

## ANTI-TUMOR ACTIVITY OF FRUIT EXTRACTS OF *MOMORDICA DIOICA* ROXB.

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### ABSTRACT

Various parts of *Momordica dioica roxb.* (MDR) have been traditionally used as ethnomedicine for a number of disorders. The aim of the present study was to examine the antitumor effect of various extracts of MDR fruits on DLA induced tumour models in mice. The crude chloroform and methanolic extract were prepared by soxhlet extraction. The marc remained after methanolic extraction was macerated with chloroform water (5:95) to yield the aqueous extract. All the three extracts were evaluated for their *in vitro* antioxidant and *in vitro* antitumor activity to select the promising ones. The promising extracts were then subjected for *in vivo* anti tumour activity against DLA induced tumour model using swiss albino mice. Cyclophosphamide was used as the standard anticancer agent.

Methanolic and chloroform extract showed best antioxidant and free radical scavenging activity owing to their high phenolic content. Chloroform extract has shown maximum activity on *in vitro* anti cancer activity among all three extracts. The activity was found to be significant and more for chloroform extract than methanolic extract. This may be due to chloroform soluble phytochemical may possess good antitumor activity. These results suggest that MDR fruit extracts possess strong antioxidant and anti tumour activity against the transplantable tumour models. However, further studies are required to support the assumption.

**KEYWORDS:** *Momordica dioica*, Anti-tumour, DLA, Brain shrimp, Solid tumor.

## INTRODUCTION

By definition, 'traditional' use of herbal medicines implies substantial historical use, and this is certainly true for many products that are available as 'traditional herbal medicines'. In many developing countries, a large proportion of the population relies on traditional practitioners and their armamentarium of medicinal plants in order to meet healthcare needs.

<sup>[1]</sup> The pharmacological treatment of disease began long ago with the use of herbs. Methods of folk healing throughout the world commonly used herbs as part of their tradition. Cancer is a generic term for a group of over hundred diseases that can affect any part of the body. An important feature of cancer is the rapid creation of abnormal cells which grow beyond their usual boundaries and can invade adjoining parts of the body and spread to other organs, a process referred to as metastasis. Metastases are the major cause of death from cancer. <sup>[2]</sup>

Every year about **85, 0000** new cancer cases are diagnosed in India resulting in about **58, 0000** cancer related deaths every year. India has the highest number of the oral and throat cancer cases in the world. Every third oral cancer patient in the world is from India. In males - oral, lungs and stomach cancers are the three most common causes of cancer incidence and death. In females - cervical, breast and oral cancers are the three main causes of cancer related illnesses and death. Overall cervical cancer is the number one cause of cancer death in India. <sup>[2,3]</sup> Cancer is caused by both external factors (tobacco, chemicals, radiation, and infectious organisms) and internal factors (inherited mutations, hormones, immune conditions, and mutations that occur from metabolism). These causal factors may act together or in sequence to initiate or promote carcinogenesis. <sup>[4]</sup> Plants have a long history of use in the treatment of cancer. <sup>[5]</sup> The search for anti-cancer agents from plant sources started in the late 1950's, with the discovery and development of the vinca alkaloids, (vinblastine and vincristine) and isolation of cytotoxic podophyllotoxins. As a result, the United States National Cancer Institute (NCI) initiated an extensive plant collection program in 1960. This led to the discovery of many other compounds such as taxanes, camptothecins and combrestatins <sup>[6]</sup>, paclitaxel (Taxol), vinorelbine (Navelbine), teniposide (Vumon) and various water-soluble analogs of camptothecin (e.g., Hycamtin) which are being used in cancer treatment with varied degrees of success. More over plant based drugs are cheap, locally available, and free from severe side effects. Over 60% of currently used anti-cancer agents are derived in one-way or another from natural sources, including plants, marine organisms and microorganisms. <sup>[7]</sup>

*Momordica dioica* Roxb. (MDR) is a perennial climbing creeper belonging to the family Cucurbitaceae and generally found in the forests of Southern India, Bengal, Maharashtra and Madhya Pradesh and occurs naturally throughout India, Sri Lanka, Burma, China and Malaya; cultivated in the Deccan. <sup>[8]</sup> The plant is reported up to an altitude of 1500 m in Assam and Garo hills of Meghalaya. Kakrol is a Cucurbitaceous crop originated in the Indo-Malayan region. <sup>[9]</sup> Alcoholic extract of MDR possess anti-allergic activity and found that it is effective to inhibit passive cutaneous anaphylaxis in mouse and rat. <sup>[10]</sup> Shreedhara *et al.* <sup>[11]</sup> reported the anti-fertility activity of aqueous and ethanolic extract of root of MDR in female rat. Ilango *et al.* <sup>[12]</sup> observed the analgesic and anti-inflammatory activities in MDR fruit pulp hexane and methanol extracts. The plant is reported for anti-feedent activity and exhibited moderate and concentration dependent anti-feedent activity. <sup>[13]</sup> The aqueous and ethanolic extracts of MDR have antioxidant and hepatoprotective activity. <sup>[14]</sup> The plant exhibited hypoglycaemic and hypolipidemic activities on alloxan-induced diabetic rats. <sup>[15]</sup> Local people routinely use this fruit as vegetables and also for the treatment of various diseases. The hypolipidemic activity of MDR was reported earlier <sup>[16]</sup> but no scientific investigation was conducted on the particular phytochemical constituent which is responsible for hypolipidemic effect. As on today there is no reference for an anti-cancer activity of MDR. With this background the present study has been undertaken to evaluate the anti-cancer activity of various extract of MDR. Recently hypolipidemic and cardioprotective activities of extract of MDR were reported from our laboratory. <sup>[17,18,19]</sup>

## METHODOLOGY

**Chemicals:** Ascorbic acid, Chloroform, Dimethyl sulfoxide, Disodium hydrogen phosphate, Ethyl acetate, Ferric chloride, Ferrous sulphate, Formalin, Hydrogen peroxide, Ketamine, Methanol, Potassium dihydrogen phosphate.

**Animals:** The experiments were carried out on 8-10 weeks old Swiss albino mice of either sex weighing 25-35 gm. Animals used in the study were procured from JSS Medical College central animal facility centre, Mysore. The animal expert protocol was approved by IAEC of JSSCP, Mysore. (IAEC Proposal number-093/2011)

Total number of animals=48

**Cell lines**

**DLA** (Dalton's ascites lymphoma) cells, obtained from JSS College of Pharmacy, Ooty, Tamilnadu were used to induce solid tumor in Swiss albino mice. The cell lines were maintained and propagated intraperitoneally by serial transplantation in adult Swiss albino mice.

**Plant material (*Momordica Dioica*)**

The fresh fruits of *Momordica dioica*, Roxb. (MDR) were collected from Karnataka, identified by Dr.K.L Krishna Asst. Professor, Dept. of Pharmacology, JSS Collage of Pharmacy, Mysore and authenticated by Dr.M.N.Naganandini, Asst. Professor, Dept. of Pharmacognosy, JSS Collage of Pharmacy, Mysore. The fruits were cleaned to remove impurities and cut into small pieces and shade dried. The coarsely powdered leaves were weighed and stored in air tight containers. A specimen sample (SAMD032) is deposited in the Dept. of Pharmacognosy of JSS Collage of Pharmacy, Mysore.

**Preparation of the Extracts**<sup>[20]</sup>

The dried powdered fruits of MDR were extracted with 100% methanol (MEMD) and chloroform (CEMD) using soxhlet extractor. The marc remained after soxhlet extraction was macerated with chloroform water (5:95) for 3 days to get aqueous extract (AEMD). All the extracts were concentrated using flash rotary evaporator and then dried under vacuum.

**Preliminary Phytochemical Screening**<sup>[20,21]</sup>

All the extracts were subjected to preliminary phytochemical studies to know the presence of various phytochemicals and their distribution in different fractions and extracts.

***In vitro* antioxidant activity**

All the extracts of MDR i.e. MEMD, AEMD and CEMD were subjected to different *in vitro* antioxidant and free radical scavenging activity like DPPH radical scavenging assay<sup>[22,23,24]</sup>, hydrogen peroxide scavenging activity<sup>[25,26]</sup> and alkaline DMSO method.<sup>[26]</sup> All the experiments were performed in triplicate and EC<sub>50</sub> values were calculated by linear regression of the plot. Ascorbic acid was used as standard antioxidant in all the methods.

***In vitro* anti tumor screening****Brine Shrimp Lethality Bioassay<sup>[27,28]</sup>**

Brine shrimp lethality assay is a preliminary cytotoxicity assay used for screening various categories of compounds. The cytotoxicity of the compounds is investigated by the ability of the compounds to cause death to the shrimps.

Brine shrimp (*Artemia salina* Leach) eggs were hatched in a beaker filled with sea water under constant aeration with a drop of yeast suspension. After 48 h, nauplii were collected by pipette against a lighted background. Ten such nauplii were transferred to each sample vial. Prior to the experiment the extracts were dissolved in Dimethyl sulphoxide DMSO (0.2 %) and diluted with sea water to obtain a stock concentration of 500  $\mu$ M.

The nauplii were treated with different concentrations of extracts (1, 10, 50, 100  $\mu$ M) in 5 ml of sea water for 24h. A drop of yeast suspension was added to each vial. The vials were maintained under illumination. After 24 h, number of surviving nauplii was counted using 3 x magnifying glasses and the percentage cytotoxicity was determined. IC<sub>50</sub> values were calculated.

***In vivo* antitumor activity of extracts of MDR against DLA induced solid tumor model.  
[29, 30]****Induction of Solid tumor**

DLA cells were aspirated from the peritoneal cavity of DLA bearing mouse, after 15 days of tumor transplantation. The ascitic fluid was drawn using an 18-gauge needle into a sterile syringe and a small amount was tested for microbial contamination. Tumor cells viability was determined by Trypan blue exclusion test and total number of viable cells were counted using haemocytometer. The ascitic fluid was suitably diluted in saline to get a concentration of 10<sup>7</sup> cells/ml of tumor cell suspension. Around 0.1 ml of this solution was injected subcutaneously to the right hind limb of mice to obtain a solid tumor. Treatment was started after 24 h of tumor inoculation and continued for 10 consecutive days as shown in Table 1. Cyclophosphamide (25 mg/kg) was used as standard drug to compare the activity of extract.

## RESULTS

Experimental design to evaluate *in vivo* anti tumour activity of MDR on DLA solid tumour model.

**Table 1. Grouping and treatment schedule of experimental animals**

Group	Treatment	Evaluation
Control + DLA cells	Sodium CMC (0.25%) p.o. (Vehicle)	Tumor volume and tumor weight were noted on 0 <sup>th</sup> , 5 <sup>th</sup> , 10 <sup>th</sup> , 15 <sup>th</sup> , 20 <sup>th</sup> , 25 <sup>th</sup> and 30 <sup>th</sup> day.
Standard + DLA cells	Cyclophosphamide as suspension in vehicle (25mg/kg) p.o.	-do-
MEMD dose I + DLA cells	As suspension in vehicle (200 mg/kg) p.o.	-do-
MEMD dose II + DLA cells	As suspension in vehicle (300 mg/kg) p.o.	-do-
MEMD dose III +DLA cells	As suspension in vehicle (400 mg/kg) p.o.	-do-
CEMD dose I +DLA cells	As suspension in vehicle (200mg/kg) p.o.	-do-
CEMD dose II +DLA cells	As suspension in vehicle (300mg/kg) p.o.	-do-
CEMD dose III +DLA cells	As suspension in vehicle (400mg/kg) p.o.	-do-

### Preliminary Phytochemical Analysis

The percentage yield of MEMD, AEMD and CEMD were found to be 15.65%, 12.67% and 9.64% respectively.

Preliminary phytochemical analysis of various extracts of MDR revealed the presence of following phytochemicals as shown below.

Extracts	Phytochemical Present
<b>MEMD</b>	-Triterpenes, tannins, carbohydrates, reducing sugars, flavonoids, alkaloid.
<b>CEMD</b>	-Cardiac glycosides, flavonoids, tannins, reducing sugars, carbohydrates.
<b>AEMD</b>	-Cardiac glycosides, flavonoids, tannins, terpenoids carbohydrates, saponins.

***In vitro* antioxidant activity:** MEMD, CEMD and AEMD, exhibited antioxidant and free radical scavenging activity in graded concentrations. Results showed MEMD and CEMD as potent free radical scavengers and antioxidants among the tested extracts and this activity may be due to presence of higher concentration of flavonoids and phenolic compounds. Since, MEMD and CEMD were found to be potent antioxidants they were selected for *in vivo* anti-cancer activity.

### DPPH free radical scavenging activity

The IC<sub>50</sub> values of MEMD, AEMD, CEMD for DPPH scavenging activity was found to be 247.83±17.87, 613.62±22.87, 389.9±18.60 µg/ml, whereas ascorbic acid used as a reference standard showed scavenging potential with an IC<sub>50</sub> value of 3.17±0.50 µg/ml as shown in Table 2. The scavenging activity was found to be dose dependent and MEMD, CEMD exhibited better DPPH scavenging potential when compared to AEMD.

### Hydrogen peroxide scavenging activity

The IC<sub>50</sub> values of MEMD, CEMD, & AEMD for H<sub>2</sub>O<sub>2</sub> scavenging activity was found to be 293.8±12.87, 393.63±16.98, 367.77±26.65 µg/ml, whereas ascorbic acid used as a reference standard showed scavenging potential with an IC<sub>50</sub> value of 16.00±0.19 µg/ml (Table 2). The scavenging activity was found to be dose dependent. MEMD & CEMD potentially scavenged H<sub>2</sub>O<sub>2</sub> radical when compared to AEMD.

### Alkaline DMSO scavenging activity

The IC<sub>50</sub> values of MEMD, CEMD, & AEMD for alkaline DMSO scavenging activity was found to be 268.01±12.76, 317.87±20.7, 314.87 ±22.01 µg/ml, whereas ascorbic acid used as a reference standard showed scavenging potential with an IC<sub>50</sub> value of 34.79±0.64 µg/ml as shown in Table 2. MEMD & CEMD exhibited better alkaline DMSO scavenging potential when compared to AEMD.

**Table 2: Anti-oxidant activity of extracts of MDR fruits (IC<sub>50</sub> values in µg/ml)**

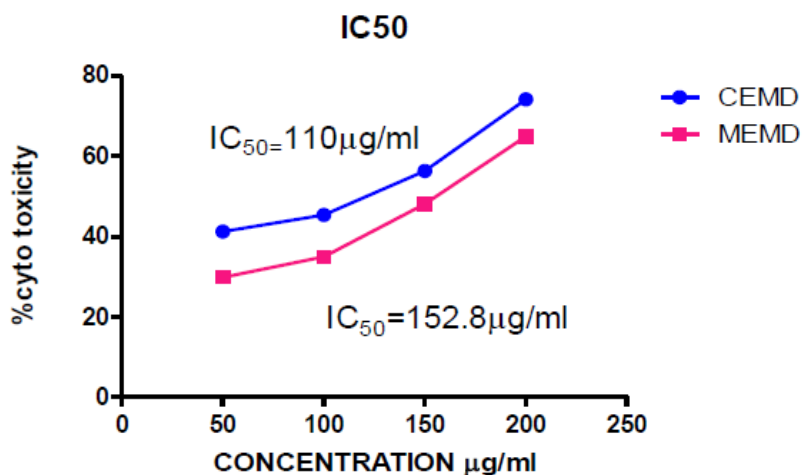
Method	IC <sub>50</sub> values (µg/ml concentration)			
	MEMD	AEMD	CEMD	Ascorbic acid
DPPH method	247.83±17.87	613.62±22.87	389.9±18.60	3.17±0.50
Hydrogen peroxide	293.80±12.87	367.78±22.87	380.63±16.98	16.00±0.19
Alkaline DMSO	268.01±12.76	317.87±20.77	314.18±16.75	34.79±0.64

Values are given as Mean±SEM, n=3

### In-vitro antitumor activity

**Brine Shrimp lethality assay (BSL):** The IC<sub>50</sub> value of chloroform extract was found to be 110.22±1.30 when compared to the methanol extract with an IC<sub>50</sub> of 152.80±12.7. The BSL assay is very preliminary method to assess the cytotoxic activity. (Graph1)

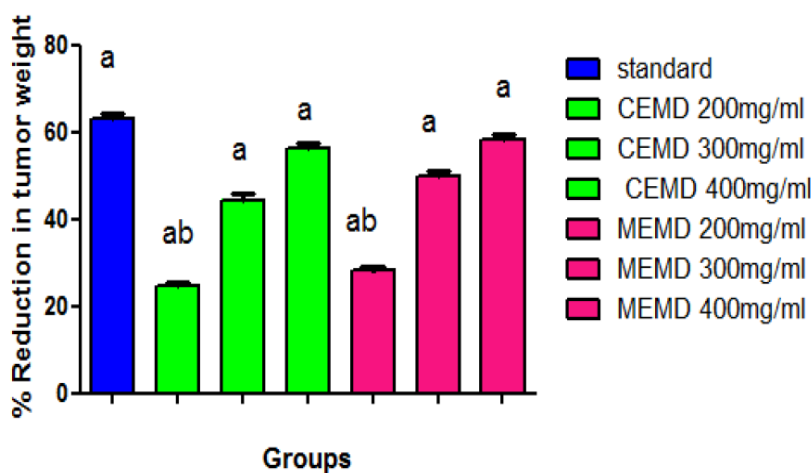




**Graph 1: Effect of various extract of MDR on Brine shrimp**

### DLA solid tumor model

At the end of fourth week, the weight of DLA solid tumor in control mice was  $10.15 \pm 0.49$ g. Standard cyclophosphamide treatment, at a dose of 25mg/kg, significantly reduced the solid tumor weight by  $62.94 \pm 1.46$  g when compared with control. CEMD treatment at all the three doses caused significant reduction in the solid tumor weight when compared with control. However, CEMD at 400 mg/kg was found most effective in reducing the tumor weight by  $56.38 \pm 0.9$ g.



**Graph 2: Effect of various extracts of MDR on tumour weight against DLA induced solid tumor in mice. (Percentage reduction in tumor weight)**

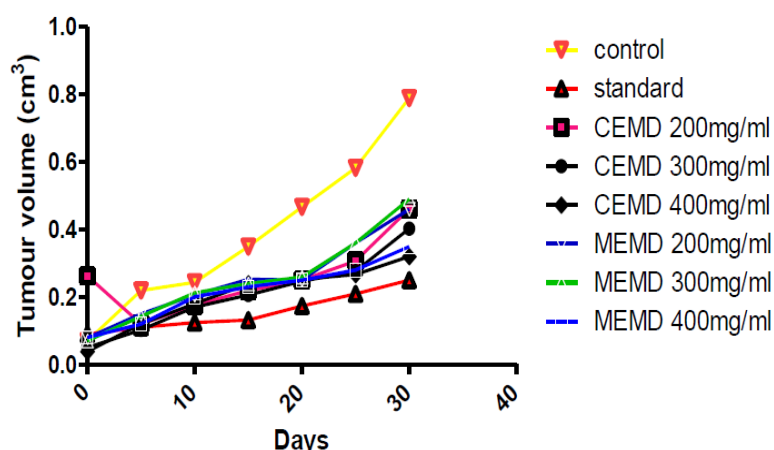
All the values are mean  $\pm$  SEM n=6

<sup>a</sup>significant when compared to control  $P < 0.05$

<sup>b</sup>Significant when compared to standard  $P < 0.05$



The DLA inoculation significantly increased the tumor volume ( $0.82 \pm 0.15$ ) in control mice on 30<sup>th</sup> day. A maximum reduction in tumor volume ( $0.24 \pm 0.01$ ) was observed in standard drug treated group on 30<sup>th</sup> day. The solid tumor development was significantly inhibited by the standard from 10<sup>th</sup> day onwards as compared to respective day control. A similar reduction in tumor volume was observed in CEMD treatment in a dose dependent manner. CEMD (except at lower dose, 200 mg/kg,) significantly reduced development of solid tumor volume from 10<sup>th</sup> day as compared to the respective day control.



**Graph 3: Effect of extracts of MDR against DLA induced Solid tumour on mice (Tumor volume).**

## DISCUSSION

In the present study we have undertaken the evaluation of MDR fruit extracts for its potential anti-tumor activity. A phytochemical is a natural bioactive compound found in plant which is known in protecting many diseases. The phytochemical tests indicated the presence of alkaloids, glycosides, tannins, and flavonoids in the MEMD as well as in CEMD as shown in result section. Such compounds were known to possess potent antioxidant activity. These compounds are known to be biologically active and therefore could be responsible for their therapeutic effect. MEMD and CEMD showed better *in vitro* antioxidant & free radical scavenging activity among all the tested extracts. However, none of the extracts were found to be more potent than the standard (ascorbic acid) since their  $IC_{50}$  values were found to be higher. Due to its natural origin and potent antioxidant ability MEMD & CEMD could be used as a potential preventive intervention for free radical-mediated diseases. In the present study MEMD and CEMD of MDR showed cytotoxic activity in the BSL bioassay. Among two, the CEMD was found most effective than MEMD compared to the an  $IC_{50}$  value. Since

the BSL assay is very preliminary method to assess the cytotoxic activity. For assessing the effect of the extracts in solid tumours, Dalton's ascites lymphoma inoculated mice were used. The DLA treated animals have developed tumor when tested by various parameters. The development of tumor was found to be significant when compared with normal animals in all respect. Treatment with MEMD and CEMD resulted in reduction in solid tumor weight and tumor volume at higher doses (400 mg/kg) as compared to solid tumor weight and tumor volume in control group mice. However observed values were not statistically significant. MEMD (400 mg/kg) showed effective in reducing the tumor weight whereas CEMD (400 mg/kg) has been decreased tumor weight by when compared to control group.

## CONCLUSION

As cancer is one of the most prevalent diseases second only to cardiovascular disease leading to the mortality. In spite of tremendous scientific investigations are making best efforts to combat this disease, the sure-shot, perfect cure is yet to be brought into world medicine. Hence the search for molecule with the selective antitumor activity devoid of many of the side effects of conventional chemotherapy is ongoing process till the goal is reached.

In this context we made an attempt to assess the possible antitumor activity of *Momordica diocia* Roxb fruits in various *in vitro* and *in vivo* models.

The extracts were subjected to *in vitro* and *in vivo* anticancer studies. In DLA model CEMD at dose of 400 mg/kg decreased the growth of solid tumor as evidenced by the reduction of solid tumor weight and volume.

Though the selected extracts have shown promising activity in all the tested models, it is very difficult to conclude at this stage the possible mechanism for its anticancer activity. Hence more experiments are required to elucidate the molecular mechanism so that we could come out with potent and selective anticancer agent derived from plant source.

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