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ANTIOXIDANT AND ANTICANCER ACTIVITY OF *MOMORDICA CHARANTIA*

Jyoti Singh[1] and Prof. R. K. Singh*[2]

[1]Institute of Pharmacy, RITM- Lucknows
[2]Ex. HOD, Toxicology & Experimental Medicine CSIR-CDRI, Lucknow, India.

***Corresponding Author: Prof. R. K. Singh**
Ex. HOD, Toxicology & Experimental Medicine CSIR-CDRI, Lucknow, India.

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ABSTRACT
Since ancient times, a variety of plants have been used as medicine and vegetable throughout the world. The combination of medicine and vegetable usage have Momordica charantia popular for thousand years. Momordica charantia (Figure 1), a valuable plant, belongs to the cucurbitaceae family; it is commonly known as bitter gourd, bitter melon, kugua or karela. Natural products are the best source for various medicinal drugs. The enzymatic and non-enzymatic antioxidative capacities of bitter gourd (Momordica charantia) were investigated in water extract .this study aimed to identify antioxidant and anticancer activity of Momordica charantia extract on different cell lines like HEPG2, HCT116 and MCF-7. Total phenolic compounds free radical DPPH scavenging activity and SOD activity were assayed in the extract. The average total phenolic compound result indicated that momordica charantia was significantly higher in antioxidant content ($P<0.05$) and the effect of momordica charantia extract was highly significant in HEPG2 cells than HCT116 as well as MCF-7 which showing the IC50 of Momordica charantia extract in HEPG2 was 0.80 ug/ml while in HCT116 was 0.87 ug/ml and was 1.50 ug/ml in MCF-7 cell respectively. Also the effect of the Momordica charantia extract was more potent in HCT116 compared to MCF-7 cells.

KEYWORDS: Phenolic compounds, DPPH, Apoptosis, MCF-7, HepG2, HCT116.

1. INTRODUCTION
Bitter gourd (*Momordica charantia* L.), also known as bitter melon, is an important vegetable grown in tropical and sub-tropical regions of Africa, India, and several Caribbean countries. *Momordica charantia* is a herbaceous plant that can grow up to 10 meters tall. The plant bears simple, alternate leaves that are 4-5 cm in width with 3-7 deeply separated lobes. The interior of the fruit is somewhat hollow, containing white pith and seeds. When ripe, the fruits become yellow and split into segments that curl back to reveal the seeds. The fruits are eaten while still green and unripe. the phenolic contents and antioxidant properties of leaf, stem, and fruit extracts by analyzing the inhibition of the free radical 1,1-Diphenyl-2-picrylhydrazyl (DPPH). They found that the fruit extract had the highest value of antioxidant activity and that gallic acid was the predominant phenolic compound in the fruit extract. Wu and Ng (2008) compared the antioxidant capacity of bitter gourd extracted in water versus ethanolic extraction and found that both water and ethanol extracts were effective in reducing the stable radical DPPH to the yellow diphenylpicryl hydrazine. They also found that both water and ethanol extracts were effective in reducing the stable radical DPPH to the yellow colored diphenylpicryl hydrazine. The seeds of *Momordica charantia* are believed to contain anticancer agents and remedies for gastrointestinal diseases. *Momordica charantia* are believed to possess enzymatic and non-enzymatic antioxidant activities against the buildup of reactive oxygen species (ROS) in the cells (Dasgupta and De, 2006; Wu and Ng, 2008; Xanthopoulou et al., 2009; Dhiman et al., 2012). ROS include the singlet oxygen $^1O_2$, the superoxide anion O_2^-, the hydroxyl ion OH^-, the peroxide O_2^{2-}, and the hydrogen peroxide H_2O_2. These free radicals cause cellular injuries and initiate peroxidation of polyunsaturated fatty acids in biological membranes. ROS are also cellular signaling agents in the regulation of plant development, stress responses, hormone regulation and programmed cell death (Palma et al., 2002; Breusegem and Dat, 2006). Organisms defend themselves against the destructive actions of ROS by using enzymatic and non-enzymatic mechanisms. One of the most simple, least expensive, reliable, short-time in-vitro anticancer activity screening tests is apoptotic, IC50, and cell viability. All of these tests can use together as different parameters useful to screen positive and negative effects of many plant extracts, potential anticancer drugs and so on (Wang et al., 2006) many societies (Indian and Turkish) used *Momordica*

charantia in their own folk medicine for treating different antidiabetic, abortifacient, anthelmintic, contraceptive, dysmenorrhea, eczema, emmenagogue, antimalarial, galactagogue, gout, jaundice, abdominal pain, kidney (stone), laxative, leprosy, leucorrhea, piles, pneumonia, psoriasis, purgative, rheumatism, fever and scabies) (Zhao et al., 2015). (Licastro et al., 1980) have investigated the toxicity of *Momordica charantia* lectin as an inhibitor for human normal and leukemic lymphocytes, while(Pongnikorn et al., 2003), studied the effect of the plant (MC) on level and function of natural killer cells in cervical cancer patients with radiotherapy. With the continued interest, many researchers showed different effect of (MC) plant on different type of cancers in vivo and in vitro. The objective of this research were to investigate the total phenolic compound of *Momordica charantia* extracts in inhibiting the free radical DPPH and to investigate the activities of the superoxidase dismutase (SOD) and β-glucosidase enzymes and the cytotoxicity effect of (MC) plant on HepG2, MCF7 and HCT116 cancer cells were evaluated by means of IC_{50}. Consequently, our study has been designed to fill the avoid data regarding the effect of (MC) plant extract.

Fig. 1: *Momordica charantia.*

2. MATERIALS AND METHODS

2.1 Plant Materials

Momordica charantia were purchased from local market lucknow and the sample was authenticated by Botany division, CSIR- Central Drug Research Institute Lucknow (U.P.), India.

2.2 Preparation of Plant extract

The aqueous plant extract was prepared by washed in distilled water and air-dried overnight then the fleshy parts were cut into small pieces and fine powdered. The aqueous plant extract was left in the water bath (Fisher Scientific-2232, USA) for 1 hr. at 60 C then leave overnight at room temperature. The extract was filtered with filter paper. The filtrates were combined and concentrated using vacuum rotatory evaporator system (HB-10, IKA) and the temperature was maintained at 45 °C. The extract was dried in an oven. The dried extract was stored at -20 C until use(Nawwar *et al.*, 2012).

2.3 Total phenols assay

The total phenolic compounds of each extract was determined as Gallic acid equivalent (GAE) using the Folin-Ciocalteu method (Zhou and Yu, 2006). The assay consisted of reacting 200 µl of each extract with 1 ml of Folin-Ciocalteu reagent in cuvets and allowing the mixtures to incubate at room temperature for 5 minutes. Following the 5 minute incubation, 1 ml of 0.5 M sodium bicarbonate solution was added to the reaction mixture and incubated at room temperature for an additional 90 minutes. Various concentrations of Gallic acid were made as standards and treated the same way as the experimental cuvets. The absorbances of the cuvets were recorded at 725 nm using a UV/VIS GENESYS spectrophotometer. The absorbances of the samples were compared to those of known concentrations of Gallic acid. The total phenolic content of each extract was expressed as µg of GAE/ml.

2.4 DPPH inhibition assay

DPPH free radical scavenging activity was measured spectrophotometrically at 517 nm according to the methods of Cheung et al. (2003) and of Wu and Ng (2008). The procedure consisted of making 3.0 ml dilutions of each sample in spectrophotometer cuvets. The dilutions consisted of 0.1 µg/µl and 0.5 µg/µl total proteins. 3.0 ml extraction buffer was used as a negative control and 3.0 ml of 0.2 µg/µl ascorbic acid was used as a positive control in 2 separate cuvets. To each cuvet, 3 ml 0.1 mM DPPH were added. The reaction mixtures were incubated at room temperature for 30 minutes and the absorbances were recorded at 517 nm. The percent inhibition of DPPH was calculated as [(Absorbance without extract – Absorbance with extract)/Absorbance without extract] x 100 (Wu and Ng, 2008).

2.5 Superoxide dismutase (SOD) activity

The abilities of the extracts to neutralize the superoxide free radical were measured spectrophotometrically according to Beauchamp and Fridovich (1971). Aliquots of 0.1 ml extracts were made in 5 ml cuvets. Three replicates were made for each sample. To each aliquot, 3.0 ml reaction mixture (50 mM sodium phosphate buffer, pH 7.8, 0.2 mM NBT, 10 mM L-methionine, 1 mM EDTA, and 5.0 µM riboflavin) were added. The second set was wrapped in aluminum foil and served as a blank. Both sets were placed in a growth chamber with light intensity of 3,000 mol^{-1} m^{-1} s^{-1} for 10 minutes. The reduction of NBT by the SOD enzymes was measured spectrophotometrically at 560 nm. A standard curve was generated using commercial Chloroplast SOD (Sigma Aldrich, St. Louis, MO, USA) according to Hamissou (2011).

2.6 Cell culture

Human colorectal carcinoma (HCT 116), Human hepatic carcinoma (HEPG-2) and Human breast cancer (MCF-7) were obtained from American type culture collection,. Cells were maintained in RPMI media supplemented with 100µ g/mL streptomycin, 100 units/ml penicillin and 10% heat-inactivated fetal bovine serum in a humidified, 5% (v/v) CO2 atmosphere at 37 ºC, the cells were sub-cultured tow times in a week.

2.7 Cytotoxicity assays against tumor cells

The cytotoxicity of the extracts was tested against the HCT116, HEPG-2 and MCF-7 tumor cell lines by sulforhodamine B (SRB). Exponentially growing cells were collected using0.25% trypsin—EDTA and plated in 96-wellplates at 1000cells/well. Cells were exposed to the extracts for 72h and subsequently fixed with TCA (10%) for 1h at 4°C. After many washing, cells were exposed to 0.4% SRB solution for 10min in a dark place and subsequently washed with 1% glacial acetic acid. After drying overnight, Tris—HCl was used to dissolve the SRB-stained cellsand the color intensity was measured at 540nm. The data are analysis using Sigma Plot version 12.0. (Skehan et al., 1990).

2.8 Cell viability

Sulforhodamine B (SRB) stain, allowed us to distinguish viable and dead cells from each other. Viable and dead cells were detected using density measured at 450 nm using microplate reader (Anthos Zenyth-200RT, Cambridge, England) (Tolba et al., 2013).

2.9 Apoptosis

Using fluorescein stain, condensed or fragmented nucleus was counted with the assistance of the fluorescent microscope (Nikon).

2.10 Statistical Analysis

The data generated during the study was analyzed using one way ANOVA test and the p- values less than 0.05 were considered to be significant.

3. RESULT

3.1 Total Phenolic Content

Phenolic phyto compounds of plants show powerful free radical scavengers activity. They have potential to inhibit the lipid peroxidation by neutralizing peroxyl radicals generated during the oxidation of lipids. *Momordica charantia* 15.12± 2.21 mg GAE/g weight in phenolic compound.

3.2 DPPH inhibition assay

The antioxidant activity of *Momordica charantia* extract was evaluated using DPPH free radical inhibition method. Ascorbic acid was used as standard compound. On average, *Momordica charantia* was 83.03% ± 7.62 as effective as ascorbic acid (%EAA) in inhibiting the free radical DPPH.

3.3 Superoxide dismutase (SOD) activity

The results of the enzymatic antioxidant capacity (SOD) of *Momordica charantia* extracts are presented in 2.25 ± 0.70 units of SOD activity per µg total proteins (ua/µg total proteins) was recorded.

3.4 Cytotoxicity profile

Sulforhodamine B assay was used to assess the anti-proliferative effect of the *Momordica charantia* extract against three different cell lines (HCT116, MCF-7 and HepG2). The extracts showed considerable anti-proliferative activity selectively against MCF-7, HCT116 and HepG2. The effect of the *Momordica charantia* extract was highly significant in HepG2 cells than HCT116 cell as well as MCF-7 which showing the IC50 of *Momordica charantia* extract in HepG2 was 0.80µg/ml while in HCT116 was 0.87µg/mland was 1.50µ g/ml in MCF-7 cells respectively. Also the effect of the *Momordica charantia* extract was more potent in HCT116 compared to MCF-7 cells (table 1).

In the other hand the cell viability parameter showed rise line with increasing dose, striking that the curve of cell viability percent gone very slowly with small concentrations (0.01, 0.01, and 1µ g/ml) while the difference between the concentration 1 µ g/ml and concentration 10 µ g/ml was highly significant in all cancer cell lines (Table 2 figure 1., 2, 3). HepG2 cells are still the most sensitive cancer cell line to (MC) plant extract.

Table 1: The IC_{50} as µ g of *Momordica charantia* extract HCT116, MCF-7 and HepG2 cancer cell.

Plant extract	HepG2	HCT116	MCF-7
Momordica charantia	0.80	0.87	1.50

Table 2: The viability % of HCT116, MCF-7 and HepG2 cancer cell after treatment with *Momordica charantia* extract.

Concentration µg/ml	Viability%		
	HCT116	MCF-7	HepG2
0	100	100	100
0.01	95.4939	98.4881	94.0010
0.1	91.4317	96.9854	86.8918
1	91.1786	95.3177	79.4896
10	2.7683	5.8396	3.5444
100	2.1576	1.8924	2.3049

4. DISCUSSION

In most plants, total phenolic compounds have been determined to be the main antioxidative compounds. Wu and Ng (2008) and Kubola and Siriamornpun (2008) independently investigated the phenolic compounds of *Momordica charantia* fruits extracted in water and obtained averages of 0.516 mg of GAE/ml per ml and 0.202 mg GAE/ ml respectively. The two independent investigations reported their data in mg GAE/ml extract not specifying the amount of fresh weight in the 1.0 ml extract. In the absence of agreeable expression of units, this study found it more informative to present data in mg of antioxidant/g fresh weight of the fruit or vegetable rather than mg antioxidant/ml extract. Therefore, this research is reporting its findings of total phenolic compounds in mg GAE/g fresh weight *Momordica charantia*. averaged 13.28 ± 1.71 mg GAE/g fresh weight. Another property useful in determining the non-enzymatic antioxidant values of vegetable is the capacity of the extracts to inhibit the

free radical DPPH. Antioxidants present in the extracts were expressed as the reduction of the purple-colored stable free radical 1,1-diphenyl-2- picrylhydrazyl (DPPH) to the yellow-colored diphenylpicryl hydrazine by donating an electron or a hydrogen. This reduction of the free radical diminishes cellular damage by allowing the neutralization of reactive oxygen species present in the solution (Wu and Ng, 2008). As a result of a buildup of ROS, lipid peroxidation of cell membranes can occur. DPPH scavenging activity is expressed in IC_5 values or in percent ascorbic acid or Vitamin E equivalents. Ascorbic acid, an antioxidant commonly known as Vitamin C, is known to scavenge 50% of DPPH at 50 µg/ml (Hsu et al., 2007; Arazo et al., 2011; Ramkumar et al., 2012). Wu and Ng (2008) reported IC_{50} values of 129.94 µg/ml in *Momordica charantia* extracted in water. In this research, the DPPH reductions by the zucchini extracts never surpassed the 50% mark. Since IC_{50} value and percent inhibition indicate the samepotential, this research found it necessary to report the present data as percent effectiveness of the extracts to inhibit the free radical DPPH compared to ascorbic acid. Bitter gourd was found to be 83.03% ± 7.62 as effective in scavenging DPPH free radial as ascorbic acid.

Enzymatic antioxidants are equally important in protecting organisms against free radical build-up. Enzymatic antioxidants, such as superoxide dismutase, protect cells and tissues from oxidative damage by reactive oxygen species. Superoxide dismutase (SOD), peroxidases (PO) and catalases (Cat) are some of the enzymatic antioxidative defense mechanisms. In this study, only the SOD activity in *Momordica charantia* were investigated. Although the magnitudes of the values were low, the one-way ANOVA indicated the existence of a significant at 5% probability level. Interestingly, there is evidence suggesting that diabetes and other health problems are complicated by oxidative stress due to generation of free radicals (Garg et al., 1996) and by a decrease in the body's natural antioxidant defenses (Oberly, 1988). In an independent study, Sathishsekar and Subramanian (2005) showed that diabetic rats had lower SOD activity in their kidneys and livers compared to diabetic rats treated with *Momordica charantia* seed extracts.

Although the way to discover new anticancer drugs seems long, hard and expensive, it is important to give people living with cancer longer survival, better quality of life, less of pain and suffering. Just as cancer has the potential to have a variety of types, the plants which produce natural products also have the potential to produce different molecules and chemical groups withstand or resist cancer.

Cancer which considered as complicated disorder or devastating disease characterized by transformation led to uncontrolled cell growth which involves, dysregulation of apoptosis, proliferation, invasion, angiogenesis and metastasis (Ichikawa et al., 2006). Consequently, there are two ways trying to keep cancer cell under control, Drug design by synthetic chemistry or natural products using plant, bacterial or algae extract. Drugs design by synthetic chemistry generally suffering from unwanted side effects because they have been produced under conditions far away from living materials. While natural products, produced inside the cell itself which may give it an advantage than synthetic products. The anticancer activity of *Momordica charantia* extract has been demonstrated in vitro and in vivo in prostate, breast, ovary and pancreatic, breast cancers in vivo. (Srinivasan et al., 2007; Pitchakarn et al., 2010; Fang et al., 2012a; Yung et al., 2015). Our study focused on the effect of crude extract of *Momordica charantia* on liver cancer (HepG2), Human colon cancer (HCT116) and breast cancer (MCF-7) in vitro because it had not been reported especially in MCF-7 and HCT116 while HepG2 used as a control to compare our results with elder research and publications. Our observations showed that the (MC)plant methanolic extract has a potential effect to decrease the proliferation rate of different cancer cell lines specially HepG2, liver cancer. The mechanism of action of MC plant extract may due to their chemical contents like triterpenoid, which confirmed as anti-proliferative ingredient (Akihisa et al., 2007). It seems that triterpenoid showed its effect on the liver, breast and colon cancer in vitro.

5. CONCLUSION

This research found that bitter gourd was 83.03% as effective as ascorbic acid in inhibiting the free radical DPPH. *Momordica charantia* has significantly higher total phenolic compounds and high antioxidant containing vegetable food. There is no doubt that this plant gained very high attention to be one of the strong candidate anticancer drugs. Despite that, needs further studies to screen and to investigate the mechanism of action in details.

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