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## Phytochemical investigation, antibacterial and anticancer activities of the extract of *Momordica charantia*

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### Abstract

Cancer cause sickness and consequently can be viably overwhelmed by a multi-constituent remedial strategy. Herbal extracts shows the example of such stratagem. However, less research have been carried out till date that portray the effect of different extraction techniques on the phyto compounds profile of plant extracts and its effect on anticancer activity. *Momordica charantia* is a member of the Cucurbitaceae. It is a popular vegetable harvest in Indian customary medicine since olden times. It has potential lipid lowering and antioxidant activity and antidiabetic. In the present study, we have evaluated the anticancer prospective of methanolic and acetone extracts of *Momordica charantia* (MCME) and (MCAE). Reported results show that (MCME) is rich in bioactive compounds shown anticancer activity with Cell lines of (IC<sub>50</sub>) with MCF 715.6 ± 1.3 and HeLa 28.2 ± 1. This study based on the presence of cytotoxic from the *Momordica charantia*, which have been further used in herbal formulations study as an anticancer activity. Our conclusion support additional indepth study of this pharmacologic activity as a malignant tumour agent.

**Keywords:** *Momordica Charantia*, Malignant Tumour, Anticancer, Phytochemical, Antibacterial

### Introduction

Cancer is a main reason of death worldwide, representing for 13% of all deaths worldwide in 2008 (WHO, 2013) [20]. At present in many developing nations, malignancy is the third foremost cause of death after contagious and cardiovascular diseases (Tanih, 2013) [16]. As indicated by the World Health Organisation, there may be 21.4 million instances of cancer and 13.2 million passing from cancer every year by 2030 (ACS, 2011). Genetically and molecular alteration such as invasion angiogenesis, transformation, deregulation of apoptosis, proliferation and metastasis are characteristics of cancer (Fimognari *et al.*, 2011) [6]. Hence there is an urge for the expansion of new anticancer medicine for its treatment and prevention. Bioactive compounds from plants play a foremost taskin the discovery of such new drugs. It has been estimated that about 60% of approved drugs were of natural origin (Douglas Hanahan and Robert, 2000) [4].

Medicinal plants are well considered in the fundamental sources of natural bioactive compounds. It has assessed that about 50% of the treatment products in United States of America and Europe countries are basically from products derived from natural or their relative derivatives (Newman *et al.*, 2003) [9]. Beyond the 250,000–500,000 plant species in the earth, just 1–10% have been examined biologically and chemically for their potential pharmaceutical value (Verpoorte, 2000) [18]. The utilizing of complementary alternative medicine has dramatically increased in India along with USA, in the last 2 decades (Pandey *et al.*, 2006) Approximately 60% of anticancer compounds are derived from medicinal plants and other natural resources; however, there are still a many of plants that have an anticancer potential but they have not yet been fully investigated (Cragg, 2005) [2]. Thus, the other solution for the harmful effects of synthetic drugs is the use of complementary alternative medicines as very few studies have been reported on the use of herbal medicine in management of prostate cancer (Rao *et al.*, 2004) [13].

In the current investigation carried out, a screening of hydro alcoholic extracts of *Momordica charantia* leaves extracts were investigated for anticancer activity against breast cancer cell

lines MCF 7 and HeLa human prostate cancer cell lines.

## Materials and Methods

### Plant collection

*Momordica charantia* plant was collected from surroundings of university the in November 2017. The plant was identified and authenticated. Fresh plant material was washed with water, air dried, and then blended to a fine powder. The powder was stored in airtight containers at 4°C for further use.

### Extraction

About 150gms of fresh leaves were powdered and subjected to extraction by cold extraction. The extraction was done with methanol and acetone solvents. All the extracts were concentrated by a rotary vacuum evaporator and the left-over solvent was evaporated to dryness using a water bath. The phytochemical analysis was carried out by Elgorashi and Staden (2004)<sup>[5]</sup> and Trease and Evans (2002)<sup>[17]</sup>.

### Antibacterial activity

The antimicrobial activity of synthesized silver nanoparticles was investigated applying the standard agar well diffusion assay Nanda and Saravanan (2009). The tested pathogens were speeded uniformly on nutrient agar plates using sterile spreader, then, five wells of 6-mm diameter (dm) were made using sterile well borer. Twenty micro litres of plant extracts with various concentrations (25, 50, 75, and 100 µg/ml) was poured into the all wells. The plates were incubated at 37 °C for 18 h for the bacterial after incubation the zone of inhibition were recorded.

### Anticancer activity

#### Cell culture

Estrogen receptor (ER)-positive cancer cells were studied in this study. The cells were cultured in DMEM- Dulbecco's Minimum Essential Medium with 10% FBS-fetal bovine serum and 50g/mL gentamicin. The cells were incubated at 37 °C in CO<sub>2</sub> incubator in an atmosphere of humidified 5% CO<sub>2</sub> and 95% air. The cancer cells growing in the exponential phase were used for cell viability assay.

**Table1:** Showing the phytochemicals present in the *Momordica charantia* leaves extracts.

Tests	Methanol Extract	Acetone Extract
Alkaloid	++	++
Glycoside	+	+
Steroid	+	++
Flavonoid	—	+
Saponin	++	++
Reducing sugars	—	—
Tannin	++	+

### Cell viability by MIT assay

After 72 h, the media of treated cells (100 µL), were detached and the cell culture were incubated with 50 µL of MTT at 37 °C for 4 h period. After incubation, the formazan produced was then solubilized by the addition of 100 µL dimethyl sulfoxide. The suspension was placed on a micro-vibrator for 5 min and then the absorbance was recorded at 540 nm by an ELISA-Enzyme-linked immunosorbent assay reader and the results were analyzed in triplicate and the percentage was calculated (Mosmann 1983).

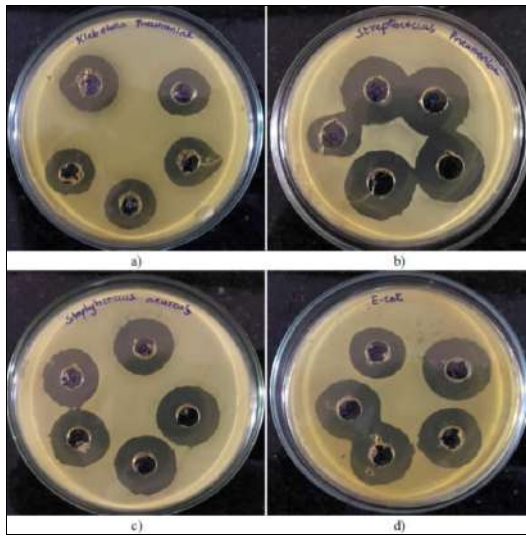
## Results and Discussion

Plants are the most important source for all kind of food and medicine. From ancient time to modern world it is not possible to manufacture the medicine without plant or it's photochemical. They are valuable source of natural active constituents that are used to maintain human health and also used for the treatment of many human diseases (Stary and Hans, 1998)<sup>[15]</sup>. The use of medicinal plants for using in diseases treatment is as old as the human species. Accepted clarification on the use and effectiveness of medicinal plants considerably contribute to the disclosure of their medicinal properties, so that they are regularly prescribed, even if their phytochemical constituents are not always completely known (Silva *et al.*, 2010)<sup>[14]</sup>. The phytochemical result tabulated in Table1 was found to be Alkaloid, Glycoside, Steroid, Flavonoid, Saponin, reducing sugars and Tannin for all the extracts. The result showed that all extracts contained appreciable amount of flavonoids and reducing sugars which was absent in both methanol and acetone extracts. (Sood *et al.* 2012)<sup>[15]</sup> reports that phytochemical analysis of the plant confirm the presence of various phytochemicals like cardiac glycosides, tannins, carbohydrates, terpenoides, saponins resins, and phytosterols. While other phytochemical like alkaloids, glycosides, flavonoids, steroidal terpenes and phylobatamins were found to be absent in all the extracts.

Plant inferred specialists are being utilized for the treatment of disease. A few anticancer specialists from plants include; vinblastine, taxol, vincristine, the camptothecin derivatives, topotecan and etoposide, and etoposide got from epipodophyllotoxin are in clinical utilize everywhere throughout the world. Various malignancy research studies about have been led utilizing customary restorative plants with an end goal to find new remedial operators that do not have the poisonous symptoms related with current chemotherapeutic specialists and the medications under clinical phytomedicines has expanded significantly over the most recent two decades. *Momordica charantia* leaves extracts were experimented to estimate their antibacterial capacity against pathogenic bacteria like two strains of Gram +ve bacteria and Gram -ve bacteria like *Klebsiella pneumoniae*, *Streptococcus pneumoniae* *Staphylococcus aureus* and *Escherichia coli* using well diffusion method. Evaluation of antibacterial activity of these *Momordica charantia* extracts was shown in Table2 and Figure1. The results were shown that the extracts were potentially powerful in inhibiting the microbial growth of pathogenic bacteria.

**Table2:** Showing the Antibacterial activity of *Momordica charantia* leaves extracts

Organisms	Methanol Extract (MCME)				
	10µg	25µg	50µg	75µg	100µg
<i>Klebsiella pneumoniae</i>	09	11	14	15	18
<i>Streptococcus pneumoniae</i>	08	10	13.5	16	20
<i>Staphylococcus aureus</i>	10	11	14	17	20
<i>Escherichia coli</i>	11	11	14	15	19
<i>B.cereus</i>	02	11	13	16	16
<i>P.aeruginosa</i>	0	—	—	—	—



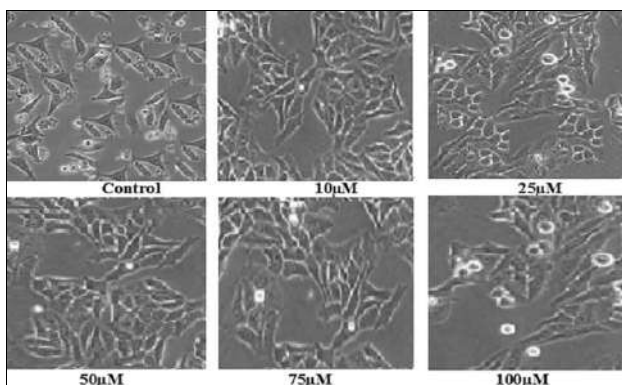
**Fig 1:** Antibacterial activity *Klebsiella pneumoniae*, *Streptococcus pneumoniae* *Staphylococcus aureus* and *Escherichia coli*.

In the present study *Momordica charantia* leaves were initially selected and tested for anticancer activity. The results on cytotoxicity of *Momordica charantia* leaves extracts on MCF 7 and HeLa cancer cell lines are shown in Table3.

**Table3:** Antiproliferative activity on human cells of fractions obtained from the *Momordica charantia* methanol- MCME and Acetone extract -MCAE.

Test Extract	Antiproliferative activity on Cell lines (IC50)	
	MCF7	HeLa
Methanol extract (MCME)	15.6±1.3	28.2±1.
Acetone extract (MCAE)	174.2±1.3	96.6 ± 1.3

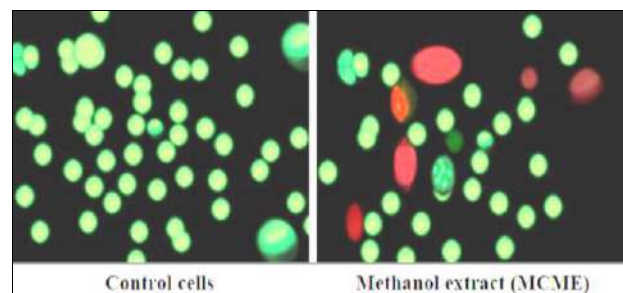
The evaluation of the anticancer activity of plant extracts is essential for safe treatment. It enables identification of the intrinsic toxicity of the plant and the effects of acute overdose (Padmaja *et al.*, 2002; Rahman *et al.*, 2016) [10, 12]. Cancerous cell lines were estimated by MTT assay. The methanol and acetone extract leaves of *Momordica charantia* has shown anticancer activity on selected cancer cell lines. Among the tested extracts, methanolic extract was found to have potent cytotoxicity against cancerous cell lines with MCF7 values ranging IC50 15.6 ± 1.3 (Figure2).



**Fig 2:** Antiproliferative activity on human cells of fractions obtained from the *Momordica charantia* methanol- MCME

The aromatic plants are a rich source of compounds with anticancer properties and deliver less harmfulness in normal cells. In this way, expanding consideration has been put on recognizing novel anticancer medication medicines from regular sources (Mukherjee *et al.*, 2001; Wang *et al.*, 2010; Desai *et al.*, 2008) [8, 3, 19]. Most against tumour drugs are proposed to dispose of rapidly multiplying dangerous cells, and along these lines, they commonly show cytotoxicity and actuate apoptosis in disease cells (Kaufmann and Earnshaw, 2000). Apoptosis is an outstandingly composed cell death procedure described by loss of plasma layer phospholipid asymmetry, enzymatic cleavage of the DNA into oligonucleosomal sections, and division of the cells into film bound apoptotic bodies (Cotter, 2009). The present examination additionally explored the induction of apoptosis in breast tumour cells upon treatment with ethanolic concentrates of WS and TC Acridine orange-ethidium bromide assay and Hoechst 33,342 assay by fluorescent microscopy revealed that the ethanolic extracts of WS and TC instigated apoptosis, but not necrosis, in breast cancer cells. DNA fragmentation is a hall mark property of apoptosis (Bortner *et al.*, 1995) and DNA fragmentation assay further corroborated the ethanolic extracts of WS and TC induced apoptosis in breast cancer cells.

Apoptosis is the procedure of induction of altered cell death, and any variations in the normal in the typical pathways ensnared in apoptosis prompts minimal apoptosis and the protection to apoptosis initiates enhanced cell progression, which is ensnared in advancing disease (Kerr *et al.*, 1972) [7]. During the induction of apoptosis, the cancer cells are subjected to beginning of events that include changes in both morphological and biochemical characteristics of the cell (Raff, 1992) [11].



**Fig 3:** AO/EtBr staining of the MCF-7 cells. (a) Control – viable cells shows green stained nuclei. (b) Extract treated MCF-7 cells showing the red colour seems dead cells.

In the present investigational study, both normal and breast cancer cells were subjected for the examination of morphological changes incited by *Momordica charantia* methanol extract separate (MCME) A major change in the cell morphology was observed in the breast cancer cells with cell shrinkage, cell wall blebbing and reduction in cell population in *Momordica charantia* methanol extract (MCME) treated cells contrasted to the untreated cells Figure3.

There were no such critical alterations seen in the *Momordica charantia* methanol extract (MCME) treated normal cells which indicated the nontoxic nature of the compound toward the normal breast cell lines. Most anti-cancer compounds or drugs are projected to remove quickly proliferating malignant cells, and therefore, they typically



indicate cytotoxicity and induce apoptosis in cancer cells (Kaufmann & Earnshaw, 2000). Apoptosis is a especially formed cell death process characterized by loss of plasma membrane phospholipid asymmetry, enzymatic cleavage of the DNA into oligonucleosomal fragments, and segmentation of the tumour cells into membrane-bound apoptotic bodies (Cotter, 2009). The present study also examined the induction of apoptosis in breast cancer cells upon treatment with Methanolic extracts (MCME). Acridine orange-ethidium bromide experiment and Hoechst 33,342 assay by fluorescent microscopy revealed that the Methanolic extracts of (MCME) instigated apoptosis, but not necrosis, in human breast tumour cells. DNA fragmentation is a trait property of apoptosis (Bortner *et al.*, 1995).

### Conclusion

Plants play a vital role in health care applications with about 80% of the world's populations depending on the use of conventional medicine which is primarily based on plants. In the present study the phytochemical characterization, Antibacterial and anticancer studies of the extract were shown remarkable results thus the identification of liable bioactive compounds and quality standards are necessary for future study.

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