

Evaluation of Anticancer, Antioxidant and Possible Anti-microbial Properties of extracts of *Momordica dioica* roots

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*Article History:

Received: 19/05/2017

Revised: 23/05/2017

Accepted: 10/06/2017

DOI: <https://doi.org/10.7439/ijpc.v7i5.4174>

Abstract

Since many years, plants were known to possess anticancer activities against different cancer cell lines. In this paper, we report a study based on anticancer, anti-bacterial and antioxidant properties plants *Momordica dioica*. The roots of plant material were collected, shade dried and extracted with methanol using soxhlet extraction procedure. Anticancer activities were assayed with standard XTT colorimetric procedure against MCF-7 and Hep-G2 cell lines. From the analysis, it was found that *Momordica dioica* showed good cytotoxic activity on MCF-7 cell line at tested dose. Further work is in progress to evaluate the chemical constituents present in these plants.

Keywords: *Momordica dioica*, XTT, anticancer, antibacterial and antioxidant.

1. Introduction

The medicines derived from plant kingdom have enormous significance in treating various disorders and diseases of the mankind, but their quality parameters and safety profile must be assured. Since the beginning of the twenty-first century, the use of herbal medicine has been increased due to the developments have taken place in herbal drug technology and the increased awareness of the people about the benefits of herbal medicines. Through the systematic evaluation of the safety and efficacy of thousands of plant metabolites are being successfully and used for the treatment of variety of ailments. 80% of the world's population relies on plants for their medication [1]. It is a welcoming note that the use of the medicinal plants is increasing in many countries, where 35% of drugs contain natural products [2, 3]. It is evident that age-related diseases like memory loss, diabetic wounds, osteoporosis, liver, and immune disorders, etc for which no modern medicine is not yet developed and only palliative therapy is available, but

certain plant-derived products were in use for these diseases and mentioned in Ancient literature [4].

Life saving drugs like aspirin, morphine, emetine, dioxin, and ephedrine were derived from medicinal plants and introduced into modern therapeutics several centuries ago [4]. As per Namdeo, about a quarter of all prescribed pharmaceuticals in advanced countries contain compounds that are directly or indirectly derived from plants [5]. Currently, there is a widespread attention in drugs derived from plants, and it is reported that green medicine is safe and dependable [4].

In developing countries like India, people under or just above poverty line such as farmers, people of small remote villages and native communities have no access to modern medicine, depends on traditional remedies and use many native plants for the treatment of common diseases. And they used to add some of the plant materials in their diet to maintain health and to prevent some of the disorders. *Momordica* fruit is one of its kind used widely in southern

Asia countries especially India. *Momordica* genus includes 187 species of those 19 are accepted species [6-8]. *Momordica dioica* (*M. dioica*) is a perennial dioecious climber creeper plant belongs to the family *Cucurbitaceae*, found throughout India. Folk medicinally, *M. dioica* is used both in the prevention and cure of various diseases [9].

All parts of the *Momordica* plant were found to possessing various pharmacodynamic activities such as antihypertensive, antidiabetic, diuretic, laxative, hepatoprotective, anti-inflammatory, antivenomous, antiasthmatic, antidepressant, antiperspirant, antifertility, spermicidal, and anti-hemorrhoidal properties [10-30]. The present study is an attempt made to elucidate the effect of methanolic fraction of *Momordica dioica* for antioxidant, antimicrobial and cytotoxic activities on Breast and Colon cancer cell lines (MCF-7 and HT-29).

2. Materials and methods

2.1 Plant Material:

Fresh roots of both male & female plants of *Momordica dioica* were collected from the surroundings of Warangal, Telangana and collected specimens in August 2016 and were authenticated by comparison with corresponding herbarium specimens.

2.2 Extraction preparation:

Root materials were air dried, cleaned from extraneous materials, mechanically ground and the coarsely powdered root of *M. dioica* was subjected to successive solvent extraction in Soxhlet extractor using methanol. The crude extracts were filtered and the filtrate evaporated using a rotary evaporator. The dissolved constituents were further dried under pressurized vacuum conditions. Extract solutions were stored at -20°C until use.

2.3 Antimicrobial activity:

2.3.1 Organisms used:

Infection causing Bacteria and fungi were selected for testing antimicrobial activity. *Staphylococcus aureus*, *Bacillus Subtilis*, *E. Coli*, and *Pseudomonas aeruginosa* (CABR-C-12) were obtained from the Centre for advanced Biological Research-Hyderabad and were maintained on nutrient agar slants. The standard antibiotic, Streptomycin was used for the antibacterial activity.

2.3.2 Preparation of inoculum

Stock cultures were maintained at 40°C on nutrient agar slants for bacteria. Active cultures for experiments were prepared by transferring a loopful of culture to 5 ml of Brain Heart Infusion broth and incubated at 37°C for 24 hours.

2.3.3 Antibacterial activity

Muller Hinton Agar was prepared according to the manufacturer's instructions. The medium was sterilized by autoclaving at 121°C for 15 minutes at 15 psi pressure and

was used to determine the antibacterial activity of *Momordica dioica* root extract. Sterile molten cool (45°C) agar was poured aseptically into sterile Petri plates (15 ml each) and the plates were allowed to solidify at room temperature in a sterile condition. After solidification and drying, the plates were seeded with appropriate microorganisms by streaking evenly on to the surface of the medium with a sterile spreader and wells (8 mm diameter) were cut out from the agar plates using a sterile stainless steel bore and filled with 0.1ml of the both *Momordica dioica* root extract solutions in respective wells. Tetracycline and double distilled water were used as positive and negative control respectively. Then the plates were incubated at 37°C for 24 hrs in the next day the zones of inhibition were measured with a measuring scale [31]. This experiment was carried out in triplicate for their confirmation. The results were read by the presence or absence of zone of inhibition.

2.3.4 DPPH Antioxidant activity

The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used to determine the free radical scavenging (antioxidant) activity of the extracts according to Ebrahimzadeh, and Bahramian, (2009) [32, 33]. The samples are kept incubation for 20 min and readings were recorded at 517 nm. Percent inhibition of antioxidant activity was calculated by using the following formula and readings of the test sample are compared with that of ascorbic acid (Vitamin C) (Positive control).

% inhibition of DPPH = ((Control OD – Test OD)/Control OD) X 100.

2.3.5 Cytotoxic activity:

Cell culture: Human cancer cell lines used in this study [Hep-G2 (Liver Cancer) and MCF-7 (Breast Cancer)] were procured from National Centre for Cell Science, Pune. All cells were grown in Minimal essential medium (MEM, GIBCO) supplemented with 4.5 g/L glucose, 2 mM L-glutamine and 5% fetal bovine serum (FBS) (growth medium) at 37°C in 5% CO₂ incubator.

XTT assay: The biochemical procedure is based on the activity of mitochondrial enzymes which are inactivated shortly after cell death. This method was found to be very efficient in assessing the viability of cells. A colorimetric method based on the tetrazolium salt, XTT (2, 3-Bis(2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carbox-anilide), was first described by P.A. Scudiero in 1988. In brief, the trypsinized cells from T-25 flask were seeded in each well of 96-well flat-bottomed tissue culture plate at a density of 5x10³ cells/well in growth medium and cultured at 37°C in 5% CO₂ to adhere. After 24hr incubation, the supernatant was discarded and the cells were pretreated with growth medium and were subsequently mixed with different concentrations of test compounds

(6.25,12.5,25,50,100 & 200 µg/ml) in triplicates to achieve a final volume of 100 µl and then cultured for 48 hr. The compound was prepared as 1.0 mg/ml concentration stock solutions in DMSO. Culture medium and solvent are used as controls. Each well then received 50 µl of fresh XTT (0.9mg/ml in RPMI along with XTT activator reagent) followed by incubation for 2hr at 37°C. At the end of the incubation shacked the 96-micro well plate for 15 sec. The Optical Density (OD) of the culture plate was read at a wavelength of 490 nm (reference absorbance at a wavelength of 630 nm) on an ELISA reader, Anthos 2020 spectrophotometer [34-37].

3. Results

The powdered, dried root material of the both male and female plants were extracted with methanol as solvent and dried under reduced pressure. Several researchers already reported that *Momordica* contains lectins, proteins, triterpenes and vitamins [38]. It also contained small quantities of essential vitamins like ascorbic acid, carotene, thiamin, riboflavin, and niacin [39]. It also contains protein in the leaves and dry weight of aerial plant parts remained higher in male as compared to female defruited, and monoecious plants [40]. In addition to that, the dry root of *Momordica dioica* reported to contain three triterpenes and two steroidal compounds, viz, α -spinasterol octadecanolate, α -spinasterol-3-O- β -D-glucopyranoside, 3-O- β -D-glucuronopyranosylgypsogenin, 3-O- β -D-glucopyranosylgypsogenin and 3-O- β -D-glucopyranosyl hederagenin. The thin layer chromatography studies revealed that the root extracts were found to contain glycosides, steroidal and alkaloidal compounds by using specific staining reagents.

The *in vitro* antibacterial properties of root extract of *Momordica dioica* are presented in Table 1. The tested extracts of *Momordica dioica* shown affective antibacterial activity against both Gram positive and Gram negative bacterial pathogens. The extract of the female plant VJ2, have shown a comparatively high zone of inhibition. The extract VJ2 exhibited highest antibacterial activity against

S. aureus with 18 mm, 13 mm against *E. Coli* and *B. Subtilis*. The male plant extract VJ1 was active against *P. aeruginosa*. But none of the extracts was not effective as standard compounds.

In the DPPH radical scavenging activity, the *Momordica dioica* root extracts were promising results. *Momordica dioica* root extracts have shown IC₅₀ values of 76.55 and 290.28 µg/ml for both male and female plants respectively, and they were not comparable with the standard ascorbic acid with IC₅₀ of 9.3 µg/ml.

The extracts were screened for their cytotoxic activity on Liver Cancer (Hep-G2) and Breast Cancer (MCF-7) cell lines using the rapid XTT assay method by dissolving in dimethylsulfoxide. XTT rapid assay was used for the initial screening of anticancer activity of the plant extracts. The secondary metabolites like alkaloid, glycoside, tannin, steroid, terpenoid and flavonoids are present in the plant extracts, are principally contributed to the cytotoxic effects. The *Momordica* plant also contains several glycosidic, alkaloidal, and terpenoidal constituents in significantly higher concentration. The basis of the XTT assay is cleavage of the tetrazolium salt to formazan occurs by the succinate reductase in the mitochondria of metabolically active cells, which can be measured by absorbance at 490 (or 450) nm in a microplate reader. Efficient reduction of XTT requires an electron coupling reagent.

The results have shown in Tables 3-4, the inhibitory data was given in terms of % cell survival (% CS) and % cell inhibition (% CI). The IC₅₀ values were determined by plotting the graph between concentration and % cell inhibition (Figure 1-2). *Momordica* root extracts were shown a significant inhibition at the tested concentrations. The IC₅₀ values of the plant extracts were found to be 116.598µg/ml and 165.852µg/ml for both male and female plant extracts respectively on liver cancer cell lines. The extracts have shown inhibition on breast cancer cell lines with IC₅₀ values of 135.9 µg/ml and 86.56 µg/ml by both VJ1 and VJ2 respectively.

Table 1: Zone of Inhibition of VJ1 and VJ2 in Antibacterial Activity

Zone of Inhibition				
	<i>Staphylococcus aureus</i>	<i>Bacillus Subtilis</i>	<i>E. Coli</i>	<i>Pseudomonas aeruginosa</i>
Standard	Ampicillin	Norfloxacin	Ceftriaxone	Ciprofloxacin
10	10 mm	10 mm	19 mm	09 mm
25	12 mm	15 mm	22 mm	14 mm
50	19 mm	13 mm	27 mm	20 mm
VJ1				
10	06 mm	0 mm	06 mm	08 mm
25	09 mm	2mm	09 mm	10 mm
50	12mm	6mm	12mm	17 mm
VJ2				
10	08 mm	10mm	12 mm	06 mm
25	15 mm	15mm	12 mm	09 mm
50	18 mm	13mm	13 mm	12mm

Table 2: Antibacterial Activity of plant extracts VJ1and VJ2

S. No	Anti-Oxidant Activity (IC ₅₀ µg/ml)		
	Sample	VJ1	VJ2
1	Methanol extract	76.55	290.28
2	Ascorbic acid		9.3

Table 3: Dose Response of VJ1and VJ2 on Hep –G2 (Liver Cancer)

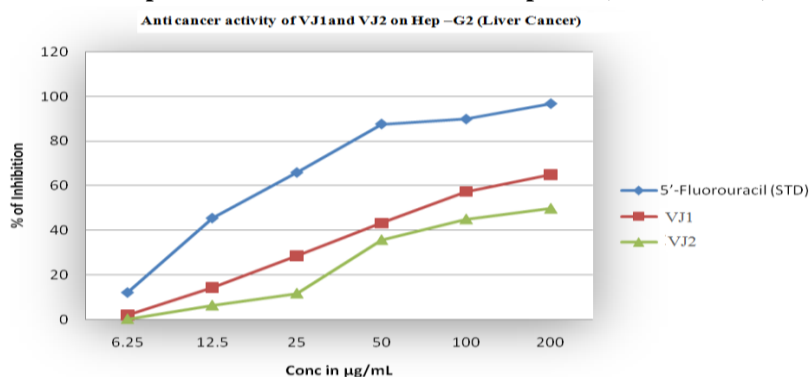
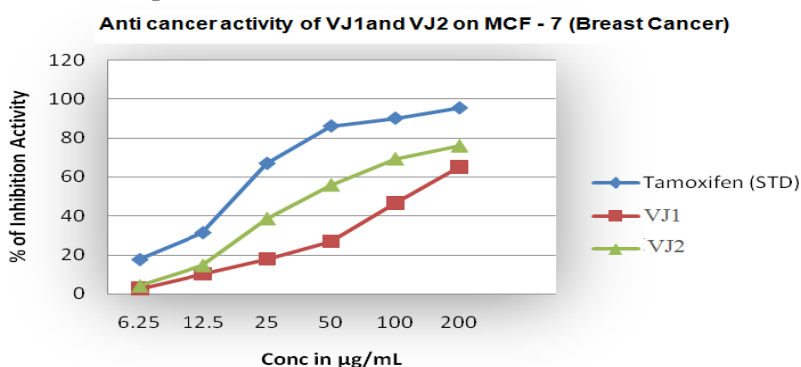
5-Fluorouracil (STD)				VJ1			VJ2		
Conc (ug/ml)	OD 490 nm	% C S	% CI	OD at 490 nm	% CS	% CI	OD 490 nm	% CS	% CI
6.25	0.482	87.7	12.3	0.534	98	2	0.542	99.6	0.4
12.5	0.314	54.4	45.6	0.471	85.5	14.5	0.511	93.5	6.5
25	0.211	34	66	0.400	71.4	28.6	0.484	88.1	11.9
50	0.102	12.3	87.7	0.326	56.7	43.3	0.363	64.1	35.9
100	0.091	10.1	89.9	0.254	42.5	57.5	0.316	54.8	45.2
200	0.056	3.2	96.8	0.216	34.9	65.1	0.291	50	50

Table 4: Dose Response of VJ1and VJ2 on MCF - 7 (Breast Cancer)

Tamoxifen (STD)				VJ1			VJ2		
Conc (ug/ml)	OD 490 nm	% C S	% CI	OD at 490 nm	% CS	% CI	OD 490 nm	% CS	% CI
6.25	0.475	82.4	17.6	0.555	97.5	2.5	0.546	95.8	4.1
12.5	0.402	68.6	31.4	0.515	90	10	0.491	85.4	14.6
25	0.214	33	67	0.474	82.2	17.8	0.364	61.4	38.6
50	0.113	13.8	86.2	0.426	73.1	26.9	0.273	44.1	55.8
100	0.092	9.8	90.2	0.322	53.4	46.6	0.202	30.7	69.3
200	0.064	4.5	95.5	0.224	34.8	65.2	0.167	24.1	75.9

Table 5: IC₅₀ Values of VJ1and VJ2 on Cancer cell lines

Cell line	IC ₅₀ Values(µg/mL)		
	Standard	VJ1	VJ2
Hep –G2 (Liver Cancer)	15.001	116.598	165.852
MCF - 7 (Breast Cancer)	21.64	135.9	86.56

Figure 1: Dose-Responsecurve of VJ1and VJ2 on Hep –G2 (Liver Cancer) cell lines**Figure 2: Dose-Responsecurve of VJ1and VJ2 on MCF-7 (Breast Cancer) cell lines**

4. Conclusion

The perennial dioecious plant, *Momordica dioica* was having broad range of biological activities. In the present investigation, we studied the antioxidant, anticancer and antimicrobial activities of the both male and female plant extracts by *in vitro* studies. The extracts have shown a cytotoxic capability on various human derived hematological and solid tumor cell lines. The overall results of the present study provided evidence for the antioxidant, anticancer and antimicrobial activities of studied plant extracts, and bring supportive data for future investigations that will lead to their use in cancer, oxidative stress, and antimicrobial therapy. Efficacy and mechanisms of action in various normal and cancer cell models *in vitro*, coupled with bio-assay guided purification in order to elucidate active anticancer compound(s) from the crude extract will be reported in due course.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgement

VK wanted to thank the Director and Principal, Janagaon Institute of Pharmaceutical Sciences, Janagaon, Warangal for their support during the research work.

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