Bull. Chem. Soc. Ethiop. **2022**, 36(3), 651-666.
DOI: https://dx.doi.org/10.4314/bcse.v36i3.14

ISSN 1011-3924
Printed in Ethiopia
Online ISSN 1726-801X

ANTIOXIDANT AND CYTOTOXIC ACTIVITIES OF DIFFERENT SOLVENT FRACTIONS FROM *MURRAYA KOENIGII* SHOOTS: HPLC QUANTIFICATION AND MOLECULAR DOCKING OF IDENTIFIED PHENOLICS WITH ANTI-APOPTOTIC PROTEINS

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Received March 29, 2022; Revised May 19, 2022; Accepted June 2, 2022

ABSTRACT. *Murraya koenigii* is known for its health benefits against constipation, diarrhea, bacterial infections, wounds and skin related diseases. Aim of this project is to determine cytotoxic aptitude of antioxidant compounds present in *M. koenigii*. The fractionation of *M. koenigii* shoots methanol extract was carried out with different solvents followed by determination of total phenolic content, radical scavenging potential along with phenolic profile. *M. koenigii* shoot fractions were analyzed for their cytotoxic potential by MTT assay besides evaluating molecular interactions between identified phenolics with Bcl-2, Bcl-xl and MCL-1. The results revealed that butanol fraction contains maximum amount of quercetin, 4-hydroxy-3-methoxy benzoic acid and trans-4-hydroxy-3-methoxy cinnamic acid. Ferulic acid is abundant in water fraction whereas *n*-hexane fractions contain sinapic and vanillic acids. The ethyl acetate fraction possess the highest level of phenolics as well as radical scavenging potential. HPLC results show that 9 organic acids are present in ethyl acetate and butanol fractions. The highest cytotoxic activity was exhibited by *n*-hexane and ethyl acetate fractions. Molecular docking studies supports that ethyl acetate and *n*-hexane fractions are the major sources of antioxidant and cytotoxic compounds. Also, molecular interactions exist between identified phenolics from plant shoots fractions with anti-apoptotic proteins Bcl-2, Bcl-xl and MCL-1.

KEY WORDS: *Morraya koenigii*, Fractionation, Antioxidant, Cytotoxic, Molecular docking

INTRODUCTION

One of the biggest challenges in the field of drug development is to treat different types of tumors and cancers which may develop within the human body. The death rate is increasing day by day due to different cancer related health effects. An estimated projection of deaths due to cancer may reach up to the alarming figure of 13 million by 2030 [1, 2]. The development and progression of cancer depends largely on the generation and interaction of free radical species as well as reactive oxygen/nitrogen species within the human body. The radicals which are free to move along with reactive species can cause deterioration of biomolecules and may induce a state of oxidative stress inside the human body which results in the development of cancer along with several other related diseases which include but are not limited to atherosclerosis, rheumatoid arthritis, aging and neuro-degenerative disorders [3, 4]. The frequent intake of antioxidant supplements may help in minimizing the risk of cancer development and progression within the human body [5]. According to several studies, the antioxidants are considered as cytotoxic in nature depending upon their mode of action which may involve antioxidant enzymatic activities [5]. Also, there are several micronutrients which are present in the daily diet of human beings in the form of vitamins and

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minerals which have the purpose of maintaining the antioxidant potential above a certain threshold level within the human body so that the body may combat with carcinogenic agents. The antioxidant abilities of compounds, therefore, can directly be attributed to their positive anticancer and cytotoxic potential [6]. Although certain synthetic compounds are being used as antioxidant supplements in several countries, some of the developed nations have refrained their citizens from using them on the basis of scientific studies which reveal the carcinogenicity of those synthesized compounds [7]. Such restrictions associated with the use of synthetic antioxidant compounds have motivated the researchers and food scientists to explore potentially safe, effective and sustainable sources of natural antioxidants and cytotoxic agents. Plants have been recognized as one of the richest source of natural antioxidants and chemo-preventive agents such as tannins, phenolics, xanthones, flavonoids, coumarins, curcuminoids and terpenoids [8].

The focus of research in the field of drug development is particularly on the identification of cytotoxic agents which may come from botanical origin [9, 10]. Although several studies have confirmed cytotoxic potential of a compound isolated from plant material, however, there is an increasing trend towards either the use of whole plant extract or its solvent fractions as medication against cancer. This practice can be attributed to the possible synergistic effect of multiple secondary metabolites which can lead towards effective chemoprevention [11-13].

Cytotoxic activity is characterized by the introduction of apoptosis that is a requirement for the control of cancer. Therefore, attention should be diverted towards screening of inducers of apoptosis originating from plants either as isolated pure compounds, enriched fractions or crude extracts [14, 15]. Apoptosis is known for causing morphology-based changes including chromatin condensation, membrane blabbing, shrinking of cells, DNA fragmentation and apoptotic bodies formation [16]. The Bcl-2 family of proteins determines the fate of cell; whether the cell survives or dies as they control the ejection of mitochondrial apoptogenic factors related to death protease known as caspases [17]. Based on protein function and structure, the subdivision of Bcl-2 protein family can be achieved as pro-apoptotic and anti-apoptotic proteins [18]. Pro-apoptotic proteins (BAX like proteins) are death factors. On activation, they oligomerize with mitochondria and increase mitochondrial permeabilization causing cell death, while the survival agents comprise of anti-apoptotic proteins like Bcl-2, Bcl-xl and MCL-1. The formation of heterodimers with BAX/BAK is reported which results in inhibition of their action and leads towards cell survival [19].

M. koenigii, (curry leaf or kari patta) in Subcontinent, is a member of family Rutaceae. It is distributed in South East Asia and Australia. It is a highly valuable plant for its characteristic aroma and medicinal value and has been widely used in several ancient systems of medicine for therapeutic applications including the treatment of bronchial abnormalities, piles, vomiting and skin infections. The medicinal values of this plant have been reported mainly due to its leaves, stem, bark and oil [20].

This research is designed to investigate antioxidant and cytotoxic ability of different solvent fractions obtained from *M. koenigii* shoots. The selection of shoots extract for this study is based on the possible presence of cytotoxic alkaloids [21] which may lead towards cytotoxic effect of shoots extract of *M. koenigii.* Also, the root extract of this plant has already been explored for its biological potential [21, 22].

A common technique usually employed in such type of plant investigations is the extraction procedure which is a time-tested mechanism to isolate bulk of organic compounds from a plant. On one side, as there is a possibility of combined positive effect of isolated compounds from plant material, the extract of whole plant can still be dangerous and may pose serious health related problems due to the presence of compounds containing contrasting characteristics coming in the extract from varying parts of a plant such as roots and shoots. Different plant parts evolve to carry out different functions for a plant and, therefore, contain different classes of compounds which do not mix with each other during the life span of a plant. Combining all these compounds from a complex plant coming from various plant parts may result on their unwanted combined effects

due to possible non-compatibility issues and may lead towards further health risks of serious nature instead of cure. Therefore, a good approach should be to extract compounds from different plant parts separately and test them separately as whole extracts in relevant bioassays in order to search for the desired compounds against selected targets.

Furthermore, identification and quantification of phenolic acids by HPLC from these fractions along with molecular docking of identified phenolics with anti-apoptotic proteins for the confirmation of cellular apoptosis is described for the first time in this study. Neither these compounds nor the described activities have previously been explored for the shoots extract of *M. koenigii*. The promising results obtained in case of this easily accessible plant species fills the gap in literature for those scientists who are looking towards new sources of lead compounds from plant origin in order to optimize low-cost anticancer drugs.

EXPERIMENTAL

Chemicals and reagents

The solvents used (ethyl acetate, chloroform, butanol, methanol and *n*-hexane) were of HPLC grade and purchased from Deajeng, Korea. Folin-Ciocalteu, sodium carbonate, DPPH and 2,2'-azino-*bis*-3-ethylbenzothiazoline-6-sulfonic acid were purchased from Merck, Germany whereas DMEM (Dulbecco modified eagle medium) and FBS (Fetal Bovine serum) were purchased from GIBCO Invitrogen, USA. Antibiotics and L-Glutamine were purchased from Invitrogen, USA, MTT reagent (3-(4,5-dimthylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide was obtained from MB cell, Korea. The buffer used for extraction, 96 well plates as well as flasks used for culturing were obtained from Oxygen Life Sciences, California. *Hela* cancer cell line and sodium dodecyl sulfate were purchased from Vivantis, USA.

Extraction scheme

The fresh shoots of *M. koenigii* were collected from soon valley, Punjab, a valley that is famous due to its herbal flora and subjected to a known extraction scheme [23] with slight modifications. After collection, the shoots sample was allowed to dry in shade. Later, this was subjected to grinding in order to obtain fine powder prior to extraction. The grinding of the shoots was carried out by using mortar and pestle. No mechanical or electrical equipment was used for grinding the heat generated from which could disrupt the compounds present in the plant. Briefly, 400 g of ground shoot material was soaked in 80% methanol and subjected to shaking for 6 h in orbital shaker at 200 rpm. The use of methanol in high percentage was necessary to isolate polar organic compounds from plant material and the extensive shaking at low rpm would ensure maximum extraction of the desired polar organic constituents from plant into the solvent. The filtration of extract was followed by drying on a rotary evaporator (Buchi, R-300) under reduced pressure at 35 °C. For fractionating the sample, 40 g crude extract was dispensed in 450 mL distilled water and solvent extraction was performed with 450 mL of *n*-hexane, chloroform, ethyl acetate and butanol, successively. The water fraction was obtained lastly. The process of solvent extraction was repeated thrice with the mentioned solvents. The volume of solvent used each time was 1.5 liter. All of the solvent extracted fractions were dried and lyophilized followed by their storage at –78 °C before using them for further analyses.

Determination of total phenolic content (TPC)

The TPC was quantified using Folin-Ciocalteu reagent assay. Required reaction mixture was prepared by mixing 100 µL of different fractions of *M. koenigii* shoots and 2.5 mL of freshly prepared 0.5 N Folin-Ciocalteu reagent. The prepared mixtures were left in dark for 15 min and

then 2 mL of 7.5% sodium carbonate was introduced. The resultant mix was placed for half an hour in dark. The absorbance measurement was carried out at 735 nm on a UV-Vis spectrophotometer. The TPC calculation as gallic acid equivalents was carried out following the preparation of standard calibration curve by running series of standard solutions.

DPPH scavenging activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity evaluation had been achieved by using reported method with few modifications [24]. Briefly, diluted methanol *M. koenigii* shoot fractions (about 2.5 mL) were added to a volume of 1.0 mL of 0.3 mM solution of DPPH. Vigorous shaking of the resultant mix was performed followed by placing it for half an hour in dark. All of the flasks were covered with aluminum foil in order to minimize the effect of foreign light on DPPH scavenging of the plant shoots extract. Absorbance measurement of the obtained solution was carried out at 517 nm. The blank solution used in this study consisted of pure methanol only. Standards such as BHT, BHA and ascorbic acid were employed for comparison purposes. The inhibitory percentage of DPPH radicals was determined by using the following formula:

$$\%\ Inhibition\ of\ DPPH = \frac{\text{Abs. of control} - \text{Abs. of sample}}{\text{Abs of control}} \times 100$$

$ABTS^+$ scavenging activity

The $ABTS^+$ (2, 2'-azino-*bis*-(3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging assay was carried out using a reported method [8]. The $ABTS^+$ radical cation was obtained in half an hour at room temperature and in dark by mixing 0.5 mM $ABTS^+$ water solution with manganese dioxide (MnO_2) which was used as an oxidizing reagent. Buffer used for this purpose was phosphate buffer (0.1 M, pH 7.0). The general procedure for preparation of phosphate buffer is to add 20.214 g of sodium phosphate dibasic heptahydrate and 3.394 g of sodium phosphate monobasic monohydrate in 800 mL of distilled water in a beaker. The desired pH should be adjusted by adding suitable volume of either HCl or NaOH solution and making the final volume up to 1 L by distilled water. The dilution of obtained $ABTS^+$ solution with ethanol was carried out until the absorbance of 0.7 ± 2 at 734 nm was achieved. Briefly, diluted solution of *M. koenigii* shoot fractions (0.1 mL) was mixed with 4.0 mL of $ABTS^+$ solution and absorbance monitored at 734 nm by using UV-*Vis* spectrophotometer. The $ABTS^+$ solution was used as reference solution whereas BHA, BHT and ascorbic acid were the standard chemicals used for comparison. The percent inhibition of $ABTS^+$ was assessed with following formula:

$$\%\ Inhibition\ of\ ABTS = \frac{\text{Abs. of control} - \text{Abs. of sample}}{\text{Abs of control}} \times 100$$

Antiproliferative activity (MTT assay)

Percent cell viability of carcinoma cell was measured by using MTT assay [2]. *Hela* cells about 5000 cells in DMEM medium were incubated at 310 K temperature in 96 well plate and in the presence of 5% carbon dioxide. The pH was maintained at 7.4 and humidity was 90%. The dissolved fractions were added in wells at concentrations of 26 µg, 52 µg, 104 µg, 208 µg and 416 µg, respectively. The incubation process of all cells at 310 K temperature was carried out for 24 hrs. A volume of 200 µL of each dose was introduced in all of the wells excluding blank as well as control. On completion of incubation period, a total volume of 25 µL of MTT solution was introduced to all wells. Again, the plates were incubated for 120 min at 310 K temperature. After drying the above protocol mix, 100 µL of buffer used for extraction was introduced in all wells followed by incubation at 310 K temperature for two days. The O.D. was measured at λmax

of 570 nm. The blank solution consisted of the buffer alone. The %age relative cell viability was determined as

$$\%\ Relative\ cell\ viability = \frac{\text{A570 of control}}{\text{A570 of sample}} \times 100$$

Phenolic acid quantification by HPLC

Identification as well as quantification of the phenolics from *M. koenigii* shoot fractions by HPLC (Aligent HPLC system) was carried out [25]. Briefly, 30 mg of all extracts were dissolved in 5 mL of 6 M hydrochloric acid. Next, addition of 12 mL methanol was carried out followed by 8 mL of distilled water. The obtained mix incubation was performed at 90 °C for 120 min followed by filtration using 0.2 mm millipore membrane filter prior to injecting the sample in HPLC system.

The HPLC analysis was carried out with the help of HPLC system with column 20 RBAX ECLIPSE, XDB-C18, (4.6 × 150 mm; 5 µm, Agilent USA), UV-*Vis* Spectra-Focus detector and injector-auto sampler. The tetrahydrofuran/acetonitrile/0.05% phosphoric acid solution (20:3:77, v/v/v) was utilized as mobile phase in isocratic elution and at a final flow rate of 1 mL/min. The filtration of mobile phase by using 0.2 mm millipore membrane filter and its degassing with a sonicator was performed before use. The UV-*Vis* detector was tuned at 280 nm and the column temperature was set at 37 °C. The volume of injected sample was 10 µL.

Molecular docking

For molecular docking studies, X-ray crystallography determined structures of anti-apoptotic proteins such as Bcl-2, Bcl-xl and MCL-1 were obtained from RCSB protein data bank with their corresponding PDB ID's 2W3L [26], 2YXJ [27] and 3KZ0, respectively [28]. All the structures were in high resolution of 2.10 Å, 2.22 Å and 2.35 Å, respectively. The binding site residues of respective anti-apoptotic proteins were investigated carefully through their bound inhibitors in crystal structures and further validated from CASTp server (Computed Atlas of Surface Topography of proteins) (http://sts.bioe.uic.edu/castp/). The potential phenolics in this study were retrieved from Pubchem in SDF format and converted into 3D Mol format with the help of ChemOffice 2012 (Cambridge Soft Corporation, Cambridge, MA, USA) [29]. In order to conduct molecular docking, removal of heteroatoms was carried out and hydrogens and charges were added. General purpose semi-empirical molecular orbital package ver.2012 was used for the energy minimization procedure for both ligands and proteins. Grids on respective crystal structures made with a dimension of 30 Å × 30 Å × 30 Å were centered covering the hydrophobic groove of anti-apoptotic proteins. Molecular docking simulations were performed using AutoDockVina (Scripps Research Institute, La Jolla, CA, USA). Dell Inspiron Core i7 workstation with Ubuntu 12 operating system was used to run AD Vina setup. Protein complex analysis of the identified phenolics was performed with the help of Ligplot and UCSF Chimera 1.10 [30, 31].

In silico drug likeness and ADMET properties

The drug likeness of the identified compounds was determined with the help of Lipinski's rule of five (RO5) [32]. Mcule and molinspiration server were used for calculation of their physicochemical properties. The ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) studies of identified phenolics were examined for their ADMET threats through blood, body fluids, tissues and excretory material of the body. The OSIRIS property explorer (http://www.organic-chemistry.org/prog/peo/) and ADMET SAR (http://1mmd.ecust.edu.cn:8000/) servers were used for ADMET assessment [33].

RESULTS AND DISCUSSION

Total phenolic contents

Figure 1 shows extracted yield of total phenolics in different fraction of *M. koenigii* shoots. According to the data obtained, the quantity of total phenolics present in different fractions decrease in the order, ethyl acetate > butanol > chloroform > water > *n*-hexane. The range of phenolics in terms of mg/GAE equivalent content is from a little over 100 mg in *n*-hexane fraction to almost 300 mg in ethyl acetate fraction. Therefore, the ethyl acetate fraction can be rendered as the potential candidate for cytosolic action of the plant's shoot extract. According to literature also, highest quantity of phenolic acids is normally present in ethyl acetate fraction [34, 35].

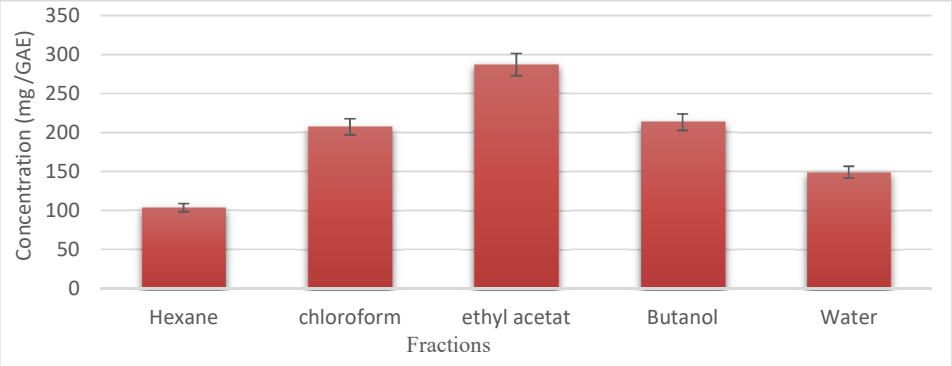

Figure 1. Total phenolic contents from all fractions of *Morraya koenigii* shoots fractions.

DPPH scavenging activity

Comparison of antioxidant activities among different fraction from *M. koenigii* shoots is shown in Figure 2. Three standard compounds namely ascorbic acid, BHT and BHA were used as positive controls for comparison purposes. A varying trend of DPPH %age inhibition of different fractions in the order of ethyl acetate > chloroform > butanol > *n*-hexane > water was noted while standers showed trend as ascorbic acid > BHA > BHT. It can be seen from the data of Figure 2 that DPPH percentage inhibition of fractions are relatively ($p < 0.05$) lower than that of ascorbic acid, BHA and BHT. However, the inhibition percentage of ethyl acetate fraction is comparable to standards. Chloroform as well as butanol fractions are also showing considerable inhibition percentage. The ethyl acetate fraction being highest scavenging ability possessing fraction may contain good quantity of cytotoxic contents as compared to rest of the fractions, *n*-hexane being the least DPPH scavenger.

$ABTS^+$ scavenging activity

Figure 3 shows $ABTS^+$ radical scavenging ability of different fractions of *M. koenigii* shoots extracts as %age inhibition of $ABTS^+$. Maximum %age inhibition of $ABTS^+$ among different fractions was observed in ethyl acetate fraction. It was comparable to the standards used (ascorbic acid, BHA and BHT) closely followed by water fraction. Chloroform, butanol and *n*-hexane fractions showed relatively lower %age inhibition of $ABTS^+$ than ascorbic acid, BHA and BHT which were used as positive standards. A similar trend in case of $ABTS^+$ scavenging assay has been observed to that of DPPH scavenging assay. This further confirms the findings and strengthens the fact that the ethyl acetate fraction holds good free radical scavenging potential.

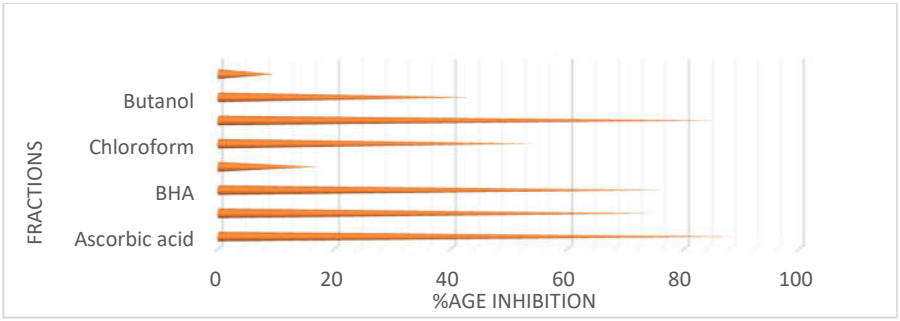

Figure 2. % DPPH inhibition of solvent fractions from *Morraya koenigii* shoots extracts.

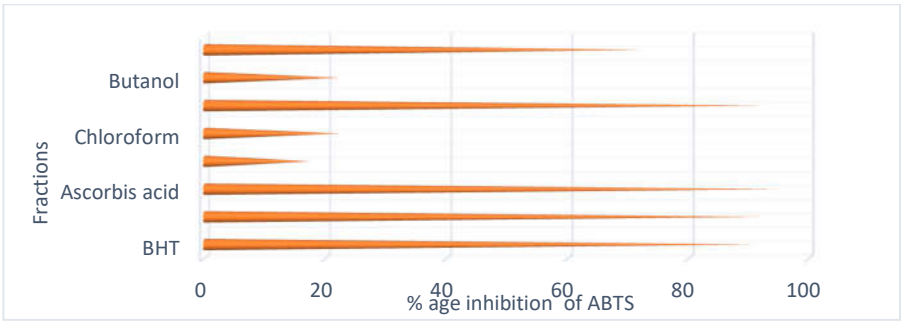

Figure 3. % ABTS inhibition from different solvent fraction of *Morraya koenigii* shoots.

Quantification of phenolic acids from M. koenigii fractions using HPLC

The HPLC identification as well as quantification of 11 different phenolics from *M. koenigii* shoots fractions was carried out (Table 1). The four phenolic compounds present in higher percentage were *trans*-4-hydroxy 3-methoxy cinnamic acid, quercetin, gallic acid and 4-hydroxy-3-methoxy benzoic acid (Figure 4). The fraction extracted with *n*-hexane consisted of eight phenolic compounds including quercetin (0.13 ± 0.035 µg/g), gallic acid (0.64 ± 0.03 µg/g), ferulic acid (2.56 ± 0.04 µg/g), sinapic acid (0.80 ± 0.055 µg/g), vanillic acid (0.52 ± 0.03µg/g), 4-hydroxy-3-methoxy benzoic acid (2.46 ± 0.035 µg/g), *p*-coumaric acid (0.44 ± 0.04 µg/g) and syringic acid (0.36 ± 0.035 µg/g). The highest amounts of phenolics found in this fraction were of ferulic acid and 4-hydroxy-3-methoxy benzoic acid. Chloroform fraction contained only two phenolics. The one with minor quantity was quercetin (0.15 ± 0.035 µg/g) and other with major quantity was gallic acid (0.69 ±0.045 µg/g). The ethyl acetate fraction contained quercetin (0.17 ± 0.025 µg/g), gallic acid (1.61 ± 0.035 µg/g), *p*-coumaric acid (0.50 ±0.05 µg/g), *trans*-4-hydroxy-3-methoxy cinnamic acid (0.96 ± 0.035 µg/g) and caffeic acid (1.39 ± 0.01 µg/g) in small quantities. However, the main ingredient of this fraction was 4-hydroxy-3-methoxy benzoic acid (6.38 ± 0.02 µg/g). Butanol fraction revealed the presence of small amount of quercetin (0.40 ± 0.055 µg/g) whereas gallic acid (6.0 ± 0.055 µg/g) and vanillic acid (7.27 ± 0.025 µg/g) were present in considerable amounts in butanol fraction. However, the butanol fraction also contained 4-hydroxy-3-methoxy benzoic acid & *trans*-4-hydroxy 3-methoxy cinnamic acid as (24.02 ± 0.08

µg/g and 23.97 ± 0.030 µg/g), respectively. These two acids majorly constitute the butanol fraction of *M. koenigii* shoots extracts. The water fraction showed the presence of quercetin (0.33 ± 0.030 µg/g), gallic acid (3.36 ± 0.06 µg/g), ferulic acid (7.45 ± 0.045 µg/g), 4-hydroxy-3-methoxy benzoic acid (19.19 ± 0.19 µg/g), *p*-coumaric acid (0.39 ± 0.05 µg/g), *m*-coumaric acid (0.47 ± 0.05 µg/g), *trans*-4-hydroxy 3-methoxy cinnamic acid (1.045 ± 0.045 µg/g) and caffeic acid (4.48 ±0.02 µg/g). Among all of the above in water fraction, as is evident from the results, 4-hydroxy-3-methoxy benzoic acid was the main ingredient. All tested *M. koenigii* shoots fractions contained quercetin (0.40 ± 0.055 µg/g), gallic acid (3.36 ± 0.06 µg/g), ferulic acid (7.45 ± 0.045 µg/g), vanillic acid (7.27 ± 0.025 µg/g), 4-hydroxy-3-methoxy benzoic acid (24.02 ± 0.08 µg/g), *p*-coumaric acid (0.50 ± 0.05 µg/g), *m*-coumaric acid (0.47 ± 0.05 µg/g), *trans*-4-hydroxy-3-methoxycinnamic acid (23.97 ± 0.030 µg/g), caffeic acid (4.48 ± 0.02 µg/g) and syringic acid (0.36 ± 0.035 µg/g), respectively.

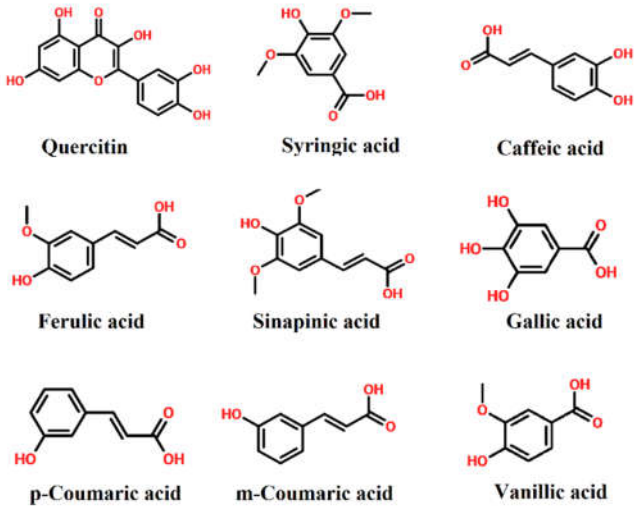

Figure 4. Identified phenolics from *M. koenigii* shoots fractions in two-dimensional structures. The 4-hydroxy-3-methoxy benzoic acid and *trans*-4-hydroxy 3-methoxy cinnamic acid are IUPAC names of vanillic acid and ferulic acid.

Antiproliferative activity (MTT assay)

Figure 5 shows that incubation of *Hela* cancer cells with different fractions obtained from *M. koenigii* shoots have significantly inhibited the cell's proliferation in a dose dependent manner over a dose rang of 26 - 416 µg. Figure 5 reveals that the *n*-hexane fraction showed lowest % cell viability (highest cytotoxic activity) compared to other fractions. The remaining decreasing order can be presented as chloroform > butanol > ethyl acetate > water. The *n*-hexane fraction, at different doses, resulted % cell viability as 26 µg (95.87 ± 0.40), 54 µg (83.00 ± 0.50), 104 µg (77.95 ± 0.50), 208 µg (68.51 ± 0.49) and 416 µg (53.43 ± 0.33), respectively. At same dose levels, other fraction resulted different % cell viability trends as chloroform (94.85 ± 0.48, 83.75 ± 0.55, 68.81 ± 0.35, 62.03 ± 0.39, 54.09 ± 0.34), butanol (89.55 ± 0.47, 82.97 ± 0.37, 79.62 ± 0.36, 71.83 ± 0.37, 71.83± 0.37), ethyl acetate (89.55 ± 0.47, 82.97 ± 0.37, 79.62 ± 0.36, 71.83 ± 0.37, 64.71 ± 0.25) and water (90.52 ± 0.35, 87.06 ± 0.36, 81.26 ± 0.37, 77.74 ± 0.36, 69.80 ± 0.37), respectively. Early dose showed no remarkable difference among % cell viability while at dose of

416 µg, the *n*-hexane fraction showed lowest % cell viability (highest cytotoxic activity) than other fractions. In agreement with results of this study, literature [36] also reports highest inhibitory effect on growth of HT-29 cells with *n*-hexane fraction from *Doenjang* (a natural fermented Korean soy paste). Similar findings were also reported in another study [37] where indication of highest HT-18 cancer cell inhibition was reported by *n*-hexane fraction of *Manda Enzyme*. Meanwhile, the *n*-hexane fraction from coconut peel is reported to possess the highest antiproliferative activity against KB cell line [2]. According to another study [38], two non-polar fractions (*n*-hexane and chloroform) of *Lawsonia inermis* are reported to present 60% growth inhibition in *Hela*, MCF-7, A-549 and C6gloima cells. Also, the *n-h*exane fraction from seeds of *Nigella sativa* was reported to be active against A-549 lung carcinoma and DLD-1 colon carcinoma with IC_{50} values of 31.0 and 63.0 µg/mL, respectively [39].

Table 1. Phenolic compounds in *Morraya koenigii* shoot fractions analyzed by HPLC.

Phenolic compounds	Fraction from *n*-Hexane (µg/g)	Fraction from chloroform (µg/g)	Fraction from ethyl acetate (µg/g)	Fraction from butanol (µg/g)	Fraction from water (µg/g)
Quercetin	0.13±0.035	0.15±0.035	0.17±0.025	0.40±0.055	0.33±0.030
Gallic acid	0.64±0.03	0.69±0.045	1.61±0.035	6.0±0.055	3.36±0.06
Ferulic acid	2.56±004	Nil	Nil	Nil	7.45±0.03
Sinapic acid	0.80±0.055	Nil	Nil	Nil	Nil
Vanillic acid	0.52±0.03	Nil	Nil	7.27±0.025	Nil
4-Hydroxy-3-methoxy benzoic acid	2.46±0.035	Nil	6.38±0.02	24.02±0.08	19.19±0.19
p-Coumaric acid	0.44±0.04	Nil	0.50±0.05	Nil	0.39±0.05
m-Coumaric acid	Nil	Nil	-	Nil	0.47±0.05
trans-4-Hydroxy 3-methoxy cinnamic acid	Nil	Nil	0.96±0.035	23.97±0.030	1.045±0.045
Caffeic acid	Nil	Nil	1.39±0.01	Nil	4.48±0.02
Syringic acid	0.36±0.035	Nil	Nil	Nil	Nil

Molecular docking studies

Molecular docking analysis was carried out on identified phenolics from *M. koenigii* shoots fractions and anti-apoptotic proteins, Bcl-2, Bcl-xl and MCL-1. All identified phenolics were checked on the basis of Lipinskis RO5 and physicochemical properties in order to assess the drug likeness of contents of *M. koenigii* shoots fractions (Table 2).

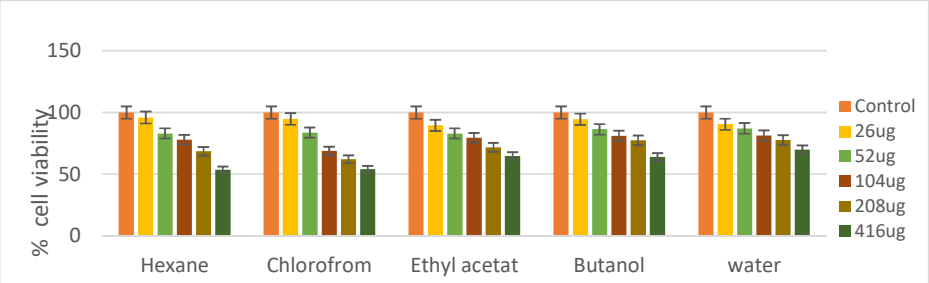

Figure 5. % cell viability of different solvent fractions from M. koenigii shoots against Hela cancer cell line at different doses of fractions.

Table 2. Physicochemical properties of identified phenolics.

Identified phenolics	Mass	Log p	HBA	HBD	Rot. Bond	PSA	RO5 violation	Refractivity	Atoms	Heavy atoms	H- Bond
Cafiec acid	180.157	1.1956	4	3	2	77.76	0	47.1578	21	13	8
Ferulic acid	194.183	1.4986	4	2	3	66.76	0	51.6268	24	14	10
Gallic acid	170.119	0.5016	5	4	1	97.99	0	39.4703	18	12	6
m-Coumaric acid	164.157	1.49	3	2	2	57.53	0	45.1348	20	12	8
p-Coumaric acid	164.157	1.49	3	2	2	57.53	0	45.1348	20	12	8
Quercitin	302.235	1.988	7	5	1	131.36	0	78.035	32	22	10
Sinapic acid	224.209	1.5072	5	2	4	75.99	0	58.1188	28	16	12
Syringic acid	198.172	1.1076	5	2	3	75.99	0	48.4083	24	14	10
Vanillic acid	168.146	1.099	4	2	2	66.76	0	41.9163	20	12	8

After molecular docking procedures using AD Vina, the conformations of ligands were categorized with respect to their binding ability with anti-apoptotic proteins. The results of the analysis were analyzed by keeping into consideration lowest energy of binding, number of H-bonds and hydrophobic interactions among each protein-ligand complex. The phenolics identified in this study from *M. koenigii* shoots fractions confirmed good binding affinity with all anti-apoptotic proteins under investigation.

Molecular docking of phenolics with apoptotic proteins proved the cytotoxic potential of *n*-hexane fraction which can be attributed to the presence of quercitin as identified by HPLC in the *n-h*exane fraction. Among all identified phenolics by HPLC, quercitin showed most promising binding energies when docked with Bcl-2, Bcl-xl and MCL-1 with binding energy values of -7.6, -7.8 and -7.6, respectively. Literature [40] reports the apoptotic potential of quercetin in nuroblastoma mouse cell line. Quercetin was also used with resveratrol for synergistic effect on HT-29 colon cancer cell line [41]. Further reports [42] present the comparison of cytotoxic activity between quercetin and its water soluble sulfated derivative on human colon cancer Lovo cell and breast cancer MCF-7 cells. Also, [43] another research on apoptotic potential of quercetin by activating caspase-3 and regulating Bcl-2 and cyclooxygenase-2 pathways in human HL-60 cells presents the importance of quercetin. All these literature reports are in direct agreement with the results obtained by the current study.

Docking results with Bcl-2, Bcl-xl and MCL-1

All phenolics docked perfectly with the binding site residues making the hydrophobic groove as determined by X-ray co-crystallized structures and predicted from CASTp server showed strong binding energies (-7.6 to -5.6 kcal/mol) (Figure 6). The docking simulations of phenolics for Bcl-2 showed the presence of one H-bond with quercitin, caffeic acid and ferulic acid with a binding energy of -7.6, -7.1, -5.8 and -5.9 Kcal/mol and H-bond lengths of 2.67 Å, 2.94 Å, 2.92 Å and 2.87 Å, respectively. These phenolics were also assumed to be taking part in hydrophobic interactions. Similar results were investigated in the docking simulations with Bcl-xl where all phenolics showed promising binding energies (-7.8 to -5.2 kcal/mol). All phenolics except quercitin and caffeic acid showed one or two H-bonds and made several hydrophobic interactions. Gallic, *m*-coumaric and sinapic acids showed two H-bonds each with a molecular bond distance between 2.70 Å and 3.21 Å. Identified phenolics with MCL-1 had good binding energies from

-7.8 to -5.6 kcal/mol and form one H-bond with gallic, ferulic and vanillic acids possessing bond lengths of 3.16 Å, 2.71 Å, 2.89 Å and 2.79 Å. Likewise, Bcl-2, Bcl-xl and MCL-1 were also observed forming number of hydrophobic interactions (Table 3). Among all phenolics, quercitin showed most promising binding energies when docked with Bcl-2, Bcl-xl and MCL-1 with binding energies -7.6, -7.8 and -7.6 kcal/mol, respectively. The docking poses of quercitin with all three anti-apoptotic proteins are shown in Figure 6 (A, C, E).

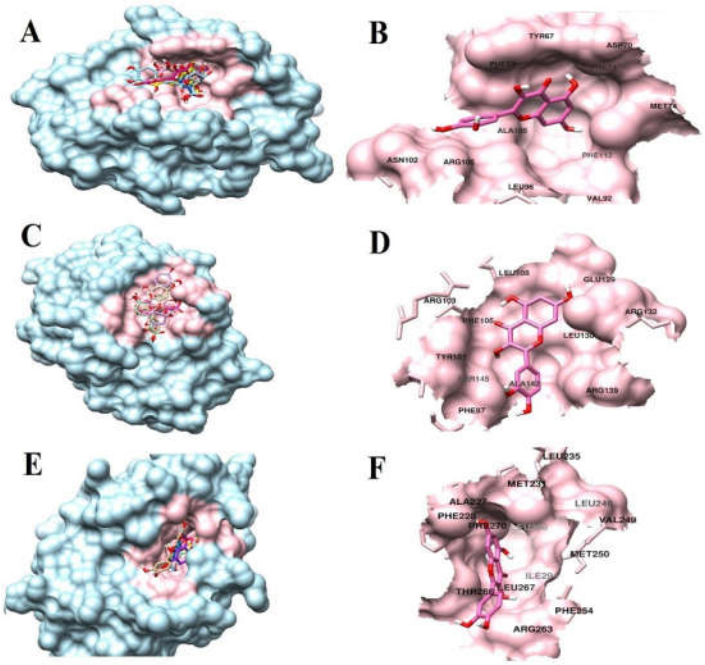

Figure 6. Molecular surface representation of hydrophobic groove of Bcl-2, Bcl-xl and MCL1 with all docked identified phenolics in stick representation (Figure 1a, 1c and 1e). Conformation of best phenolic (quercitin in sticks) inside the binding pocket of Bcl-2 (b), Bcl-xl (d) and MCL-1 (f). Binding residues molecular surface are colored in pink and labeled black.

Predictions of ADMET profile

The ADMET analysis was performed by subjecting the phenolics to OSIRIS property explorer and admetSAR servers. These servers predicted the ADMET properties such as blood brain barrier (BBB) penetration [44], human intestine absorption (HIA), aqueous solubility [45] and CYP450 inhibition [46]. Analysis of ADMET properties is a crucial step in drug designing [47]. The ADMET profile of identified phenolics in *M. koenigii* shoots fractions are shown (Table 4). All phenolics showed very promising absorption through BBB except a few. All compounds showed positive results for HIA and similar results were seen in Caco-2 permeability expect quercitin and gallic acid. For P-gp substrate, only quercitin was proven as a substrate whereas all other phenolics were non-inhibitors for P-gp inhibitor. The most important phases in drug designing are the drug metabolism as well as CYP450. Various CYP450 substrates and CYP450 inhibitor models were calculated during ADMET prediction of fractions obtained from *M. koenigii* shoots extractions.

All phenolics were in non-substrate form for all CYP450 enzymes and same results were obtained with CYP450 inhibitors where all phenolics were non-inhibitors for all CYP450 enzymes except quercitin which was an inhibitor for CYP450 1A2 and 3A4 enzymes. The most interesting result was that, all phenolics revealed low CYP inhibitory proximity as these compounds were non-inhibitors for all CYP450 inhibitors except quercitin. Also, a lead compound must also have promising PD properties; that is, a compound must also not show toxicity threats upon consumption [48]. In the current study, identified phenolics showed favorable result in MTT assay and revealed as very promising cytotoxic compounds. When the phenolics were analyzed *in silico* for toxicity risks, all compounds were negative for AMES toxicity and showed non-carcinogenic and had no substantial toxicity threats that can be harmful for humans.

Table 3. Binding interactions of phenolics with Bcl-2, Bcl-xl and MCL-1.

Antiapoptotic proteins	Docking analysis	Quercitin	Gallic acid	Syringic acid	m-Coum aric acid	Caffeic Acid	*p*-Coum aric acid	Ferulic acid	Sinapinic acid	Vanillic acid
BCL-2	Docking energy	-7.6	-6.4	-6.3	-6.2	-5.8	-5.9	-5.9	-5.8	-5.6
	H-Bonds	Ala108 (2.67Å)	0	0	0	Tyr67(2.92Å)	0	Tyr67(2.87Å)	0	0
	Hydrophobic bonds	8	9	9	5	4	5	5	9	9
BCL-XL	Docking energy	-7.8	-6.4	-6.4	-6.2	-6.1	-5.9	-5.9	-5.8	-5.2
	H-Bonds	0	Tyr101(3.11Å) Glu129(3.10Å)	Ser145(2.71Å)	Ser145(2.70Å) Ser145 (2.90Å)	0	Glu129(3.01Å)	Gly138(3.12Å)	Ser145(2.70Å) Ser145 (2.98Å)	Ser145 (2.74Å)
	Hydrophobic bonds	10	3	8	6	7	6	6	8	7
MCL-1	Docking energy	-7.6	-6.5	-6.4	-7.2	-7.2	6.9	-7.4	-7.3	-6.5
	H-Bonds	0	Leu267 (2.71Å)	0	0	0	0	Arg263 (2.89Å)	0	Arg263 (2.79Å)
	Hydrophobic bonds	6	7	9	7	7	7	8	11	7

Table 4. ADMET predictions of identified phenolics.

ADMET Properties	Querci-tin	Gallic acid	Syringic acid	m-Coumaric acid	Caffeic Acid	p-Coumaric acid	Ferulic acid (trans-cinnamic acid)	Sinapinic acid	Vanillic acid
Blood-Brain Barrier	BBB-	BBB-	BBB+	BBB+	BBB-	BBB+	BBB-	BBB+	BBB-
Human Intestinal Absorption	HIA+	HIA+	HIA+	HIA+	HIA+	HIA+	HIA+	HIA+	HIA+
Caco-2 Permeability	Caco2-	Caco2-	Caco2+	Caco2+	Caco2+	Caco2+	Caco2+	Caco2+	Caco2+
P-glycoprotein Substrate	Yes	Not a substrate	Not a substrate	Not a substrate	Not a substrate	Not a substrate	Not a substrate	Not a substrate	Not a substrate

P-glycoprotein Inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor
Renal Organic Cation Transporter	Not a transporter	Not a transporter	Not a transporter	Not a transporter	Not a transporter	Not a transporter	Not a transporter	Not a transporter	Not a transporter
CYP450 2C9 Substrate	Not a substrate	Not a substrate	Not a substrate	Not a substrate	Not a substrate	Not a substrate	Not a substrate	Not a substrate	Not a substrate
CYP450 2D6 Substrate	Not a substrate	Not a substrate	Not a substrate	Not a substrate	Not a substrate	Not a substrate	Not a substrate	Not a substrate	Not a substrate
CYP450 3A4 Substrate	Not a substrate	Not a substrate	Not a substrate	Not a substrate	Not a substrate	Not a substrate	Not a substrate	Not a substrate	Not a substrate
CYP450 1A2 Inhibitor	Yes	Not an inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor
CYP450 2C9 Inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor
CYP450 2D6 Inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor
CYP450 2C19 Inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor
CYP450 3A4 Inhibitor	Yes	Not an inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor	Not an inhibitor
CYP Inhibitory Promiscuity	High CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity
Human Ether-a-go-go-Related Gene Inhibition	Inhibition is Weak	Inhibition is Weak	Inhibition is Weak	Inhibition is Weak	Inhibition is Weak	Inhibition is Weak	Inhibition is Weak	Inhibition is Weak	Inhibition is Weak
AMES Toxicity	Non AMES toxic	Non AMES toxic	Non AMES toxic	Non AMES toxic	Non AMES toxic	Non AMES toxic	Non AMES toxic	Non AMES toxic	Non AMES toxic
Carcinogens	Not a carcinogen	Not a carcinogen	Not a carcinogen	Not a carcinogen	Not a carcinogen	Not a carcinogen	Not a carcinogen	Not a carcinogen	Not a carcinogen
Fish Toxicity	High FHMT	High FHMT	High FHMT	High FHMT	High FHMT	High FHMT	High FHMT	High FHMT	High FHMT
Tetrahymena Pyriformis Toxicity	High TPT	High TPT	High TPT	High TPT	High TPT	High TPT	High TPT	High TPT	High TPT
Honey Bee Toxicity	High HBT	High HBT	High HBT	High HBT	High HBT	High HBT	High HBT	High HBT	High HBT
Biodegradation	Not ready bio	Ready bio	Ready biodegradable	Ready bio	Ready bio	Ready bio	Ready bio	Ready bio	Ready bio

	degradable	degradable		degradable	degradable	degradable	degradable	degradable	degradable
Acute Oral Toxicity	II	III	II	III	IV	III	Iv	III	III
Carcinogenicity (Three-class)	Non-required	Non-required	Non-required	Non-required	Non-required	Non-required	Non-required	Non-required	Non-required

CONCLUSION

Present study showed antioxidant and cytotoxic potential of *M. koenigii* shoot fractions. Overall, the ethyl acetate fraction showed maximum total phenolics content, maximum DPPH radical scavenging and $ABTS^{+}$ activity among other tested fractions. The *n*-hexane fraction proved highly cytotoxic in nature by showing minimum %cell viability than other fractions thereby proving that some non-polar compounds are responsible for *Hela* cell death from *M. koenigii* shoots fractions. The HPLC quantification showed that the ethyl acetate and butanol fractions are rich with phenolic acid contents. With the help of computational chemistry, the results of apoptosis induction in *Hela* cell line as experimentally determined by different *M. koenigii* solvent fractions were verified. It has been analyzed that molecular interactions exist between identified phenolics from *M. koenigii* shoots fractions with anti-apoptotic proteins Bcl-2, Bcl-xl and MCL-1. The results of molecular docking in current research suggest that promising binding energy, hydrogen bonding and hydrophobic interaction residues are potential inhibitor sites for Bcl-2, Bcl-xl and MCL-1 anti-apoptotic proteins and these results correlate with the carried out MTT assay thereby indicating that the expression of anti-apoptotic proteins was decreased.

REFERENCES

1. Dutt, R.; Garg, V.; Madan, A. Can plants growing in diverse hostile environments provide a vital source of anticancer drugs?. *Cancer Ther.* **2014**, 10, 13-37.
2. Khonkarn, R.; Okonogi, S.; Ampasavate, C.; Anuchapreeda, S. Investigation of fruit peel extracts as sources for compounds with antioxidant and antiproliferative activities against human cell lines. *Food Chem. Toxicol.* **2010**, 48, 2122-2129.
3. Rohman, A.; Riyanto, S.; Yuniarti, N.; Saputra, W.; Utami, R.; Mulatsih, W. Antioxidant activity, total phenolic, and total flavaonoid of extracts and fractions of red fruit (*Pandanus conoideus* Lam). *Int. Food Res. J.* **2010**, 17, 97-106.
4. Zahin, M.; Aqil, F.; Ahmad, I. The in vitro antioxidant activity and total phenolic content of four Indian medicinal plants. *Int. J. Pharm. Pharm. Sci.* **2009**, 1, 88-95.
5. Hu, M.-L. Dietary polyphenols as antioxidants and anticancer agents: Mmre questions than answers. *Chang Gung Med. J.* **2011**, 34, 449-460.
6. Wu, X.; Cheng, J.; Wang, X. Dietary antioxidants: Potential anticancer agents. *Nutr. Cancer* **2017**, 69, 521-533.
7. Fan, L.; Li, J.; Deng, K.; Ai, L. Effects of drying methods on the antioxidant activities of polysaccharides extracted from (*Ganoderma lucidum*). *Carbohydr. Polym.* **2012**, 87, 1849-1854.
8. Iqbal, S.; Bhanger, M.; Anwar, F. Antioxidant properties and components of bran extracts from selected wheat varieties commercially available in Pakistan. *Food Sci. Technol.* **2007**, 40, 361-367.
9. Kim, S.H.; Cui, C.B.; Kang, I.J.; Kim, S.Y.; Ham, S.S. Cytotoxic effect of buckwheat (*Fagopyrum esculentum* Moench) hull against cancer cells. *J. Med. Food* **2007**, 10, 232-238.

10. Hwang, J.H.; Kim, J.Y.; Cha, M.R.; Park, H.R. Effect of methanolic extract from silkworm droppings on proliferation and caspase activity in HT-29 human colon cancer cells. *J. Med. Food* **2007**, 10, 467-472.
11. Nobili, S.; Lippi, D.; Witort, E.; Donnini, M.; Bausi, L.; Mini, E.; Capaccioli, S. Natural compounds for cancer treatment and prevention. *Pharmacol. Res.* **2009**, 59, 365-378.
12. Russo, G.L. Ins and outs of dietary phytochemicals in cancer chemoprevention. *Biochem. Pharmacol.* **2007**, 74, 533-544.
13. Ju, H. K.; Lee, L.W.; Chung, K.S.; Choi, J.H.; Cho, J.G.; Baek, N.I.; Lee, K.T. Standardized flavonoid-rich fraction of *Artemisia princeps* Pampanini cv. Sajabal induces apoptosis via mitochondrial pathway in human cervical cancer Hela cells. *J. Ethnopharmacol.* **2012**, 141, 460-468.
14. Taraphdar, A.K.; Roy, M.; Bhattacharya, R. Natural products as inducers of apoptosis: Implication for cancer therapy and prevention. *Curr. Sci.* **2001**, 80, 1387-1396.
15. Ramasamy, S.; Wahab, N.A.; Abidin, N.Z.; Manickam, S. Effect of extracts from *Phyllanthus watsonii* Airy Shaw on cell apoptosis in cultured human breast cancer MCF-7 cells. *Exp. Toxicol. Pathol.* **2013**, 65, 341-349.
16. Wyllie, A.H. Apoptosis: An overview. *Br. Med. Bull.* **1997**, 53, 451-465.
17. Oltval, Z.N.; Milliman, C.L.; Korsmeyer, S.J. Bcl-2 heterodimerizes in vivo with a conserved homolog, bax, that accelerates programmed cell death. *Cell* **1993**, 74, 609-619.
18. Youle, R.J.; Strasser, A. The BCL-2 protein family: opposing activities that mediate cell death. *Nat. Rev. Mol. Cell Biol.* **2008**, 9, 47-59.
19. Sermeus, A.; Genin, M.; Maincent, A.; Fransolet, M.; Notte, A.; Leclere, L.; Michiels, C. Hypoxia-induced modulation of apoptosis and BCL-2 family proteins in different cancer cell types. *Plos One* **2012**, 7, 1-16.
20. Balasubramanian, S.; Dama, S.N.G.; Reddy, P.S. GC-MS analysis of the curry leaves (*Murraya koengii*). *Glob. J. Biol. Agric. Health Sci.* **2014**, 3, 8-10.
21. Lyer, D.; Devi, P.U. Plant review phyto-pharmacology of *Murraya koenigii* (L.). *Pharmacogn. Rev.* **2008**, 2, 180-184.
22. Manvi, M.; Renu, S. Antimicrobial efficacy of *Murraya koenigii* (Linn.) Spreng. root extracts. *Indian J. Nat. Prod. Resour.* **2011**, 2, 48-51.
23. Bukhari, S.M.; Feuerherm, A.J.; Boulfrad, F.; Zlatković, B.; Johansen, B.; Simić, N. Anti-inflammatory and antioxidant activities of *Sclerochloa dura* (Poaceae). *J. Serb. Chem. Soc.* **2014**, 79, 779-791.
24. Wijekoon, M.; Bhat, R.; Karim, A.A. Effect of extraction solvents on the phenolic compounds and antioxidant activities of bunga kantan (*Etlingera elatior* (Jack.) inflorescence. *J. Food Compost. Anal.* **2011**, 24, 615-619.
25. Rababah, T.M.; Ereifej, K.I.; Esoh, R.B.; Al-u'datt, M.H.; Alrababah, M.A.; Yang, W. Antioxidant activities, total phenolics and HPLC analyses of the phenolic compounds of extracts from common Mediterranean plants. *Nat. Prod. Res.* **2011**, 25, 596-605.
26. Porter, J.; Payne, A.; Candole, B.D.; Ford, D.; Hutchinson, B.; Trevitt, G.; Whitcombe, I. Tetrahydroisoquinoline amide substituted phenyl pyrazoles as selective Bcl-2 inhibitors. *Bioorg. Med. Chem. Lett.* **2009**, 19, 230-233.
27. Lee, C.; Chandrasekaran, V.; Duke, R.; Perera, L.; Pedersen, L. A proposed structural model of human protein Z. *J. Thromb. Haemost.* **2007**, 5, 1558-1561.
28. Dutta, S.; Gullá, S.; Chen, T.S.; Fire, E.; Grant, R.A.; Keating, A.E. Determinants of BH3 binding specificity for Mcl-1 versus Bcl-x L. *J. Mol. Biol.* **2010**, 398, 747-762.
29. Wang, Y.; Li, M.; Stadler, S.; Correll, S.; Li, P.; Wang, D.; Grigoryev, S.A. Histone hypercitrullination mediates chromatin decondensation and neutrophil extracellular trap formation. *J. Cell Biol.* **2009**, 184, 205-213.
30. Wallace, A.C.; Laskowski, R.A.; Thornton, J.M. LIGPLOT: A program to generate schematic diagrams of protein-ligand interactions. *Protein Eng.* **1995**, 8, 127-134.

31. Pettersen, E.F.; Goddard, T.D.; Huang, C.C.; Couch, G.S.; Greenblatt, D.M.; Meng, E.C.; Ferrin, T.E. UCSF Chimera-A visualization system for exploratory research and analysis. *J. Comput. Chem.* **2004**, 25, 1605-1612.
32. Lipinski, C.A. Lead-and drug-like compounds: The rule-of-five revolution. *Drug Discov. Today Technol.* **2004**, 1, 337-341.
33. Cheng, F.; Li, W.; Zhou, Y.; Shen, J.; Wu, Z.; Liu, G.; Tang, Y. admetSAR: A comprehensive source and free tool for assessment of chemical ADMET properties. *J. Chem. Inf. Model.* **2012**, 52, 3099-3105.
34. Prasad, K.N.; Hao, J.; Yi, C.; Zhang, D.; Qiu, S.; Jiang, Y.; Chen, F. Antioxidant and anticancer activities of wampee (*Clausena lansium* (Lour.) Skeels) peel. *BioMed Res. Int.* **2009**, 2009, 612805.
35. Nho, J.W.; Hwang, I.G.; Kim, H.Y.; Lee, Y.R.; Woo, K.S.; Hwang, B.Y.; Jeong, H.S. Free radical scavenging, angiotensin I-converting enzyme (ACE) inhibitory, and in vitro anticancer activities of ramie (*Boehmeria nivea*) leaves extracts. *Food Sci. Biotechnol.* **2010**, 19, 383-390.
36. Park, J.M.L.K.-Y.; Moon, S.H.; Jung, K.O. Inhibitory effect of doenjang (fermented Korean soy paste) extracts and linoleic acid on the growth of human cancer cell lines. *J. Food Sci. Nutr.* **2000**, 5, 114-118.
37. Kim, D.C.; Hwang, W.I. In vitro antioxidant and anticancer activities of extracts from a fermented food. *J. Food Biochem.* **2003**, 27, 449-459.
38. Kumar, M.; Kaur, P.; Kumar, S.; Kaur, S. Antiproliferative and apoptosis inducing effects of non-polar fractions from *Lawsonia inermis* L. in Cervical (HeLa) cancer cells. *Physiol. Mol. Biol. Plants* **2015**, 21, 249-260.
39. Bourgou, S.; Pichette, A.; Marzouk, B.; Legault, J. Antioxidant, anti-inflammatory, anticancer and antibacterial activities of extracts from *Nigella sativa* (black cumin) plant parts. *J. Food Biochem.* **2012**, 36, 539-546.
40. Priya, E.S.; Selvakumar, K.; Bavithra, S.; Elumalai, P.; Arunkumar, R.; Singh, P.R.; Arunakaran, J. Anti-cancer activity of quercetin in neuroblastoma: an in vitro approach. *Neurol. Sci.* **2014**, 35, 163-170.
41. Del, A. F.-M.; Banerjee, N.; Li, X.; Safe, S.; Mertens-Talcott, S. Resveratrol and quercetin in combination have anticancer activity in colon cancer cells and repress oncogenic microRNA-27a. *Nutr. Cancer* **2013**, 65, 494-504.
42. Zhang, H.; Zhang, M.; Yu, L.; Zhao, Y.; He, N.; Yang, X. Antitumor activities of quercetin and quercetin-5′,8-disulfonate in human colon and breast cancer cell lines. *Food Chem. Toxicol.* **2012**, 50, 1589-1599.
43. Niu, G.; Yin, S.; Xie, S.; Li, Y.; Nie, D.; Ma, L.; Wu, Y. Quercetin induces apoptosis by activating caspase-3 and regulating Bcl-2 and cyclooxygenase-2 pathways in human HL-60 cells. *Acta Biochim. Biophys. Sin.* **2011**, 43, 30-37.
44. Oldendorf, W.H. Lipid solubility and drug penetration of the blood brain barrier. *Exp. Biol. Med.* **1974**, 147, 813-816.
45. Cheng, A.; Merz, K.M. Prediction of aqueous solubility of a diverse set of compounds using quantitative structure-property relationships. *J. Med. Chem.* **2003**, 46, 3572-3580.
46. Susnow, R.G.; Dixon, S.L. Use of robust classification techniques for the prediction of human cytochrome P450 2D6 inhibition. *J. Chem. Inf. Comp. Sci.* **2003**, 43, 1308-1315.
47. Alavijeh, M.S.; Chishty, M.; Qaiser, M.Z.; Palmer, A.M. Drug metabolism and pharmacokinetics, the blood-brain barrier, and central nervous system drug discovery. *NeuroRx* **2005**, 2, 554-571.
48. Tuntland, T.; Ethell, B.; Kosaka, T.; Blasco, F.; Zang, R.X.; Jain, M.; Hoffmaster, K. Implementation of pharmacokinetic and pharmacodynamic strategies in early research phases of drug discovery and development at Novartis Institute of Biomedical Research. *Front. Pharmacol.* **2014**, 5, 174.