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# Structural elucidation, *in vitro* cytotoxicity evaluation and mechansim study of newly secluded bioactive compound from the leaf extracts of *Basella rubra* L.

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ABSTRACT

A bioactive compound, BR-1 was isolated from the methanolic extract of the leaves of *Basella rubra* L. Compound BR-1 was characterized and structurally elucidated using different spectroscopic techniques like UV–vis, FTIR, NMR and MS-ESI-TOFM. The compound was identified as an isovitexin (C-glycosylflavone). Isovitexin exhibited dose-dependent cytotoxicity against human colon cancer (HT-29) cells with $IC_{50}$ value of 29.44 μg/mL. The possible reasons behind the anticancer activity of isovitexin were studied. Morphological observations revealed that isovitexin induces pro-apoptotic changes including warping of cells, clustering, and destructive fragmentation of cancer cells. Further, isovitexin induced the loss of intracellular LDH, loss of membrane integrity and fragmentation of DNA of HT-29 cells. Isovitexin triggered the apoptosis of cancer cells by upregulating the caspase-3 and Bax levels with simultaneous down regulation of procaspase-3 and Bcl-2 protein expression. Molecular docking studies revealed that isovitexin bound at the catalytic cleft of Bcl-2 by the way of six H-bonding interactions by accepting electrons from Arg-109, Glu-136, Leu-137, Arg-146, Ala-149 and Glu-152 and thereby effectively inhibited the Bcl-2. Isovitexin gratifies pharmacokinetic bioavailability parameters as evidenced by Lipinski rule and ADMET profile. The results of the present study support the development of natural bioactive compounds as cancer chemotherapeutics.

## 1. Introduction

Cancer is the most dreadful and feared disease in the world today. Cancers do occur in different stages of life and can spread across different organs of human body. According to cancer research institute UK, 8.2 million cancer patients succumbed to death in 2012. The year 2012 has registered 14.1 million new cases of cancer. It is estimated to reach an unwholesome figure of 23.6 million cases per year by 2030 [1,2]. Cancer hits the countries – with low to medium human development index (HDI) – the worst. Colorectal cancer is one of the most commonly diagnosed diseases in developing countries that affect both men and women. In India, the occurrence of colon cancer has been increased from 0.7 to 4 per lakh among men and 0.4–3 per lakh among women [3]. The incident rates of colon cancer sharpen at the age of 40, while 90% of other cancers increase sporadically at the age of 65 years [4]. The death rate is more than 50%, and patients with metastatic colorectal cancer under 10% will survive more than 5 years after diagnosis [5,6]. Cancer treatment generally includes surgical therapy, chemotherapy, radiotherapy or a combination of these treatments. Although these treatments are usually beneficial, considerable side effects are apparent [7,8]. A plethora of anti-cancer drugs are available to treat various cancers. However, cancers gain resistance to these drugs over time [9]. Genomic alterations and epigenetic modifications are at heart of this acquired resistance, since cancer is a genetic disease [10]. Therefore, the development of new drugs with high specificity, safe over prolonged periods and prone less to resistance – is highly essential and crucial. Medications based on plant origin can efficiently prevent/inhibit tumour cell proliferation, would be a beneficial option [9–11].

Indigenous folklore has long used plant based medicines for the treatment of various diseases. It is anticipated that almost three fourths of the herbal drugs used worldwide were discovered from local medicine. Nearly 70% of modern medicines in India are a result of natural

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products [12]. Taxol, a long used anti-cancer drug is a plant based compound. Plant based compounds will continue as future therapeutic agents, and also as raw material base for constructing new compounds [13]. Several studies were performed using plant based products for treating cancers, and are under way to determine new therapeutic agents with minimal to no side effects with enhanced specificity. There are more than a thousand plants that produce compounds with anti-cancer properties, and some of the modern day anti-cancer drugs are plant derivatives [14,15]. It has been identified that 75% of the modern day cancer drugs are based on plant derivatives. In an *in silico* study spanning across 5728 compounds 346 have shown anti-cancerous properties on at least 60 cell lines [16].

*Basella rubra* L. is an edible perennial vine belongs to the family Basellaceae. *B. rubra* is native to the Indian subcontinent and widely found in Southeast Asia and Africa [17]. It is a fast growing vine with heart shaped leaves. *B. rubra* is a leafy vegetable which is rich in vitamins A and C, calcium and iron. The leaves of *B. rubra* are rich in phenolic phytochemicals and it has potential antimicrobial, antifungal and antioxidant properties [18,19]. The present study reports the isolation, structural elucidation and anticancer activity of potential bioactive compound(s) from traditional medicinal plant, *B. rubra*.

## 2. Materials and methods

### 2.1. Extraction, isolation and purification

The air-dried and finely powdered whole plant leaves (2.5 kg) of *B. rubra* was used for extraction with *n*-hexane (3 × 5 L) and methanol (3 × 5 L). The concentrated methanol extract (73 g) was segregated into hexane soluble (13 g) and ethyl acetate (60 g) soluble fractions with soxhlet apparatus. The concentrated hexane soluble fractions under reduced pressure gave a dark yellow mass and further workup of hexane soluble fraction did not yield any crystalline principle.

#### 2.1.1. Column chromatography

The concentrated ethylacetate soluble fraction (60 g) was subjected to column chromatography (100–200 mesh, 400 g) over silica gel using chloroform and methanol ($CHCl_3$:$CH_3OH$) as eluents in a step gradient manner (10, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, 10) which yielded a total of 62 fractions of 100 mL each, were collected by slow elution.

#### 2.1.2. Thin layer chromatography (TLC)

The silica column eluted fractions were monitored by TLC (Silica gel-G; solvent system: $CHCl_3$-MeOH) and the spots were visualized under UV–vis light using Iodine vapors and spraying with alcoholic $FeCl_3$. This procedure yielded one pure compound designated as BR-1 which was obtained in the fraction 9:1.

#### 2.1.3. High performance liquid chromatography (HPLC)

HPLC analysis was carried out to determine the purity of the isolated compound BR-1. HPLC analysis was carried out by using Shimadzu HPLC system which is a fully automated system (system controller CBM-20A) with high flow rate accuracy, high sensitivity, minimized sample carry over and analysis stability. The specifications of HPLC system including Autosampler SIL-20A, solvent delivery unit LC-20A, online degassing unit DGU-20A, column oven CTO-20A, UV–vis detector SPD-20A and high pressure switching valve. HPLC analysis of compound BR-1 was carried out by using methanol–water (1:1,v/v) as mobile phase, eluted with the flow rate of 1.0 mL/min at 30 °C, and UV detection was done at 254 nm.

### 2.2. Characterization of bioactive compound

The melting point of compound BR-1 was determined using a Kofler hot stage apparatus.

#### 2.2.1. Ultraviolet-Visible spectroscopy (UV–vis) analysis

The compound was dissolved in methanol and absorption was measured using UV–vis1800 spectrophotometer (Schimadzu, Kyoto, Japan). The value of utilizing UV–vis spectroscopy is to detect functional groups like ketones, esters and amides, as well as the presence or absence of unsaturated functional groups.

#### 2.2.2. Fourier transform infrared spectroscopy (FTIR) analysis

FTIR has proven to be a valuable tool for the identification and characterization of compounds or functional groups (chemical bonds) present in an unknown compound. In addition, FTIR spectra of pure compounds are usually so unique that they are like a molecular "fingerprint". For most common plant compounds, the spectrum of an unknown compound can be identified by comparison to a library of known compounds. In the present study, FTIR analysis of the compound BR-1 was carried out using FTIR, ALPHA interferometer, Bruker, Ettlengen, Germany.

#### 2.2.3. Nuclear magnetic resonance (NMR) spectroscopy

$^1H$ and $^{13}C$ NMR spectra were recorded to determine the molecular structure of the compound using Bruker Advance spectrometers. A proton nuclear magnetic resonance ($^1H$ NMR) spectrum tells us about the environments of the various hydrogen atoms in a molecule; a carbon-13 nuclear magnetic resonance ($^{13}C$ NMR) spectrum does the same for the carbon atoms (Carey, 2000). Together, $^1H$ and $^{13}C$ NMR was used in determining a substance's molecular structure.

#### 2.2.4. Mass spectrometry (MS) ESI-TOFMS

MS is used for the determination of molecular weight and elemental composition of a molecule or ion. MS is used for the identification of unknown compounds and help to elucidate the chemical structure of analytes, or quantification of the amount of a known compound present in the sample, based on the production of gas-phase ions that are subsequently separated or filtered according to their mass-to-charge ratios (*m/z*) and finally detected. MS-ESI-TOFMS of the compound BR-1 was recorded in positive mode on an API Q-STAR PULSA of Applied Biosystem (Ontario, Canada).

### 2.3. In vitro cytotoxic activity

#### 2.3.1. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay

Human colon cancer cell lines (HT-29) and normal Chinese hamster ovary cell lines (CHO) were obtained from National Centre for Cellular Sciences, Pune, India. Cell lines were cultured in Dulbecco's Modifed Eagle's medium (DMEM) supplemented with 10% (v/v) heat-inactivated fetal bovine serum, 2 mM glutamine, 1 mM Sodium bicarbonate ($NaHCO_3$), 2 mM Glutamine, 100 µg/mL streptomycin and 100 units/mL penicillin. Cell lines were incubated in a humidified atmosphere of $CO_2$ (5%) at room temperature. The cytotoxic effect of isovitexin against HT-29 and CHO cells was measured by MTT assay [20]. Briefly, the actively growing cells ($1 \times 10^4$/100 µL) were seed in 96-well plates. After 12 h of incubation, the cells were treated with different concentrations of isovitexin (6, 12, 25, 50 and 100 µg/mL) and incubated for 24 h. After incubation, the cell viability was assessed by adding 10 µL of MTT (5 mg/mL) in phosphate-buffered saline (PBS) (pH 7.4) and incubated additionally for 3 h at 37 °C. Then added 100 µL of dimethyl sulfoxide to dissolve the formazan blue formed and absorbance was measured at 570 nm. Cyclophosphamide (15 µg/mL) was used as a positive control. $IC_{50}$ values were calculated using linear regression curves with best straight line fit.

#### 2.3.2. Lactate dehydrogenase (LDH) assay

The cytotoxic effect of isovitexin at different concntrations (6, 12, 25, 50 and 100 µg/mL) was further evaluated by measuring the LDH assay [21]. Quantification of LDH leakage in culture supernantant is