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# Anticancer activities of phenolic compounds from *Moringa oleifera* leaves: in vitro and in silico mechanistic study

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## Abstract

**Background:** *Moringa oleifera* is a common vegetable in many countries since ancient times, possesses numerous phenolic compounds having a wide array of biological activities. It possesses anticancer activity that can be used to develop new drugs for treatment of various types of cancers. The current study was conducted to evaluate the composition of phenolic compounds and in vitro and in silico anticancer activities of *M. oleifera* leaves extracts. The leaves of *M. oleifera* were subjected to extraction for solvent fraction using n-hexane, chloroform, ethyl acetate, butanol, and aqueous solvents. The solvent fractions were tested for anticancer activity in vitro against *Hela* cancer cell line and screened for phenolic compounds through reversed-phase high-performance liquid chromatography. The molecular docking approach was employed to check binding conformations of phytochemicals against the target protein.

**Result:** The result revealed that all the solvent fractions possess in vitro anticancer activity against *Hela* cancer cell line. The n-hexane fraction showed a 50% reduction in *Hela* cancer cell viability at 416  $\mu\text{g mL}^{-1}$  as compared to control. The extracts of solvent-fraction contained 10 phenolic compounds viz. quercetin, gallic acid, sinapic acid, vanillic acid, 4-hydroxy-3-methoxy benzoic acid, *p*-coumaric acid, *m*-coumaric acid, 4-hydroxy-3-methoxy cinnamic acid, caffeic acid, and syringic acid. Molecular docking studies revealed that the ligands bind within the active site of target protein have good binding energy values.

**Conclusion:** This study shows that *M. oleifera* leaves may have the potential to inhibit cancer cell growth and improving human health in addition to food ingredient innovations. Based on in vitro and in silico results, the phytochemicals from *M. oleifera* leaves can be used as leading drugs to treat cancer.

**Keywords:** Anticancer activity, *Hela* cancer line, Molecular docking, *Moringa oleifera*, Phenolic acids, Solvent fractions

## 1 Background

Medicinal plants are the richest resource of natural compounds having a wide range of applications for the well-being of human population. In the modern age, the dependence of humans on plants for the majority of commercial products including pharmaceutical, healthcare, food, beverages, textiles, cosmetics, and aromas is being obtained from plants. Therefore, plants are and will

remain economically, industrially, environmentally, spiritually, historically, and aesthetically important for survival, sustenance, and prosperity of life on Earth [1–4].

*Moringa oleifera* (MO) is the most commonly grown species that belongs to the monogeneric family: *Moringaceae* that is native to the Himalayan and sub-Himalayan areas cultivated throughout Pakistan, India, Afghanistan, Bangladesh, and other tropical and subtropical countries of the world [5, 6]. The MO has been utilized for flowers, fruit, and leaves as a vegetable particularly in India and Pakistan and many other parts of

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Asia and Africa [7–9]. In tropical areas of developing countries, MO is consumed by livestock as a forage, moringa micronutrient liquid, natural anthelmintic, and adjuvant against endemic diseases [5]. In many countries, roots, leaves, flowers, and seeds of MO are used in folk medicine for ailments and treatment of arthritis, asthma, blood pressure, body pain, cough, diabetes, diarrhea, dropsy, epilepsy, fever, headaches, hysteria, irritations, paralytic, skin infection, sores, tumors, weakness, wound, and employed as emmenagogues, expectorants, and mild diuretics, etc. [10–12]. They are of pungent taste and promote digestion [13]. The MO is a well-known miracle tree enriched with important minerals (calcium, potassium, zinc, magnesium, iron, and copper), vitamins (vitamin A, B, C, D, and E), carbohydrate, crude protein, ash, sugar, fat, glutelin, albumin, and globulins which can be used by doctors, nutritionists, and community health cautious persons to cure illness [5, 14–16].

The MO possess several phytochemicals such as ascorbates, beta-sitosterol, carotenoids, flavonoids, kaempferol, moringine, omega fatty acids, phenolic, quercetin, tocopherols, and vanillin, that have various biological activities including antiatherosclerotic, anticancer, antidiabetic, anti-inflammatory, antimicrobial, antioxidant, antiulcer, antiviral, and immune-boosting [8, 17–23]. Among these phytochemicals, phenolic acids are the most abundant secondary metabolites of plants and are involved in many biological activities. They are of benzoic acid and cinnamic acid derivatives including caffeic, chlorogenic, coumaric, ferulic, gallic, and quinic acids, tannins, and many others having more than 8000 phenolic structure found in various fruits and vegetables [24, 25]. Prabakaran et al. [26] reported the presence of total phenolic and total flavonoids contents in leaves as compared to roots, barks, and flower extracts of MO.

Cancer is the largest group of diseases causing death of 9.6 million people worldwide [27]. The most common type of cancer in females are breast, cervical, colorectal, lung, and thyroid cancers. Among them, cervical cancer is the fourth most leading cause of death in women and causing 270,000 deaths annually [28]. It is well known that 74% of anticancer medicines are being derived from various plant species [29]. The MO leaves extracts showed remarkable effects against various cancerous cells [30]. Balamurugan et al. [31] reported the anticancer effect of MO leaf extracts against hepatic cancer cell line (HepG2). The phytochemicals showed a dynamic role in treatment and prevention of cancer by hindering cancer cells through activating hormones and enzymes, stimulation of DNA repair mechanism, enhancing the production of protective enzymes that induce antioxidant action and enhance immunity [32–34]. Because of these facts, the current study was conducted to evaluate the composition of phenolic compounds and anticancer

activities of different solvent fractions of MO leaves extracts. To achieve the purpose of the current study, solvent fractions of MO leaves extracts were prepared using n-hexane, ethyl acetate, butanol, and water to their polarities. For phenolic compounds, various solvent-fractions (SF) were analyzed using reversed-phase high-performance liquid chromatography. The SF extracts were analyzed on *Hela* cancer cell lines for their antiproliferative activity.

## 2 Methods

### 2.1 Plant material

*Moringa oleifera* (MO) leaves were collected in June (three months after spring season) from Baagh-e-Jinnah Park Lahore, Pakistan, and transported within 1 h to Plant Biotechnology Laboratory. Leaves were washed three times with distilled water to remove unnecessary material and dust particles and dried under shade for a month. Leaves specimen were identified by consulting medicinal plant name services (<https://www.kew.org/mpns>) and by comparing herbarium specimens for taxonomic and botanical authentication. The specimen was deposited within the institute having voucher specimen numbers 845 was assigned. The dried leaves were ground into a fine powder using a mortar/pestle to maximize the extraction yield and sieved through 0.3 mm mesh size. The pulverized fine powered (100 g w/v) was extracted with 80% methanol (Sigma-Aldrich, USA) (200 mL v/v) at 25 °C for three times to retrieve more of the active compounds. After 90 min, the percolated extract was concentrated by a rotary vacuum evaporator (V700, BUCHI AG, Switzerland). The extracted solution was filtered twice with Whatman No. 1 filtering paper to remove insoluble matrices. Then, the filtrate was successively partitioned using a solvent of grade polarities: n-hexane (Daejung, Korea), chloroform (Merk, Germany), ethyl acetate (Daejung, Korea), butanol (Merk, Germany), and distilled water by using orbital shaker at 200 rpm for overnight [35]. The solvents were removed from crude leaves extract under reduced pressure using a rotary evaporator (Hei-VAP, Heidolph, Germany) and vacuum pump with the refrigerated circulator.

### 2.2 Anticancer activity

The anticancer activity of MO leaves extracts was evaluated through 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide tetrazolium (MTT) reduction assay [36, 37]. The sample of each extract was diluted in Dimethyl sulfoxide (DMSO) to obtain a concentration of 26, 52, 104, 206, and 416  $\mu\text{g mL}^{-1}$  by microdilution assay [38]. The cytotoxicity assay was performed by incubating samples in 96-well plates containing *Hela* cancer cell lines (American Type Culture Collection, ATCC) maintained in a culture medium made of Dulbecco's modified

Eagle's medium (DMEM) (Caisson Labs, USA) with 2 mM L-glutamine (Lonza) augmented with 10% fetal bovine serums (Sigma-Aldrich, USA), 100 units of antibiotics (penicillin; Sigma-Aldrich, USA). The cells were cultured in the medium up to the log phase under 5% CO<sub>2</sub>, 37 °C temperature, 7.4 pH, and 90% humidity conditions. The MTT solution was prepared by using 2.5 mg MTT and extraction buffer (prepared by using 20% sodium dodecyl sulfate and 50% DMSO in distilled water). The 0.2 mL of DMEM medium was taken in 5000 cells/well in 96-well plates and incubated at 37 °C for 24 h. Then, the medium was aspirated at 37 °C and dried on tissue paper. Further, 200 µL of samples, 25 µL of MTT solution, and 100 µL extraction buffer were added in each well and incubated at 37 °C for 24 h. The extraction buffer was used as a blank. The optical density was taken at 570 nm using enzyme-linked immunosorbent assay (ELISA) reader (PR4100, BioRad, USA). The relative cell viability was calculated by adopting a formula reported by Bendale et al. [39]. The data were statistically analyzed by SPSS 21.0 package program for Windows. Statistical analysis was carried out by employing a one-way analysis of variance (ANOVA) and the least significant difference (LSD) multiple comparison test at a 5% level of probability [40]. Values were reported as a mean of three replications along with standard error.

### 2.3 Determination of phenolic compounds through reversed-phase high-performance liquid chromatography (RP-HPLC)

The phenolic compound in different solvent extract samples was quantified through reversed-phase high-performance liquid chromatography (RP-HPLC). The samples were prepared through take 25 mg of crude extract in 5 mL of HCl (6 M), 12 mL of methanol and 8 mL of distilled water and incubated at 90 °C for 2 h. After incubation, the mixture was filtered with a 0.2-mm MF-Millipore<sup>TM</sup> membrane filter (Merck, Germany) and injected in HPLC. The HPLC separation was done using the HPLC system with column 20RBAX ECLIPSE, XDB-C18, (5 µm: 4.6 × 150 mm, Agilent, USA) and UV-VIS spectra-focus detector. The isocratic mobile phase (tetrahydrofuran, acetonitrile, and 0.05% phosphoric acid solution in the ratio of 20:3:77 v/v) was injected at a flow rate of 1 mL min<sup>-1</sup>. Before use, the mobile phase was filtered through 0.2 mm MF-Millipore<sup>TM</sup> membrane filters and degassed by sonication in an ultrasonic bath. The detection wavelength was set at 280 nm and the column temperature was maintained at room temperature with an injection volume of 10 µL.

### 2.4 Retrieval of protein structure and designing of ligands

The Bcl-2-associated X (BAX) protein human (*Homo sapiens*) was accessed from Protein Data Bank (PDB) having PDBID: 1F16 (<https://www.rcsb.org/structure/1F16>) and utilized as a target molecule. The selected protein further undergoes energy-minimization through UCSF Chimera v1.12 at 1000 steepest and 1000 conjugate gradient runs with Amber force field parameters [41]. Based on in vitro analysis, four chemical compounds were selected for the computational study. Four chemical compounds quercetin, gallic acid, *p*-coumaric acid, and 4-hydroxy 3-methoxy cinnamic acid were retrieved in PDB through sketching in ChemDraw [42]. The refinement and gematory optimization of these selected compounds were confirmed by Chem3D Pro [43] and UCSF Chimera v1.12, respectively. the molecular properties of selected compounds were predicted through an online tool such as Molsoft (<http://molsoft.com/mprop/>)

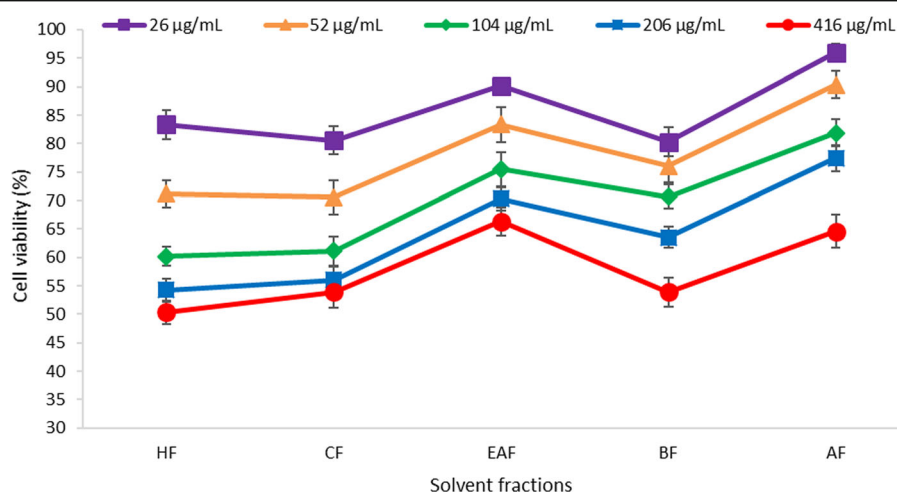
### 2.5 Molecular docking

To check the binding behavior of quercetin, gallic acid, *p*-coumaric acid and 4-hydroxy 3-methoxy cinnamic acid against BAX protein, a computational docking approach was utilized through PyRx [44]. Initially, protein grid parameters were fixed in all three dimensions by taking a binding pocket as core-site. Finally, docking experiments were performed against the minimized phyto-compounds and targeted proteins, separately using AutoDock-Vina [44]. The grid box values of selected center  $x = 39.86$ ,  $y = 25.22$ ,  $z = 38.88$  and size of  $x = 36.17$ ,  $y = 40.50$ , and  $z = 35.62$ , respectively. The default exhaustiveness value = 8 was also used for better interaction behavior of compounds against target protein in docking. The generated docked complexes were analyzed based on binding energy values as interactive behaviors (hydrogen/hydrophobic interactions) of ligands. Furthermore, the binding conformation within the active site of the target protein was also observed for all three ligands against best-selected proteins. The graphical depiction of docked complexes was investigated and visualized by UCSF Chimera v1.12. and Discovery Studio 2017.

## 3 Results

### 3.1 Solvent-fractions of *M. oleifera* leaves showed anticancer activity in vitro

The cytotoxicity of different SF of MO leaves against *Hela* cancer cells line was performed using MTT assay. A range of different doses (26, 52, 104, 208, and 416 µg mL<sup>-1</sup>) of five SF (n-hexane, chloroform, butanol, ethyl acetate, and water) of MO leaves was evaluated for their potential anticancer activity. The data were collected in terms of percent cell viability of cancer cells given in Fig. 1 and percent inhibition in cancer cells given in Table 1. Among



**Fig. 1** Cytotoxic effect of *Moringa oleifera* leaves extracts of various solvents viz. n-hexane, chloroform, ethyl acetate, butanol, and water on *HeLa* cancer line. Data of relative cell viability represented as means  $\pm$  error bars of three replications at  $p \leq 0.05$  versus control which give 100% cell viability

SF, chloroform-fraction (CF) followed by n-hexane-fraction (HF) and butanol-fraction (BF) showed their highest anticancer activities in terms of cell viability and inhibition in cancer cells. While aqueous-fraction (AF) followed by ethyl-acetate fraction (EAF) was lowest to demonstrate their anticancer activity. The inverse relationship was found between the increase in the dose of SF and cancer cell viability (Fig. 1), while, there was a direct relationship between the increase in the dose of SF and inhibitions in cancer cell growth (Table 1). Various doses of SF showed variable anticancer activity with maximum activity was recorded with the application of  $416 \mu\text{g mL}^{-1}$  of all tested SF, while,  $26 \mu\text{g mL}^{-1}$  of tested SF showed minimum anticancer activity. Application of  $416 \mu\text{g mL}^{-1}$  of HF showed maximum inhibition in cancer cells up to 50% with 50% cell viability followed by CF and BF both having 46% inhibition in cancer cells growth and 54% cancer cell viability. While EAF followed by AF at  $416 \mu\text{g mL}^{-1}$  showed the lowest inhibition in cancer cells up to 34 and 35%, respectively, with 66 and 65%, respectively, of cancer cells viability.

**Table 1** HeLa cancer cells inhibition due to the application of various solvent fractions of *Moringa oleifera*

| Doses  | HF            | CF            | EAF           | BF            | AF            |
|--|---------------|---------------|---------------|---------------|---------------|
| <i>HeLa</i> cancer cells inhibition (% $\pm$ SE) |               |               |               |               |               |
| $26 \mu\text{g mL}^{-1}$                         | $17 \pm 2.61$ | $19 \pm 3.38$ | $10 \pm 1.59$ | $20 \pm 2.07$ | $4 \pm 2.01$  |
| $52 \mu\text{g mL}^{-1}$                         | $29 \pm 3.54$ | $30 \pm 3.93$ | $17 \pm 3.52$ | $24 \pm 2.24$ | $10 \pm 2.83$ |
| $104 \mu\text{g mL}^{-1}$                        | $33 \pm 1.34$ | $39 \pm 2.06$ | $25 \pm 3.09$ | $29 \pm 2.08$ | $18 \pm 2.46$ |
| $206 \mu\text{g mL}^{-1}$                        | $46 \pm 2.52$ | $44 \pm 2.94$ | $30 \pm 3.64$ | $37 \pm 2.83$ | $23 \pm 1.55$ |
| $416 \mu\text{g mL}^{-1}$                        | $50 \pm 1.53$ | $46 \pm 2.34$ | $34 \pm 2.33$ | $46 \pm 2.25$ | $35 \pm 2.91$ |

### 3.2 Phenolic acids quantification from solvent-fractions of *M. oleifera* leaves through RP-HPLC

Reversed phased-high performance liquid chromatography (RP-HPLC) was employed for the identification and quantification of phenolic acids present in the SF of MO leaves. Results revealed that 10 phenolic compounds: quercetin, gallic acid, sinapic acid, vanillic acid, 4-hydroxy benzoic acid, *p*-coumaric acid, *m*-coumaric acid, 4-hydroxy-3-methoxy cinnamic acid, caffeic acid, and syringic acid were identified and quantified in HF, CF, EAF, BF, and AF of MO leaves. The amounts of phenolic acids present in SF of MO leaves are given in Table 2 and their chemical structural formulas are provided in Fig. 2. The value of total phenolic contents (TPC) varied and ranged from  $5.81$  to  $24.68 \text{ mg g}^{-1}$  of dry matter. Out of all of SF, BF had the highest TPC ( $24.68 \text{ mg g}^{-1}$  of dry matter). Following BF, AF ( $21.55 \text{ mg g}^{-1}$  of dry matter) and CF ( $16.11 \text{ mg g}^{-1}$  of dry matter) exhibited the highest TPC. While EAF extracted lowest amount of TPC ( $5.81 \text{ mg g}^{-1}$  of dry matter). The quercetin, gallic acid, and *p*-coumaric acid were detected in all the tested SF with highest contents of quercetin in AF ( $4.71 \pm 0.01 \text{ mg g}^{-1}$  of dry matter) and gallic acid ( $10.18 \pm 0.03 \text{ mg g}^{-1}$  of dry matter) and *p*-coumaric acid ( $10.18 \pm 0.03 \text{ mg g}^{-1}$  of dry matter) in BF. The 4-hydroxy-3-methoxy cinnamic acid was also detected in all the tested SF except HF having highest contents of  $7.81 \pm 0.01 \text{ mg g}^{-1}$  of dry matter in BF. The ferulic, sinapic, and syringic acids were detected from HF and AF. While vanillic, *m*-coumaric, and caffeic acid were only detected in a single fraction of HF, AF, and EAF, respectively. However, the extracted concentration of TPC ( $24.7 \text{ mg g}^{-1}$ ) was higher in BF that showed gallic acid ( $10.2 \text{ mg g}^{-1}$ ), 4-hydro 3-methoxy cinnamic acid ( $7.8 \text{ mg g}^{-1}$ ) and *p*-coumaric acid ( $5.5 \text{ mg g}^{-1}$ ) as major metabolites. Followed by

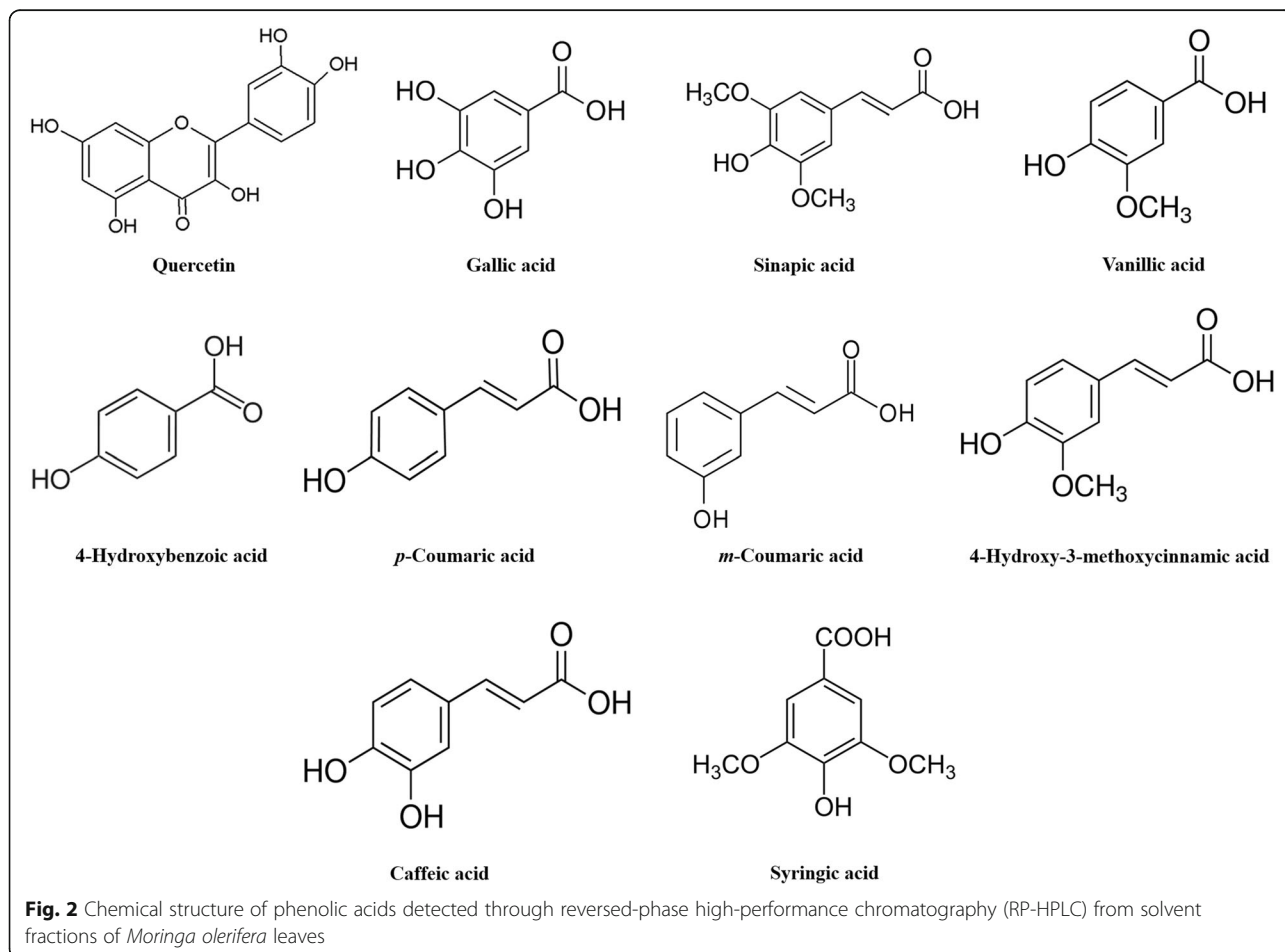
**Table 2** Phenolic compounds in solvent extract fraction of *Moringa oleifera* analyzed through reversed-phase high-performance liquid chromatography (RP-HPLC)

| Phenolic acids                    | HF                               | CF          | EAF         | BF           | AF           |
|-----------------------------------|----------------------------------|-------------|-------------|--------------|--------------|
|                                   | mg g <sup>-1</sup> of dry matter |             |             |              |              |
| Quercetin                         | 0.17 ± 0.02                      | 0.97 ± 0.02 | 0.19 ± 0.02 | 1.22 ± 0.02  | 4.71 ± 0.01  |
| Gallic acid                       | 0.64 ± 0.01                      | 8.77 ± 0.03 | 0.19 ± 0.01 | 10.18 ± 0.03 | 2.74 ± 0.01  |
| Sinapic acid                      | 0.86 ± 0.03                      | ND          | ND          | ND           | 0.37 ± 0.01  |
| 4-Hydroxy benzoic acid            | 2.43 ± 0.03                      | 2.69 ± 0.01 | ND          | ND           | 5.31 ± 0.01  |
| <i>p</i> -Coumaric acid           | 0.48 ± 0.03                      | 0.72 ± 0.02 | 1.25 ± 0.01 | 5.47 ± 0.03  | 0.43 ± 0.02  |
| <i>m</i> -Coumaric acid           | ND                               | ND          | ND          | ND           | 0.715 ± 0.02 |
| 4-hydroxy 3-methoxy cinnamic acid | ND                               | 2.96 ± 0.01 | 2.05 ± 0.01 | 7.81 ± 0.01  | 1.21 ± 0.01  |
| Caffeic acid                      | ND                               | ND          | 2.13 ± 0.02 | ND           | ND           |
| Syringic acid                     | 0.33 ± 0.02                      | ND          | ND          | ND           | 2.76 ± 0.02  |
| Total phenolic acids              | 7.92                             | 16.11       | 5.81        | 24.68        | 21.17        |

BF, CF extracted maximum TPC concentration (16.1 mg g<sup>-1</sup>) having gallic acid (16.1 mg g<sup>-1</sup>) as a major metabolite. The HF was able to extract 7.9 mg g<sup>-1</sup> of TPC concentration which tells us the possibility of other phytochemical responsible for its excellent anti-cancer activity.

### 3.3 Chemoinformatics analysis

The chemo-informatics properties were evaluated by computational approaches to check the basic biochemical properties. Results exposed that all compounds quercetin, gallic acid, *p*-coumaric acid, and 4-hydroxy 3-methoxy cinnamic acid have better-predicted values of





molecular weight (g/mol). The molecular weight (g/mol) of all compounds was also comparable with standard value (< 5000 g/mol). Moreover, the Lipinski's rule of five (RO5) results showed that compounds (quercetin, gallic acid, *p*-coumaric acid, and 4-hydroxy 3-methoxy cinnamic acid) possessed good hydrogen bond acceptors (HBA) and hydrogen bond donar (HBD) values which significantly justified their drug-like behavior. The overall predicted results values of all compounds are mentioned in Table 3.

### 3.4 Docking energy evaluation of selected compounds

To predict the best conformational position within the active region of the target protein, the selected compounds were docked against selected targeted protein. All the generated docked complexes were examined based on minimum energy values (Kcal/mol) and bonding interaction pattern (hydrogen, hydrophobic, and electrostatic). The quercetin possesses − 7.80 whereas other, gallic acid, *p*-coumaric acid, and 4-hydroxy 3-methoxy cinnamic acid exhibited − 5.8, − 5.6, and − 5.7 Kcal/mol, respectively. Docking results justified that compounds exhibited good docking energy values Kcal/mol (Fig. 3).

### 3.5 Binding conformations of phytocompounds

The binding interaction behavior of selected compounds was evaluated based on hydrogen and hydrophobic interactions. Figure 4 showed that all four compounds were bound at the same conformations inside the binding pocket of the target protein. The ligands (quercetin, gallic acid, *p*-coumaric acid, and 4-hydroxy 3-methoxy cinnamic acid) were binds with different binding poses with little conformational configurations.

### 3.6 Hydrogen and hydrophobic binding analysis of docked complexes

In gallic acid-BAX docking complex, four hydrogen bonds were observed at different residual positions such as Asp98, Asp99, Phe64, and Thr263 at different bind distance. The carboxylic group of gallic acid forms two hydrogen bonds 2.13 and 2.61 Å. The hydroxyl groups

at *meta* and *para* positions in gallic acid form hydrogen bonds having bond length 3.00 and 2.21 Å at Phe64 and Thr263, respectively (Fig. 5). In *p*-coumaric acid-BAX docking complex, a couple of hydrophobic interactions were observed at Leu62 and Pro63 with different conformation positions. The hydroxyl groups in *p*-coumaric acid form two hydrophobic interactions with bond lengths 5.32 and 5.42 Å, respectively (Fig. 6).

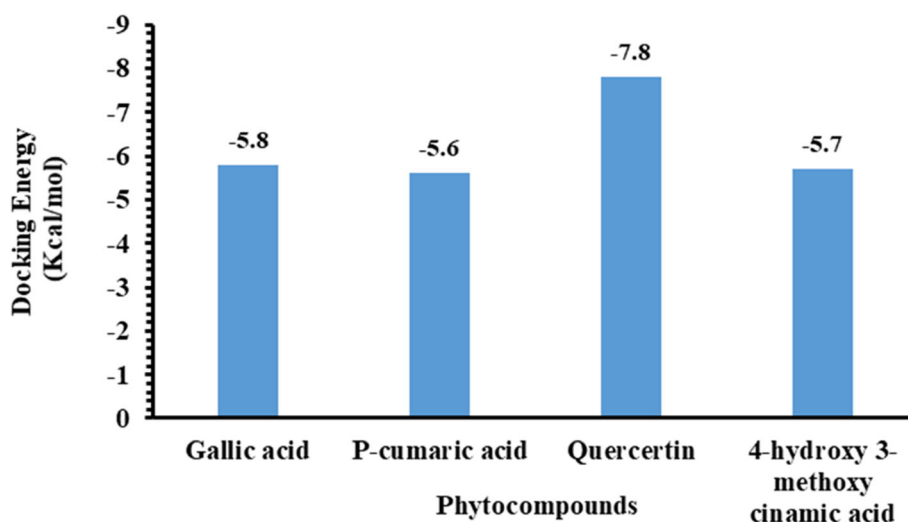
In quercetin-BAX docking complex, three hydrogen bonds Gln190, Asp99, and Glu259. The hydroxyl groups in quercetin form three hydrogen bonds with bond distances 2.46, 2.30, and 2.56 Å, respectively (Fig. 7). Quercetin showed best docking energy values and good interaction behavior compared to all other compounds. In 4-hydroxy 3-methoxy cinnamic acid-BAX docking complex, two hydrogen bonds were observed at Glu259 and Ile257 with bond distances 2.45 and 2.50 Å, respectively (Fig. 8).

## 4 Discussion

*Moringa oleifera* (MO) is a common vegetable plant in many Asian countries and is known as “the Miracle Tree” due to its uses in herbal medicine for treating over 300 various diseases [45]. Its leaves and pods possess numerous compounds with excellent health benefits that are being consumed by doctors, healers, nutritionists, and community persons to address under-nutrition and anemia conditions in children and infants [15, 46]. The MO possesses numerous compounds with excellent health benefits. Keeping in view the therapeutic activity of MO, we postulated that SF of MO leaves could be effective in the treatment of cancer disease. To the best of our knowledge, this is the first study reporting anticancer activities by using various MO leaves SF based on polarities. We assessed the SF of MO leaves for the anticancer property against *Hela* (cervical) cancer cell line. Our prime aim was to obtain numerous phenolic contents of MO leaves partitioned using five solvent of grade polarities including hexane, chloroform, ethyl acetate, butanol, and aqueous. The RP-HPLC analyses of SF of MO leave revealed numerous phenolic compounds including 4-hydroxy 3-methoxy cinnamic, 4-

**Table 3** Drug likeness analyses of phytocompounds

| Properties            | Gallic acid | <i>p</i> -Coumaric acid | Quercetin | 4-Hydroxy 3-methoxycinnamic acid |
|-----------------------|-------------|-------------------------|-----------|----------------------------------|
| Mass (g/mol)          | 170         | 164                     | 302       | 194                              |
| Logp                  | 0.55        | 2.07                    | 2.11      | 2.04                             |
| HBA                   | 5           | 3                       | 7         | 4                                |
| HBD                   | 4           | 2                       | 5         | 2                                |
| PSA (Å <sup>2</sup> ) | 77          | 46                      | 102       | 52                               |
| RO5 violations        | No          | No                      | No        | No                               |
| Drug Score            | 0.07        | − 0.74                  | 0.93      | − 0.44                           |

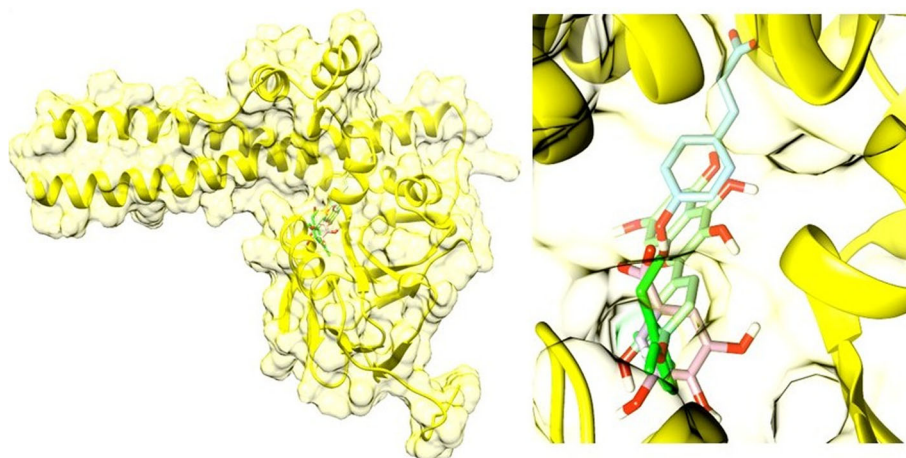


**Fig. 3** Docking energy values of compounds

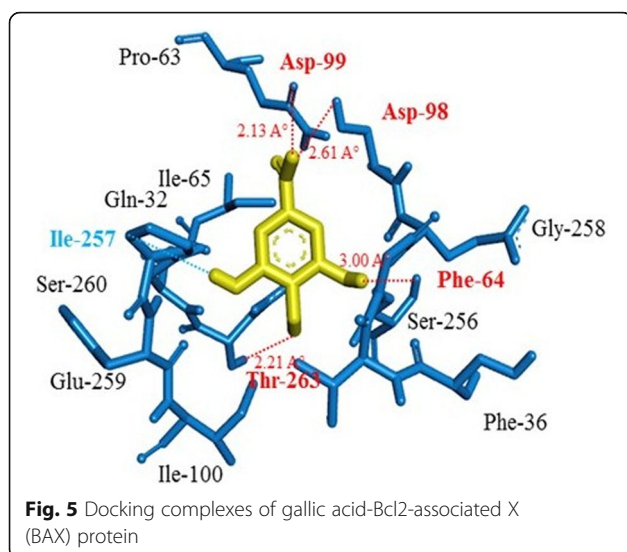
hydroxybenzoic, caffeic, ferulic, gallic, *m*-coumaric, *p*-coumaric, quercetin, sinapic, syringic, and vanillic acids in the chromatogram (Table 2).

In the present study, the anticancer activity by SF of MO leaves was observed in terms of cell viability in a dose depended manner. All the SF of MO leaves showed a significant reduction in *Hela* cancer cell viability. The concentration of SF and reduction in cell viability were directly proportional as increased concentration of SF showed maximum reduction in cell viability. A maximum reduction in cell viability was obtained from HF at  $416 \mu\text{g mL}^{-1}$  which was non-significant to CF and BF (Fig. 6). Tiloke et al. [47] reported that plants exhibit anticancer potential by interfering with the signal transduction cascade that promotes cancer cell proliferation. The inhibition of cancer cell viability could be due to phenolic compounds especially eugenol [48], D-allose [49],

and isopropyl isothiocyanate [50]. Eugenol plays a significant role in the apoptosis of breast cancer MDA-MB231 cells through the overexpression of Bax protein [51]. Eugenol could also trigger apoptosis through the downregulation of E2F1/survivin in breast cancer cells [48]. In the present study, the decreased cell viability in *Hela* cancer cells could be due to the ability of SF of MO leaves to cause an increase in apoptosis through downregulation of E2F1 and upregulation of Bax protein. Phenolic compounds present in SF of MO leaves could also cause a reduction in cell viability through shutting down of cancer survival pathway including NF- $\kappa$ B signaling cascade through downregulation of p65 component. Jung [52] reported AF of MO leaves as a novel potential anticancer candidate that induced apoptosis in lung cancer cells. The current study opens new prospective research to elucidate the molecular mechanism

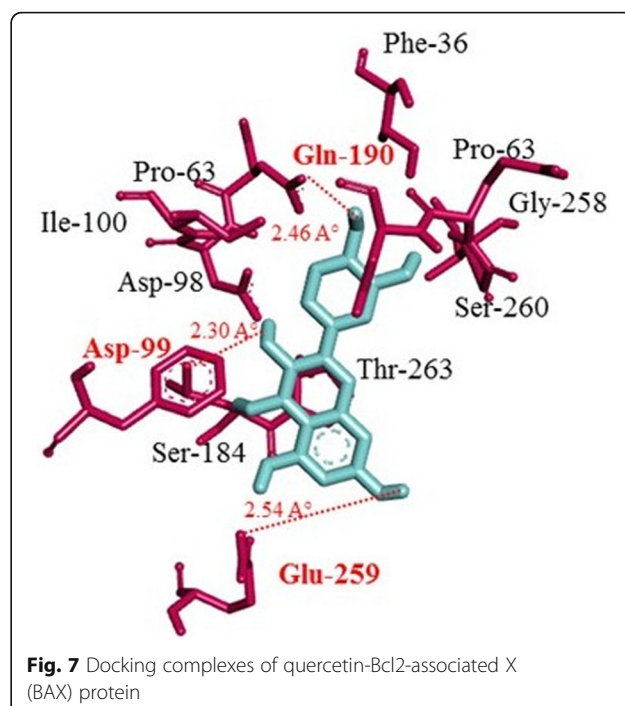
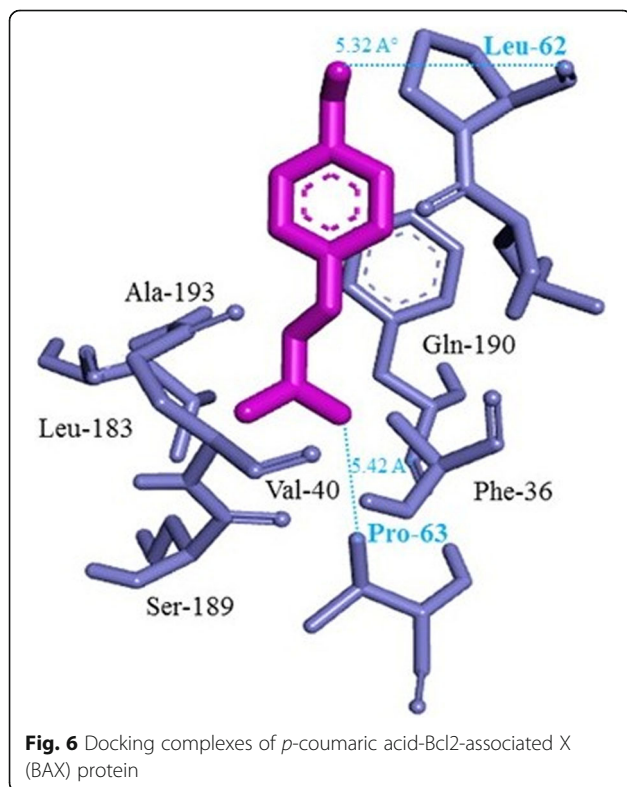


**Fig. 4** Binding poses of ligands

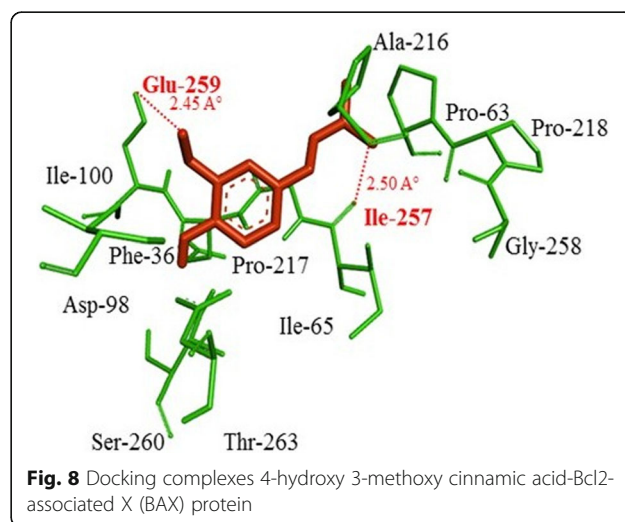


after treating with variable extracted concentration through SF of MO leaves. The future investigation needs to explore the expression of targeted protein P13K/AKT pathway and inhibition of phosphorylation of focal adhesion kinase (p-FAK) which is important in cell migration and cell invasion.

In the present study, SF of MO leaves was subjected to identification and quantification of phenolic acid



through RP-HPLC analysis. Phenolic compounds including quercetin, gallic acid, sinapic acid, vanillic acid, 4-hydroxy benzoic acid, *p*-coumaric acid, *m*-coumaric acid, 4-hydroxy-3-methoxy cinnamic acid, caffeic acid, and syringic acid were quantified in various SF of MO leaves (Table 2). Highest TPC was obtained through BF followed by AF while EAF extracts lowest TPC. Among phenolic compounds, highest concentrations of gallic acid, 4-hydroxy-3-methoxy cinnamic acid, and *p*-coumaric acid were detected in BF of MO leaves. The AF possessed all the determined phenolic contents, while, phenolic compounds *viz.* *m*-coumaric acid, 4-hydroxy 3-





methoxy cinnamic acid, and caffeic acid were not present in HF. However, HF showed higher anticancer activity as compared to AF that may be due to the presence of other phytochemicals in higher concentrations. The derivatives of hydroxybenzoic and hydroxycinnamic acid extract in SF of MO leaves could have a possible role in the prevention and treatment of cancer as reported in various studies [18, 53]. Phenolic acid from plant extract could reduce tumor initiation through adapting various mechanism *viz.* inhibiting genotoxic molecules, hindering mutagens-transforming enzymes activity, regulating enzymes such as heme-containing phase I and carcinogen-detoxifying phase II enzymes [54–56]. In the present study, highest anticancer activity was obtained from HF followed by CF and BF. The detection of phenolic acid in BF supports the findings of anticancer activity as BF reported highest TPC; however, there must be some other phytochemicals in HF and CF that are maximizing their anticancer activity. The current study is suggesting the future investigation on quantification of other phytochemicals in HF and CF of MO leaves that can define their anticancer activity.

Based on in vitro results, four phenolic compounds including quercetin, gallic acid, *p*-coumaric acid, and 4-hydroxy 3-methoxy cinnamic acid were selected to evaluate their chemo-informatics properties. The predicted results showed that compounds possessed good HBA and HBD values that significantly justified their drug-like behavior. In prior results, it has been observed that compounds with more HBA are more likely associated with poor absorption. However, there are plenty of examples available for RO5 violations amongst the existing drugs [57]. Furthermore, a molecular docking approach was utilized for the binding behavior of quercetin, gallic acid, *p*-coumaric acid, and 4-hydroxy 3-methoxy cinnamic acid against BAX protein docking, and results justified that all compounds exhibited good docking energy values. Our generated results showed that selected compounds were bind at same conformations inside the binding pocket of target protein. Among selected phenolic compounds, quercetin showed best docking energy values and good interaction behavior compared to all other compounds.

## 5 Conclusion

The current study is performed to recognize the therapeutic effect of *M. oleifera* based compounds against cancer. The in vitro and in silico results showed that quercetin, gallic acid, *p*-coumaric acid, and 4-hydroxy 3-methoxy cinnamic acid displayed potent anticancerous activity and good therapeutic potential against cancer. Based on the aforementioned results, it can be concluded that these phytochemicals could be used as leading drugs to treat cancer.

## Abbreviations

MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; ATCC: American Type Culture Collection; ANOVA: Analysis of variance; AF: Aqueous-fraction; BAX: Bcl-2-associated X; BF: Butanol-fraction; CF: Chloroform-fraction; DMSO: Dimethyl sulfoxide; DMEM: Dulbecco's modified Eagle's medium; ELISA: Enzyme-linked immunosorbent assay; EAF: Ethyl acetate-fraction; HBA: Hydrogen bond acceptors; HBD: Hydrogen bond donor; LSD: Least significant difference; RO5: Lipinski's rule of five; MO: *Moringa oleifera*; HF: n-Hexane-fraction; PDB: Protein Data Bank; RP-HPLC: Reversed-phase high-performance liquid chromatography; SF: Solvent-fraction; TPC: Total phenolic contents

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## Authors' contributions

MZM and FK conceived the study. MH, SJ, and AM helped to design and coordinate the study. FK and MZM carried out the experimental studies. SJ and AM gave input throughout the study. MZM, MH, and FK analyzed the data. MZM and MH wrote, organized, and revised the manuscript. All authors have read and approved the final manuscript.

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## Availability of data and materials

All data generated or analyzed during this study are included in this published article.

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

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## Competing interests

No potential conflict of interest was reported by the author(s).

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