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ANTIMICROBIAL, ANTHELMINTIC AND CYTOTOXIC ACTIVITIES OF METHANOLIC EXTRACTOF *CUCUMIS MELO* LINN (F1 HYBRID)

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ABSTRACT

Cucumis melo Linn (C.melo) is a fruit used in a different part of the world with many medicinal properties. The present study describes the anthelmintic, antimicrobial and cytotoxic activity of *Cucumis melo* Linn. Adult earthworms (Eudrilus eugeniae) of approximately the same length were used for anthelmintic activity. The methanolic extract *Cucumis melo Linn* (MECM) used in the present study showed significantly less anthelminthic activity. Anti-bacterial activity was performed on *S. aureus*, and *E. coli* as test organism and anti-fungal on *Candida albicans* and *Aspergillus niger*. How ever there was no significant antibacterial activity. The *in vitro* cytotoxic potential of MECM was studied on THP-1 cell lines(Human Monocytic leukaemia), A-549 cell lines (Lung cancer), HeLa cell lines (Cervical carcinoma), MG-63 cell lines (Human osteosarcoma).The results of anticancer activity of MECM against various cell lines showed that it possesses antiproliferative in all human tumour cell lines subjected to test in a dose-dependent manner.

KEYWORDS: *Candida albicans* and *Aspergillus niger*.

INTRODUCTION

Cucumis melo Linn (Muskmelon) is a member of the Cucurbitaceae family Genus Cucumis

that is having about 118 genera and 825 species. In China the fruit is cultivated since 2000 BC.[1] Wide variety of fruit forms have evolved around the world widely spread in the tropical and sub- tropical region. The highest producers of muskmelon are China and the USA. *Cucumis melo* Linn is a fruit used in a different part of the world.[2] It possesses various medicinal properties. The present study mainly focuses on the Antimicrobial, Anthelmintic and Cytotoxic activities of methanolic extract of *Cucumis melo Linn*.

The herb is widely cultivated throughout India, especially in dry and hot North- Western areas.[3] It is a climbing or creeping herb, which is annual. The herb is with angular, scabrous stem, orbicular-reniform leaves which is simple soft hairy with tendrils. Bright yellow flowers are unisexual, with 5 wrinkled petals which may be oval to egg-shaped. Its aerial parts, fruit pulp, seeds, seed oil, and roots are used medicinally for the treatment of various diseases. Its nephroprotective, antimicrobial, Anthelmintic, antioxidant, cytotoxic activity, anti-hyperlipidemic, analgesic, anti-inflammatory, diuretic, thyroid stimulatory activity have been established by research studies.[4]

The ethanolic seed extract of C.*melo* possesses anthelmintic activity which might be due to the presence of certain phytochemicals like saponins which are present exclusively in seeds, which were identified by HPTLC method.[5] Earlier studies suggest that saponins like Dioscin and Polyphyllin D exhibited significant activity against *D. intermedius.*[6]

In a study on the antimicrobial and antifungal activity of *C.melo* the possibility of containing metabolites which act against resistant bacterial pathogens were investigated and the crude extract of *C.melo* against fungal strain *C.albicans* as well as bacterial strains like *E. coli, K. pneumoniae, S. paratyphi, S. aureus* was investigated. The whole extract in acetone with *S. Paratyphi and E. coli* showed maximum activity. The response was feeble with aqueous extract in all bacterial and fungal strains. Also, acetone extract of *C.melo* shows the highest antimicrobial activity against the bacteria *E. coli*. The whole fruit extract was reported to be effective against gram-positive and gram-negative bacteria. In contrary, no anti-bacterial or anti-fungal activity was observed up to a concentration of 1000 µg/ml, when tested using agar gel diffusion assay in *E. coli, A.niger* and *C.albicans.*[7]

The MTT assay is a colorimetric assay for assessing cell metabolic activity. Tetrazolium dye reduction is generally assumed to be dependent on NAD(P)H-dependent oxidoreductase enzymes largely in the cytosolic compartment of the cell.[8] The advantages with celllines

anlysis include, easiness to handle, the important characteristic can be manipulated, genetically/epigenetically and pharmacologically. A high degree of homogeneity is the disadvantage since tumor cells are naturally heterogeneous. However, this can be avoided by using a panel of cancer cell lines, which represent the heterogeneity seen in the primary tumours.[9]

MATERIALS AND METHODS

Chemicals

The chemicals and drugs used for the study were of Pharmacopoeia/ analar or HPLC grade as required by the nature of experiment or extraction or as the case may be. They were purchased from licensed distributors of Central Drug House, Himedia Labs or Sigma-Aldrich chemicals.

Procurement of research raw material

The material used for the study was fruits of *Cucumis melo* Linn (Family: Cucurbitaceae) were purchased from Vadanerkunam, Tindivanam T.K, Villupuram Dt, Tamilnadu and identified and authenticated by experts of Department of Botany, St. Berchmans College, Changanacherry, Kottayam-686101.

Preparation of fruits for extraction

The fruits of *C. melo* were cut into pieces with the stainless steel knife. Weighed approximately 100 g, (including the seeds) and the fruit pieces together with the seeds were dried in an oven at 60^0C. The dried fruits were then comminuted to a coarse powder, and the powder passed through No.10 sieve and was used for the extraction process.

Preparation of extract

The coarse powder prepared from the fruits and seeds were extracted with methanol (absolute) by hot continuous percolation process. For extraction, portions of 100 g of the dry powder were packed in filter paper thimble each time and extracted in a Soxhlet extractor (5 cycles a day for three consecutive days). The marc was then further extracted with 95% methanol till the extractive became almost colorless. The process of extraction was repeated with fresh powder, and the entire alcoholic extractives were combined, and most of the solvent was recovered by distillation under reduced pressure.

The combined extracts were then evaporated under vacuum to form a soft extract. The final

extract was weighed, re-heated and again weighed to make sure that it was free from alcohol. The yield was calculated as percentage weight per weight of powder used, by dividingthe total weight of extract obtained by total weight of dry material used for extraction and multiplying the result with 100.

The extract was then stored in vacuum desiccators over dry silica gel and used for further studies. The methanol extract of *Cucumis melo* will be from now referred to as MECM.

Evaluation of anthelmintic activity[10]

Adult earthworms (*Eudrilus eugeniae)* of approximately the same length were used for the study. MECM was solubilised in a minimum amount of DMSO and dilutions containing 100, 250 and 500 mg% solutions in normal saline were used for the test. Piperazine citrate (GSK) solutions in normal saline having the same concentrations served as the standard. Normal saline containing DMSO was kept as the control.

All dilutions were pipetted into petri dishes of the same diameter. Carefully placed six earthworms of same sizes to each petri dish. Maintained all the worms at room temperature and carefully observed for symptoms of paralysis or death. The time taken for paralysis or death was noted. If the worm was found to be non-motile for at least 3 min and its body colour faded away, was considered as dead.

Antibacterial and antifungal activity[11,12]

To assess the ability of MECM to inhibit bacteria, the antibacterial activity was determined by agar well diffusion method. Nutrient Agar was used as the culture medium and *S. aureu*s (ATCC NO.6538 P), and *E. coli* (ATCC NO 8739) was used as the test organism. Nutrient agar plates were inoculated with bacterial strain under aseptic conditions and wells were filled with 150 µl of the extract (1 mg/ml), which was incubated at 37°C for 24 hours. After the period of incubation, the diameter of the growth zone was measured. Methanol was the control used, and Ampicillin was used as standard.

Different species of fungus such as *Candida albicans* and *Aspergillus niger* were swabbed in potato dextrose agar plates and grown overnight. Wells of about 10 mm was boredusing a well cutter, and different concentration of samples of MECM was added. The plates were incubated overnight to measure the zone of inhibition and compared with that of standard Clotrimazole.

Evaluation of cytotoxicity of MECM- MTT assay[13-14]

The *in vitro* cytotoxic potential of MECM was studied on THP-1 cell lines (Human Monocytic leukaemia), A-549 cell lines (Lung cancer), HeLa cell lines (Cervical carcinoma), MG-63 cell lines (Human osteosarcoma). Cells were initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained in Dulbecco's modified Eagles medium, DMEM (Sigma Aldrich, USA).

The cells line was sub-cultured in RPMI 1640 media (Thermo Fischer) supplemented with 20% heat-inactivated foetal bovine serum (Sigma). Bacterial contamination was prevented by antibiotics Penicillin and Streptomycin (100 units) and incubated at 37°C with 5% CO_2 in a carbon dioxide incubator (Eppendorf) for 24 h. MECM was added to the 96 well plates containing cells in concentrations of 6.25, 12.5, 25, 50 and 100 µg/ml in 0.1% Dimethyl sulfoxide (DMSO) and incubated for 24 h. The standard drug was 5-Fluoro Uracil (5-FU) 25 µg/ml for all cell lines except THP-1 cell lines. Taxol 10, 25, 50, 100 µg/ml concentration was used for THP-1 cell lines, and MECM in the same concentrations were used as test.

The cell culture suspension was added with 200 µl MTT solution. (MTT-5 mg/ml dissolved in PBS). Then it was incubated at 37ºC for 3 h and removed excess MTT reagent and washed with 1x Phosphate Buffered Saline (PBS) and added 300 µl Dimethyl sulfoxide (DMSO) to each culture tube. The tubes were kept at room temperature for 30 min until the cells are lysed and the colour was obtained. The solution was transferred to centrifuge tubes and centrifuged at 3000 rpm for 2 min to precipitate cell debris. Optical density (OD) of the supernatant was read at 540 nm using DMSO as blank in a microplate reader The % viability was calculated from the OD as follows.

$$\% \text{ of viability} = \frac{\text{Mean OD Samples x 100}}{\text{Mean OD of the control group}}$$

RESULTS AND DISCUSSIONS

Antibacterial activity and antifungal activity

For determining the antimicrobial activity, of MECM agar well diffusion method was used. Anti-bacterial activity was performed on *S. aureu*s (ATCC NO.6538 P), and *E.coli* as test organism and anti-fungal on *Candida albicans* and *Aspergillus niger*. How ever no significant antibacterial activity (fig-1) or antifungal activity (Fig-2) was shown by MECM.

Evaluation of anthelmintic activity

Evaluation of the anthelmintic activity of MECM in comparison with Piperazine in varying concentrations was done by monitoring paralysis/death occurred in earthworm with respect to time of exposure to the test material (Table-1)control constituted 1% CMC in normal saline. Even though there were previous report on Anthelmintic activity on other extracts, the methanolic extract used in the present study showed no activity.(Fig-3)

Evaluation of cytotoxicity of MECM: MTT assay

Evaluation of the effect of MECM on cell viability by MTT assay on THP-1

The effect of MECM on the viability of cultured THP1 cells was studied at different concentrations of 10, 25, 50 and 100 µg/ml. (Table 2).Taxol in similar concentrations was used as the standard. It was observed that Taxol showed significant ($p< 0.001$) reduction in cell viabilityat the concentrations employed. (Fig 4)The observed viability for 10,25,50 and

100 µg/ml concentrations of MECM were (Mean ± SEM) 89.63 ±0.43, 88.4 ± 0.89, 83.83 ± 0.33 and 80.99 ±1.08 respectively. In similar concentrations with exposure to Taxol exhibited viability of 79.63 ± 2.63, 74.7 ± 0.214, 69.59 ±0.215 and 66.3 ± 1.7 in comparison with the control, which is taken as100 % viable, the reduction of viability was significant at $p<0.001$. (Fig-5).

Evaluation of the effect of MECM on cell viability by MTT assay on A-549 cells

The effect of MECM on the viability of cultured A-549 cells was studied at different concentrations of 6.25, 12.5, 25, 50 and 100 µg/ml. 5-Fluorouracil in a concentration of 25 µg/ml was used as a standard. It was observed that 5-Fluorouracil showed significant ($p< 0.001$) reduction in cell viability at the concentrations employed. The observed viability for 6.25, 12.5, 25, 50 and 100 µg/ml concentrations of MECM were (Mean ± SEM) 98.37 ± 0.5378*,96.75 ±0.2033**, 95.32 ± 0.2689***, 93.90 ± 0.3520***, 90.04 ±1.172***, respectively. In concentration of 25µg/ml, 5- Fluorouracil exhibited viability of 32.72 ± 1.497***. The results are tabulated in Table 03.The results of MTT assay to evaluate the anticancer activity of MECM against various cell lines showed that it possesses antiproliferative in all human tumour cell lines subjected to test in a dose-dependent manner. (Fig 6). Among all cell lines tested maximum activity was observed in human leukaemia cells. Moreover, it is reported that[15] the aqueous extract of Cucumis melo repressed the cell proliferation in a dose-dependent manner in PC-3 cell lines also. New compounds with anticancer properties, which are less toxic and more effective than synthetic drugs, are the main

motivating factor for research on plant-based drugs. The substances currently used in therapy include alkaloids, flavonoids, phenolics and terpenes. Similar compounds have been identified in MECM also. (Fig-7)The combined effect of all or any of these compounds may be responsible for the cytotoxic activity. Our results were supporting the reports of other researchers. Further studies are essential to find the mechanism of action and also the components accountable for its effect.

Evaluation of the effect of MECM on cell viability by MTT assay on HeLa cells

The effect of MECM on the viability of cultured HeLa cell lines was studied at different concentrations of 6.25, 12.5, 25, 50 and 100 µg/ml. It was observed that 5 Fluorouracil showed significant ($p< 0.001$) reduction in cell viability at the concentrations of 25µg/ml. (fig 8) The observed viability for 6.25, 12.5, 25, 50 and 100 µg/ml concentrations of MECM were (Mean ± SEM) 98.75 ± 0.2506, 96.24 ± 0.7519, 92.73 ± 0.5462, 91.6 ± 0.5462, 88.6 ± 0.5462 respectively. (Fig-9)In concentration of 25 µg/ml, 5- Fluorouracil exhibited viability of 35.21 ± 0.5462. The results are tabulated in Table 04.

Evaluation of the effect of MECM on cell viability by MTT assay on MG-63cells

The effect of MECM on the viability of cultured MG63 cells was studied at different concentrations of 6.25,12.5,25,50 and 100 µg/ml.(Fig-10) It was observed that 5- Fluorouracil showed significant ($p< 0.001$) reduction in cell viability at the concentration of 25µg/ml. The observed viability for 6.25,12. 5,25,50 and 100 µg/ml concentrations of MECM were (Mean ± SEM) 98.8 ± 0.300, 97.1±0.454, 94.8± 0.600, 92.6 ± 1.28, 91.2 ±1.04 respectively. In concentration of 25µg/ml. 5- Fluorouracil exhibited viability of 33.6 ± 0.494.(Fig-11). The results are tabulated in table 05.

Tables and figures

Fig-1: Antibacterial Activity.

1-Standard (ampicillin 0.1mg/ml) zone diameter-28mm

2-Control(methanol) zone diameter-nil

3-MECM zone diameter-11mm

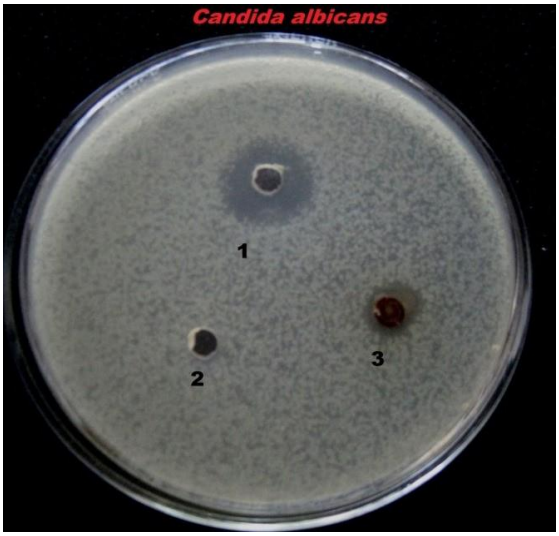


Fig-2: Antifungal Activity

1-Standard (clotrimazole 0.1mg/ml) zone diameter-22mm

2- Control(methanol) zone diameter-nil

3- MECM zone diameter-10mm

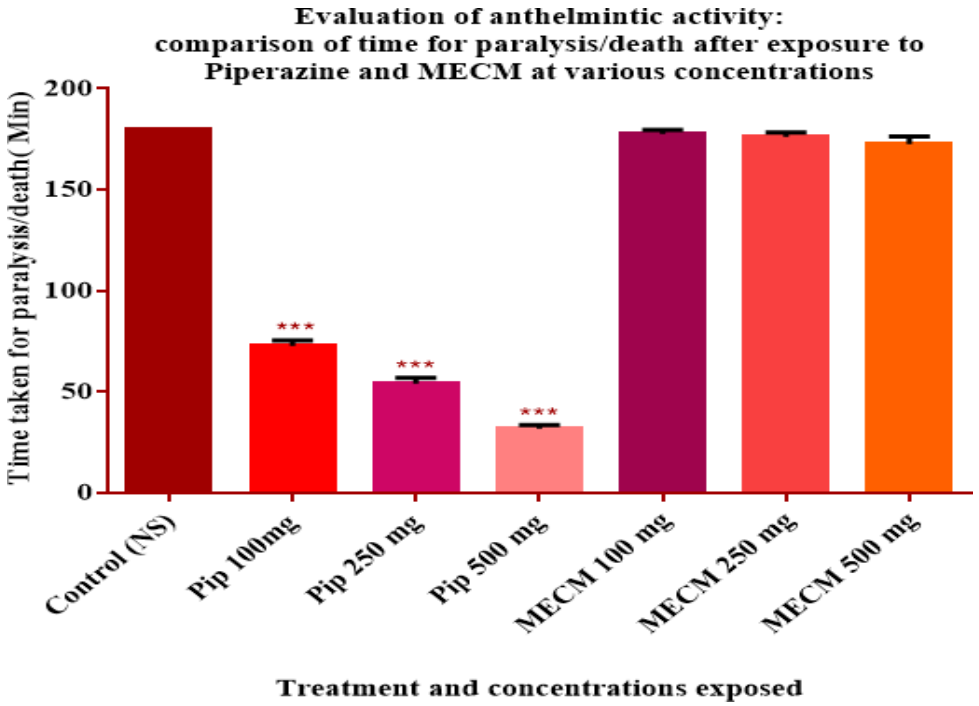


Figure.03: Evaluation of the anthelmintic activity of MECM: Comparison of time (Minutes) required for paralysis/death after exposure of earthworms to varying concentrations of Piperazine and MECM.

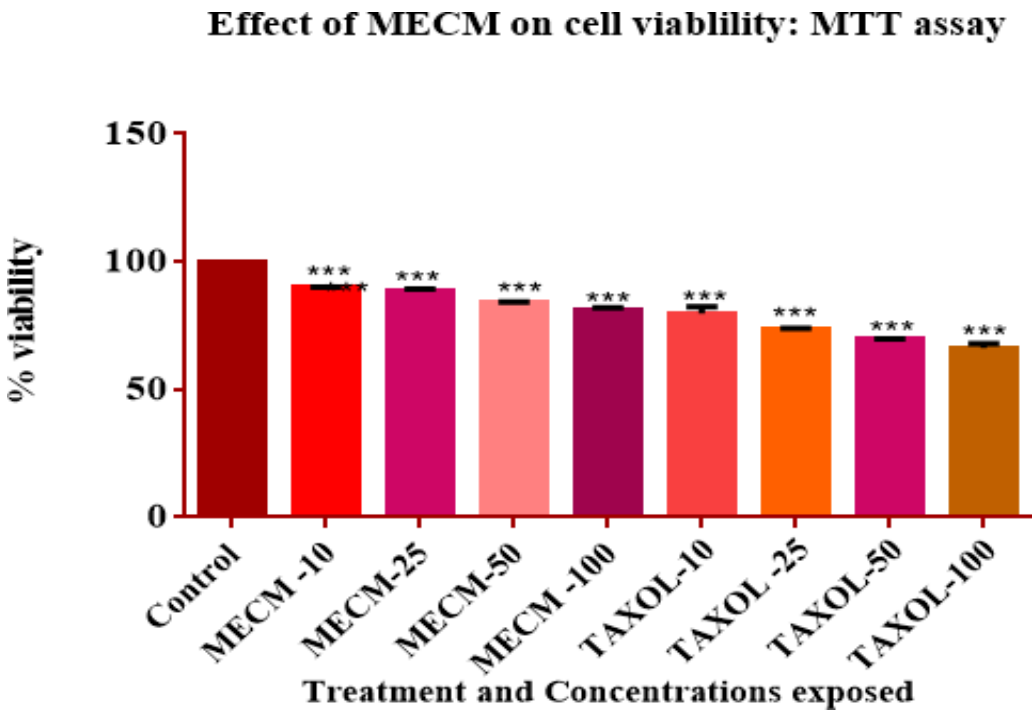
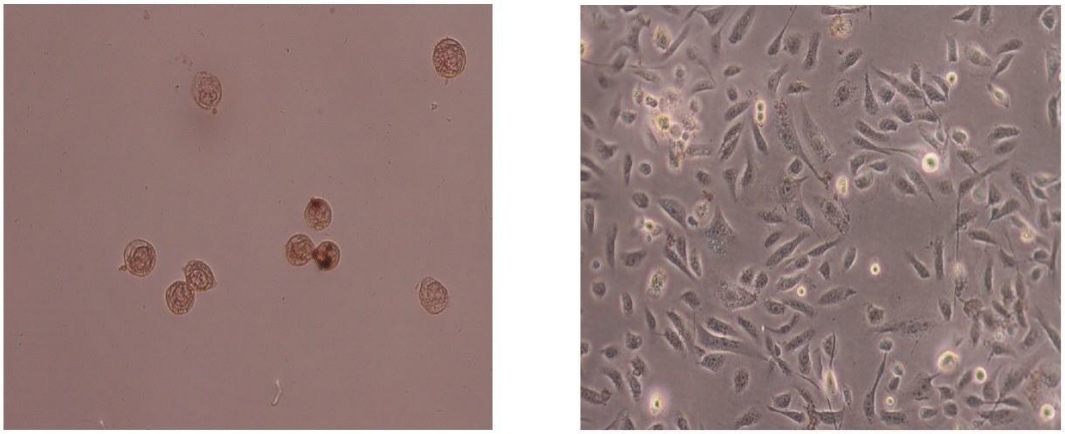


Figure.4: Comparison of reduction of cell viability of THP-1 cell lines after exposure to MECM andTaxol at concentrations of 10, 25, 50 and 100 µg/ ml.



(a) (b)

(a) MTT assay THP-1 cell lines 100µg/ml concentration of MECM

(b) controlFigure 5

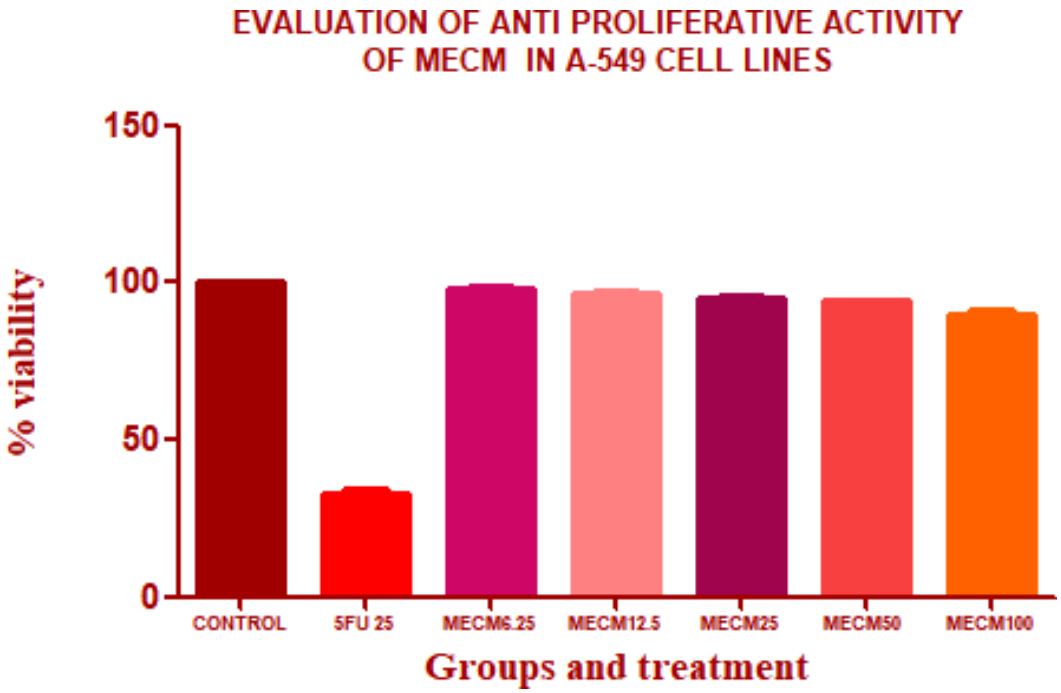


Figure.6: Evaluation of the anti-proliferative activity of MECM in A-549 cell lines.

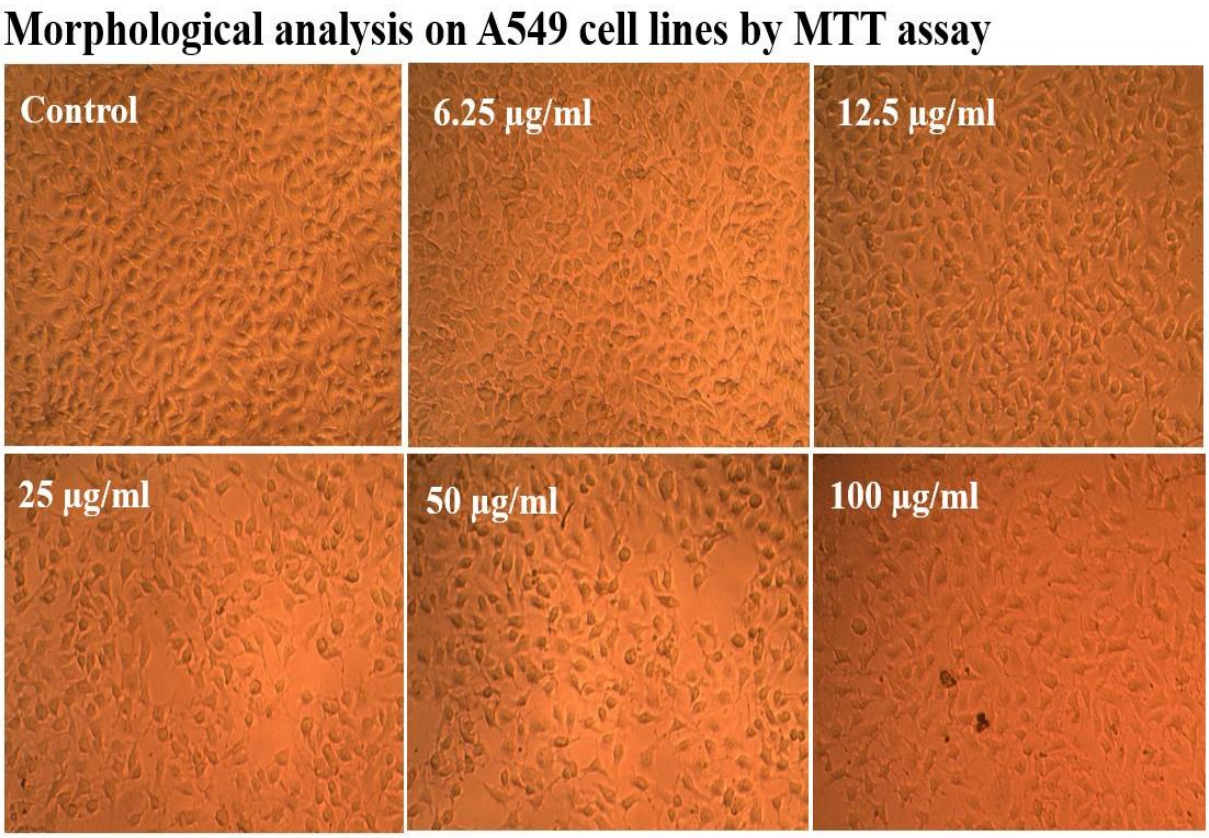


Figure. 7: Anti-proliferative activity of MECM in A-549 cell lines.

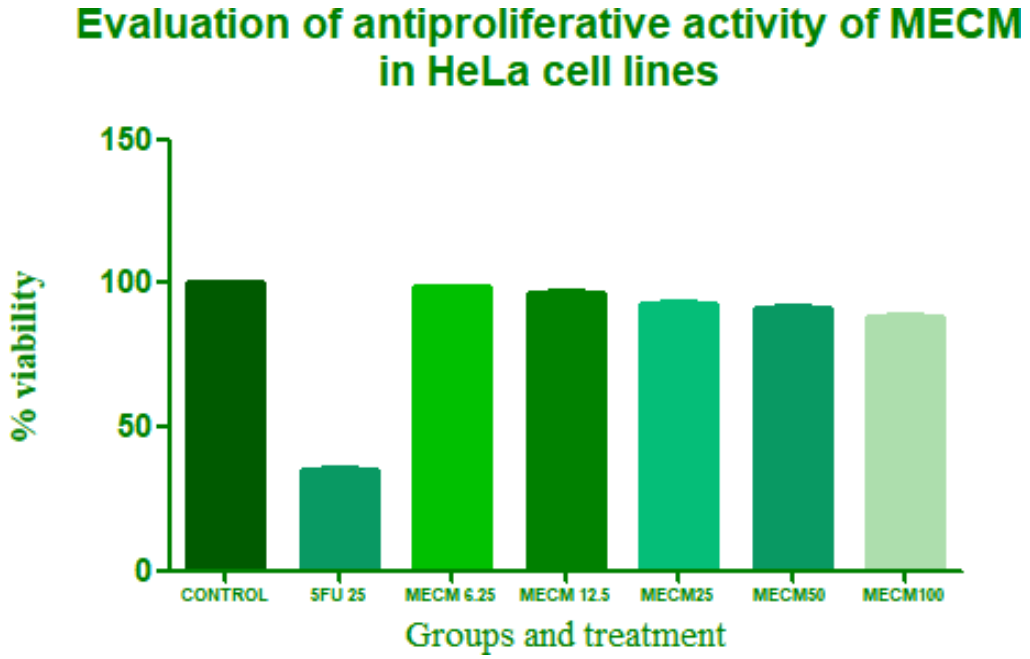


Figure.8: Evaluation of the antiproliferative activity of MECM in HeLa cell lines.

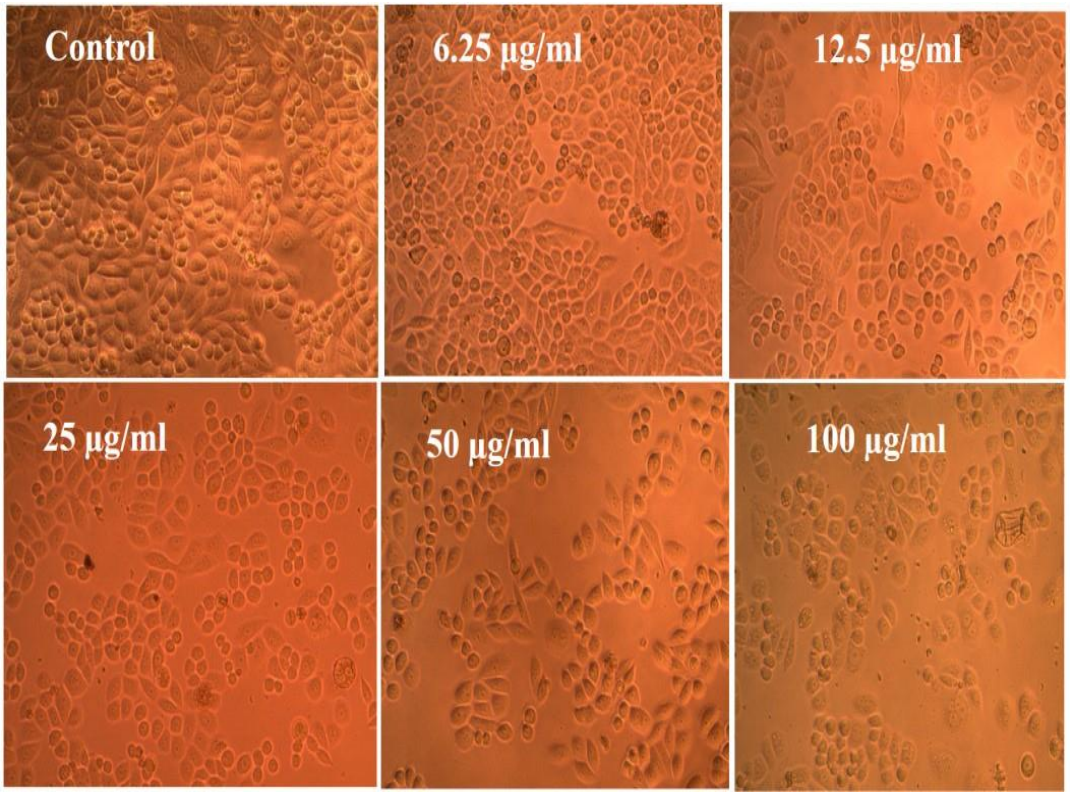


Figure.9: Antiproliferative activity of MECM in HeLa cell lines.

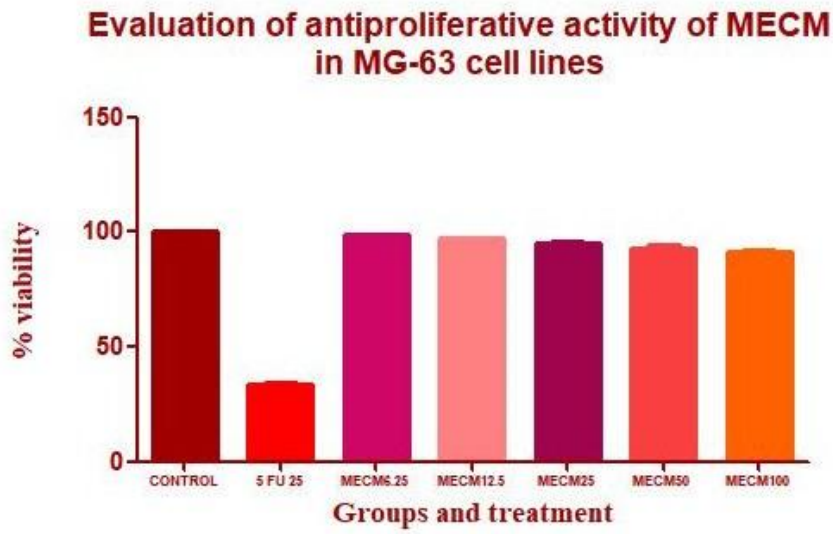


Figure.10: Evaluation of the antiproliferative activity of MECM in MG-63 cell lines.

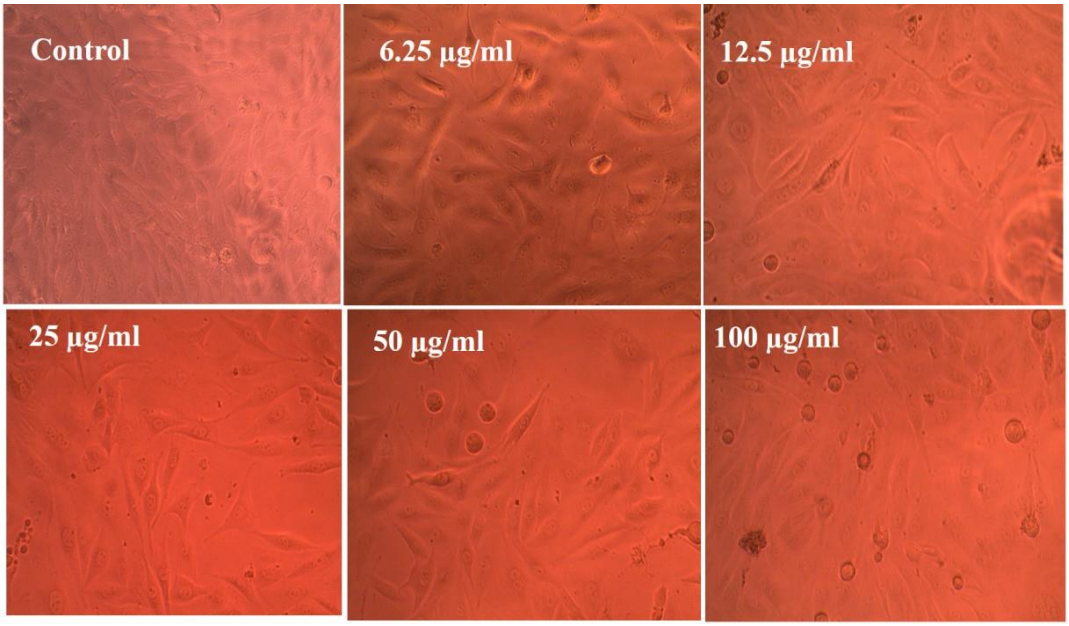


Figure.11.

Table 1: Evaluation of anthelmintic activity of MECM.

SL	Group	Exposed Concentration(mg/dl)	Time for paralysis In minutes (Mean ± SEM)
1.	**Control**	**1% CMC + NS**	180 ± 0.00
2.		100	72.67 ± 2.67***
3.		250	54.17 ± 2.903***
4.		500	31.83 ± 1.701 ***
5.		100	177.2 ± 1.961^{NS}
6.		250	176.2 ± 2.072^{NS}
7.		500	172.7 ± 3.748^{NS}
*** Significant<0.001, Ns: Not significant, p<0.001, For N=6, .with respect to control group, ANOVA with Bartlets corrected statistics, F=768.6.			

Table 2: Evaluation of the effect of MECM on cell viability by MTT assay on THP-1.

Sl no	Exposed concentration (µg/ ml)	Treatment group and % viability (Mean ±SEM)		
		Std (i) TAXOL	MECM (ii)	Control (iii)
1.	**10 (A)**	79.63± 2.63 *	89.63 ±0.43*	
2.	**25 (B)**	74.7 ± 0.214*	88.4 ± 0.89*	
3.	**50 (C)**	69.59 ±0.215*	83.83 ± 0.33*	
4.	**100 (D)**	66.3 ± 1.7*	80.99 ±.1.08*	
Significant, P<.001, ANOVA, multiple comparison, all column against control (Dunnets), N=3. F= 83.32				

Table 03: Evaluation of the anticancer activity of MECM by MTT assay on A-549 cell lines.

Sl No	Concentration(µg)	% viability among groups		
		Control	5-FU	MECM
1.	6.25			98.37 ± 0.5378*
2.	12.5			96.75 ±0.2033**
3.	25		32.72± 1.497***	95.32 ± 0.2689***
4.	50			93.90 ± 0.3520***
5.	100			90.04 ±1.172***
*Significant, p<0.05,** significant, p<0.01, *** P< 0.001, ANOVA,& Dunnets multiple comparison with control, F= 976.				

Table.04: Evaluation of the antiproliferative activity of MECM in HeLa cell lines.

Slno	Concentration(µg)	% viability among groups		
		Control	5-FU	MECM
1.	6.25			98.75 ± 0.2506***
2.	12.5			96.24 ±0.7519***
3.	25		35.21 ± 0.5462***	92.73 ± 0.5462***
4.	50			91.6 ± 0.5462***
5.	100			88.6 ± 0.5462***
*** P< 0.001, ANOVA, & Dunnets multiple comparison with control, F= 2002.				

CONCLUSION

Many research works are being done today to evaluate the therapeutic benefits of plants drugs and to isolate the active phytochemical entity responsible for the pharmacologic response. Several phytochemical constituents are being identified in the methanolic extract of F1 hybrid of *C.melo* in the present study. The methanolic extract *Cucumis melo* Linn (MECM) extract used in this study had no anthelminthic activity. *S. aureus* and *E. coli* were used as test organisms for antibacterial activity, and *Candida albicans* and *Aspergillus niger* were used for antifungal activity. However, no substantial antibacterial or antifungal activity activity was detected. But THP-1 cell lines (Human Monocytic Leukemia), A-549 cell lines (Lung cancer), HeLa cell lines (Cervical carcinoma), and MG-63 cell lines (Human osteosarcoma) were used to assess MECM's in vitro cytotoxic capacity MECM's anticancer activity against different celllines revealed that it has antiproliferative properties in all human tumor cell lines tested in a dose-dependent manner. It's hard to attribute this to a single compound. As a result, one or more natural phytochemicals with potentially beneficial medicinal properties could be discovered and extracted from the fruits.

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