcoholic extract of *Oxalis corniculata* was therefore considered to have significant levels of antioxidant capabilities in this investigation, which were comparable to the synthetic antioxidant standard used.

The amount of malondialdehyde formed in each sample was measured by measuring the absorbance at 535 nm against a reagent blank. The lipid peroxidation was measured in terms of thiobarbituric acid reactive substances (TBARS) formed in AAPH treated liver homogenate. The extract's percentage reduction in lipid peroxidation when compared to BHA as the accepted standard.

For a treatment to be effective and safe, the anticancer activity of plant extract must be assessed. It makes possible to determine the plant's intrinsic toxicity and the effects of an acute overdose. The crude extract is tested using MTT assay to determine potential toxicity. Furthermore, it indicated to any possible cytotoxic properties of the investigated plant extract. The MTT assay is based on the reduction of MTT by purple formazan product produced by mitochondrial dehydrogenase. It is commonly employed as an in vitro model system to assess the cytotoxic effects of various toxic agents and plant extracts against cancer cell lines. Human Hepatocarcinoma (Hep-G2) cell line in-vitro cytotoxicity test was carried out to screen potentially harmful substances that influence fundamental cellular activities and morphology, and there is no literature by employing this cell line. The hydroalcoholic extract and ethyl acetate fraction (EAF) of *Oxalis corniculata* showed in-vitro growth inhibition effects on the cancer cell line (Hep-G2). According to concentration, these selective effects were depending on both incubation time and concentrations (10, 20, 30, 40 and 50 µg/ml) of extract and EAF were evaluated in triplicates by serial dilution. Among that EAF showed at IC_{50} value 30.245 ± 0.58 (mean ± SD) µg/ml of the cells were dead at the concentration of 50 µg/ml and hydroalcoholic extract showed IC_{50} value at 34.494 ± 0.42 (mean ± SD) µg/ml as compared to standard doxorubicin at IC_{50} value 24.8939 ± 0.25 (mean ± SD) µg/ml (Fig. 5) and Cell viability in terms of growth inhibition of the extract and EAF was found to be dose dependent (Fig. 4).

Conclusion

Based on the experiments performed, it was concluded that the plant *Oxalis corniculata* contains a wide range of secondary metabolites that holds the strong antioxidant activity. The *oxalis corniculata* plant extract showed good anticancer activity based on in-vitro and in-silico analysis. This study provides the scientific evidence to conduct additional research, look into the lead compounds present in the plant, assess its anticancer potential on in-vivo animal models and put forward an attempt to carry out trials on humans.

Abbreviations

OC	*Oxalis corniculata*
EAF	Ethyl acetate fraction
Hep-G2	Human Hepatocarcinoma cell line
DPPH	*α, α* -Diphenyl-*β*-picrylhydrazyl
NO	Nitric oxide scavenging activity
ABTS	2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
FRAP	Ferric reducing antioxidant power
NBT	Nitroblue tetrazolium
TBA	Thiobarbituric acid assay

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Author contributions

We have assured that "all authors have read and approved the manuscript." All the authors have equal contribution and participation in this research work. SG has reviewed all manuscripts on Antioxidant properties and cytotoxic effects of *Oxalis corniculata* on human Hepatocarcinoma (HepG2) cell line: An in vitro and in silico evaluation and he had completed his work under the supervision of KP. KP also helped him in their research work and guides to resolve the complications.

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The research work has been carried out by us, and we assure you that it can be provided to you whenever required.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication
Not applicable.

Competing interests
No competing interests to declare.

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