

IN VITRO CYTOTOXIC ACTIVITY OF NOVEL OLEANANE TYPE OF TRITERPENOID SAPONIN FROM STEM BARK OF *MANILKARA ZAPOTA* LINN

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ABSTRACT

Cancer is a disease in which there is an uncontrolled multiplication and spread of abnormal forms of the body's own cells. It is a major public health problem worldwide with millions of new cancer patients diagnosed each year and many deaths resulting from this disease. Chemotherapeutic agents though effective against various types of cancers, are not totally free from side effects and not cost effective. Drug discovery from medicinal plants has played an important role in the treatment of cancer and most clinical applications of plant secondary metabolites and their derivatives over the half century have been applied towards combating cancer. Saponins are a group of naturally occurring plants secondary metabolites i.e. glycosides, characterized by their strong foam-forming properties in aqueous solution. There are more than 11 distinguished classes of saponins including dammaranes, tirucallanes, lupanes, hopanes, oleananes, taraxasteranes, ursanes, cycloartanes, lanostanes, cucurbitanes and steroids. Due to the great variability of their structures, saponins always display anti-carcinogenic effects through varieties of anticancer pathways. Manilkoraside, a new saponin is first time isolated from the ethanolic extract of stem barks of *Manilkara zapota* Linn. In the present study, anticancer activity of ethanolic extract and manilkoraside investigated on different cancer cell lines such as HL-60, HT-29, A 549, A 431 and MCF-7. The activity was assessed by trypan blue dye exclusion assay for cell viability and MTT based cytotoxicity assay. The results of the MTT based cytotoxicity assay revealed that ethanolic extract and manilkoraside demonstrated potent inhibition of cancerous cell growth against HL-60 and HT-29 with EC₅₀ Value 24µg/ml and 64µg/ml for ethanolic extract and 20µg/ml and 48µg/ml for manilkoraside respectively. A moderate cytotoxic activity was observed against A 549, A 431 and MCF-7 with EC₅₀ values greater than 80µg/ml.

Keywords: *Manilkara zapota*, Manilkoraside, Anticancer, HL-60, HT-29, A 549, A 431, MCF-7

INTRODUCTION

Cancer is a serious clinical problem that possesses significant social and economic challenges to the healthcare system. Cancer is the major public difficulty and one of the top cause of death in the prosperous countries. Despite improved imaging and molecular diagnostic techniques, cancer continues to affect millions of people globally. In many countries, cancer is the second leading cause of death after heart diseases. Overall survival rate has only improved slightly despite advances in surgery, radiotherapy, and chemotherapy. Most of the anticancer drugs currently used in chemotherapy are cytotoxic to normal cells and cause immunotoxicity which affects not only tumour development, but also aggravates patient's recovery. The discovery and identification of new antitumor drugs with low side effects on immune system has become an essential goal in many studies of immunopharmacology¹.

Conventional plants are valuable source of novel cytotoxic agents and are still in performance better role in health concern. Throughout history and crosswise the world, the plant kingdom has provided a diversity of medicines for cancer treatment. The World Health Organization (WHO) has estimated that approximately 80% of the world's population depends on traditional medicines for meeting their primary health care needs. The areas of cancer and infectious diseases have a leading position in utilization of medicinal plants as a source of drug discovery. Among FDA approved anticancer and anti-infectious preparations drugs of natural origin have a share of 60% and 75% respectively².

Molecular targeted agents are currently being studied in all treatment settings including that of chemoprevention, which is defined as the use of natural or synthetic non-essential dietary agents to interrupt the process of carcinogenesis and to prevent or delay tumour growth. Plants have been a prime source of highly effective conventional drugs for the treatment of many forms of cancer. There is considerable interest in the screening of plant and other natural product extracts in modern drug discovery programs, since structurally novel chemotypes with potent and selective biological activity may be obtained³. Phytoconstituents like saponins^{4,5}, flavonoids⁶ and coumarins⁷ have been used to possess potential anticancer activity as well as another mutual activity.

Manilkara zapota Linn. (Sapotaceae), is an evergreen, glabrous tree, 8-15 m in height. It is cultivated throughout Indian subcontinent including Maharashtra and Bangladesh, though it is native to Mexico and Central America⁸. The seeds of *Manilkara zapota* are aperients, diuretic tonic and febrifuge. Stem bark is astringent and febrifuge. The leaves and barks are used to treat cough, cold, dysentery and diarrhoea⁹. Antimicrobial¹⁰ and antioxidant^{11,12} activities are also reported from the leaves and stem barks of *Manilkara zapota* Linn. Phytochemical screening showed presence of carbohydrate, saponin, tannin and flavonoid¹³.

Saponin glycosides are amphiphilic in nature with highly surface active property. Their biological activity is closely related to chemical structures that determine the polarity, hydrophobicity and acidity of compounds. Because of heterogeneity in their chemical structures, saponins always display anti-carcinogenic effects through varieties of anticancer pathways. Hence this work is aimed to isolate and characterize one of the active phytoconstituent i.e. saponin from ethanolic extract of stem barks of *Manilkara zapota* Linn. and screen it for its anticancer potential.

MATERIALS AND METHODS

MATERIALS

Solvents

95% Ethanol, Methanol, Diethyl ether, Chloroform, DMSO (Dimethyl sulphoxide)

Reagents

Trypan blue (Hyclone, Lot no: JRH27098), EDTA (MP Biomedicals, Lot No: 6941H), Trypsin (Invitrogen, Lot No: 1376596), MTT (Roche applied sciences, Cat. No: 11465 007 001)

Media

RPMI-1640 (Sigma Aldrich Ltd. Mumbai), FBS (Fetal Bovine Serum) (Bioclot, Lot No: 07310)

Equipments

Fluorescence inverted microscope (Leica DM IL), CO₂ incubator (RS Biotech, mini galaxy A), ELISA plate reader (Lab system Multiscan),

Melting point apparatus (Veego Melting Point apparatus-VMP-PM), IR (Shimadzu-8400S FTIR spectrophotometer), APCI-MS (Atomic Pressurized Chemical Ionization-Mass Spectroscopy) (Varian Inc, USA-410 Prostar Binary LC with 500 MS IT PDA Detectors spectrophotometer), ^1H NMR (Varian Mercury YH-300 MHz ^1H NMR spectrophotometer)

Cell lines and culture conditions

Five cell lines, HL-60 (Human leukemia cell lines), HT-29 (Human colon cancer cell lines), MCF-7 (Human breast cancer cell lines), A 431 (Human skin cancer cell lines) and A 549 (Human lung cancer cell lines) were procured from the NCCS, Pune. These cell lines were cultured in RPMI-1640 medium. The media were supplemented with 10% fetal bovine serum, glutamine (2 mM), penicillin (100 units/ml) and streptomycin (100g/ml). The cultures were maintained in a humidified 5% CO_2 incubator at 37°C and the cells were sub cultured every 3-4 days to maintain logarithmic growth and were allowed to grow for 24 h before use.

Subculture of adherent cell lines (HT-29, MCF-7, A-549 and A-431)

Cultures were viewed using an inverted microscope to assess the degree of confluency and the absence of bacterial and fungal contaminants was confirmed. Cell monolayer was washed with FBS without $\text{Ca}^{2+}/\text{Mg}^{2+}$ using a volume equivalent to half the volume of culture medium. Trypsin/EDTA was added on to the washed cell monolayer using 1ml per 25 cm^2 of surface area. Flask was rotated to cover monolayer with trypsin. Flask was returned to the CO_2 incubator and left for 2- 10 mins. The cells were examined using an inverted microscope to ensure that all the cells were detached and floated. The cells were resuspended in a small volume of fresh serum containing HT-29, MCF-7, A 549 and A 431 medium respectively. 100-200 μl was removed to perform a cell count. The required numbers of cells were transferred to a new labeled flask containing pre-warmed HT-29, MCF-7, A 549 and A 431 medium and incubated as appropriate for the cell line.

Collection of plant material

The plant specimen of *Manilkara zapota* Linn. was collected from Ghodegaon (Ambegaon taluka), Pune district, Maharashtra, India in the month of October 2010. It was identified and authenticated at Botanical Survey of India, Pune with voucher specimen no. "MANIZS1".

METHODS

$$\% \text{ Cell Viability} = \frac{\text{Total number of viable cells}}{\text{Total number of cells}} \times 100$$

Cytotoxicity assay

MTT assay^{15,16,17}

MTT Colorimetric assay is based on the capacity of Mitochondrial succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) into an insoluble, colored formazan product which is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells. MTT assay was employed to assess cell proliferation. Viable cells were seeded into 96- well microtitre plates at 2×10^4 cells/well in RPMI-1640 medium supplemented with FBS (fetal bovine serum), 100units/ml penicillin, 100 $\mu\text{g}/\text{ml}$ streptomycin, and were cultured in a humidified atmosphere of 5% CO_2 and 95% air at 37°C. 150 μl of cell suspension was cultured with 10 μl of various concentrations i.e. 10, 20, 40 and 80 $\mu\text{g}/\text{ml}$ ¹⁸ of the ethanolic extract and manilkoraside from stem barks of *Manilkara zapota* respectively dissolved in DMSO (dimethyl sulphoxide) as solvent and incubated for 48h. Similar solutions containing the same concentrations of cyclophosphamide were also prepared and served as standard solutions. Control cells were incubated in RPMI-1640 medium only. Wells containing only media were considered as a blank. All cyclophosphamide,

Extraction and isolation

Stem barks of *Manilkara zapota* were shade dried for a week and powdered. Powdered material (800 g) was extracted using soxhlet apparatus with 95% ethanol for about 36 h. the extract was filtered and concentrated in vacuum under reduced pressure using rotary flash evaporator to get syrupy liquid. The syrupy liquid was transferred in petri dishes and allowed to dry in air at room temperature for about 24 hrs. A dark brown coloured mass weighing 68 g (8.50%) was obtained. 20 g of this extract was taken into the minimum amount of methanol and was stirred until it gets well dissolved into the methanol. Cold diethyl ether was slowly added into the methanolic solution and crude saponins were obtained as dull brown coloured precipitate which was then separated by filtration and dried under vacuum. A dull brown coloured powder mass weighing 9.2 g (46.00%) was obtained. Further purification was done on silica gel column chromatography using solvent system CHCl_3 : CH_3OH with increasing polarity. At CHCl_3 : CH_3OH (20:80) fraction a brown coloured amorphous compound weighing 1.472g (16.00%) was obtained, which was dried and subjected to spectroscopical analysis¹⁴.

Viability and characterization of cell lines

Trypan blue dye exclusion assay¹⁵

It is based on the principle that live cells possess intact cell membranes that exclude certain dyes, such as trypan blue, whereas dead cells do not. Trypan blue is a blue acid dye that has two azo chromophores group. The percentage of viable and non-viable cells were determined, using trypan blue exclusivity stain. The cell lines (HT-29, HL-60, MCF-7, A 549 and A 431) were grown at 37°C at humidified 5% CO_2 in RPMI 1640 medium. The RPMI 1640 medium was filtered using 0.45 μm membrane filter. Various concentrations i.e. 10, 20, 40 and 80 $\mu\text{g}/\text{ml}$ ¹⁸ were prepared by dissolving known weight of ethanolic extract and manilkoraside in DMSO. Similar solutions containing the same concentrations of cyclophosphamide were also prepared and served as standard solutions. Control cells were incubated in culture medium i.e. RPMI-1640 medium only. Cancer cells were seeded at a density of 2×10^4 cells/well and they were treated with different concentrations of the ethanolic extract and manilkoraside for 48 h at 37°C in the presence of 5% CO_2 . After 48h, 20 μl of medium and equal volume of 0.4% trypan blue were mixed, incubated for 5 min at 37°C in the presence of 5% CO_2 . Viable and dead cells were examined and counted under light microscope at 100X by Neubauers haemocytometer. The percent cell viability determined by following formula

ethanolic extract and manilkoraside dilution doses were tested in triplicates. The cell proliferation is based on the ability of the mitochondrial succinate-terazolium reductase system to convert 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) to a blue colored formazan. The test denotes the survival cells after toxic exposure. 10 μl of MTT labeling mixture was added and incubated for 4 h at 37°C and 6.5% CO_2 . After 4h, 100 μl of solubilization solution was added in each well. After 48h incubation at 37°C temperature and 5% CO_2 , the absorbance of soluble formazan product produced by viable cells was measured at 450nm using ELISA plate reader. Reference wavelength used was 630 nm. Percentage inhibition of the cell proliferation by cyclophosphamide, ethanolic extract and manilkoraside against all cell lines was calculated using the following formula,

$$\% \text{ Cell survival} = \frac{(\text{At} - \text{Ab})}{(\text{Ac} - \text{Ab})} \times 100$$

where, At = Absorbance of Test, Ab= Absorbance of Blank (Media), Ac= Absorbance of control (cells)

Statistical analysis

Statistical analysis was done by using INTA Software. Data were analysed by one way analysis of variance (ANOVA) followed by Dunnet's test. Results were presented as Mean± SEM. Values of *p<0.05, **p<0.01 were regarded as statistically significant.

RESULT AND DISCUSSION

Manilkoraside was obtained as brown coloured amorphous compound having the melting point 235-240°C. It shows absorption maxima (λ_{max}) at 281nm. FT-IR (KBr) (cm^{-1}) shows peak at 3352, 2943, 1751, 1728, 1631 and 1045 cm^{-1} which were consistent with the presence of hydroxyl, C-H aliphatic stretching, carbonyl group, C=C stretching and C-O-C group. APCI-MS spectra in negative ion mode showed quassimolecular ion peak at m/z 997.40 Da. $[M+H]^+$ indicating a molecular weight of 998.0 Da. ^1H-NMR (300MHz, TMS as reference compound and DMSO d_6 as solvent, chemical shifts written in ppm): 1.09[-6.3][d,6H,CH₃(59,70)], 0.99[s,15H,CH₃(60,61,62,63,67)], 1.60[m,1H,C H(58)], 4.29[t,7H,CH(4,5,12,13,18,19,20)], 3.56[s,2H,O-CH-O(2,9,15)], 1.99[s,11H,OH(21,22,28,29,31,32,33,64,65,66,69)], 5.20[t,1 H,CH(50)], 3.66[s,2H,CH₂OH(68)], 3.99[q,1H,CHOH(47,53)], 3.88[-7.8][d,1H,CHOH(54)], 3.8[m,3H,CH(6,11,17)], 1.14[-5.4][d,9H,CH₃(23,27,30)], 1.19[t,2H,CH(57)]. (Numbers in bracket represents the respective protons in structure). Molecular formula: C₅₂H₈₆O₁₈; Elemental analysis: C-62.50%, H-8.67%, O-28.09%. From these spectral studies the compound isolated thus confirms of Manilkoraside, a new pentacyclic triterpenoid saponin.

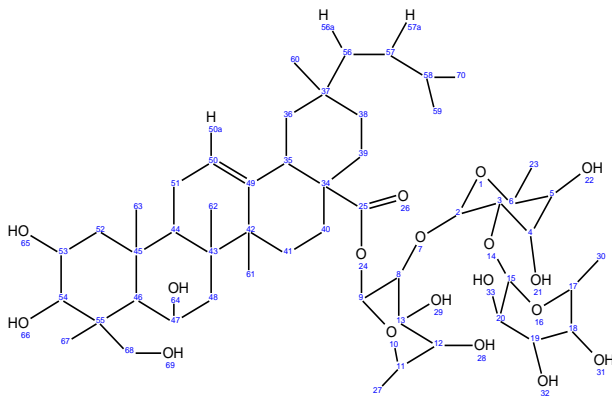


FIG. 1: NUMBERING STRUCTURE OF MANILKORASIDE ISOLATED FROM ETHANOLIC EXTRACT OF STEM BARKS OF MANILKARA ZAPOTA LINN.

In-vitro confirmation of toxicity of ethanolic extract and Manilkoraside from stem barks of *Manilkara zapota* Linn. on HL-60, HT-29, A431, A549 and MCF-7 cell lines. Percentage of viable cell and cytotoxicity activity on cells after control, cyclophosphamide

(standard), ethanolic extract and manilkoraside treatment can be obtained by performing trypan blue dye exclusion technique and MTT colorimetric assay respectively.

Viability and characterization of cell lines

Cell lines derived from NCCS, Pune were free from any kind of bacterial and fungal contamination. Percentage cell viability of cell lines were carried out by using trypan blue dye exclusion technique. Treatment of ethanolic extract and manilkoraside from stem barks of *Manilkara zapota* Linn. against HT-29, HL-60, MCF-7, A549 and A431, in all concentration range (10, 20, 40 and 80µg/ml) showed decrease in percent cell viability, as compared to that of negative control i.e. RPMI 1640 media containing cells. Percentage cell viability of all these cell lines decreases with increase in concentration from 10-80µg/ml. In overall variation of test samples of ethanolic extract and manilkoraside from stem barks of *Manilkara zapota* Linn., showed their best activity in the concentration of 80µg/ml. When compared with activity of standard drug cyclophosphamide, Manilkoraside showed better activity than ethanolic extract. Therefore, it is concluded from cell viability assay that ethanolic extract and manilkoraside from stem barks of *Manilkara zapota* Linn. exhibited very good anticancer activity.

TABLE 1: EFFECT OF STANDARD, ETHANOLIC EXTRACT AND MANILKORASIDE ON % CELL VIABILITY OF HL-60

GROUPS	CONCENTRATIONS (µg/ml)			
	10	20	40	80
Control	96.03±1.93	96.03±1.93	96.03±1.93	96.03±1.93
Standard	47.82±4.28**	45.39±4.80**	27.80±4.66**	13.82±2.60**
EE	61.03±5.56**	53.38±3.71**	37.86±5.01**	23.46±4.16**
Manilkoraside	56.97±4.75**	50.96±4.67**	31.99±4.62**	21.31±3.82**

All values are expressed as Mean±SEM. Data were analysed by One-way ANOVA followed by Dunnet's test

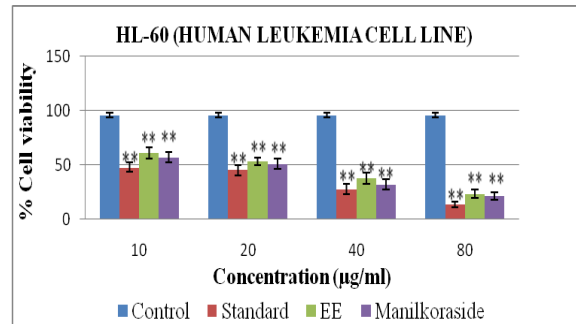


FIG. 2: EFFECT OF EE AND MANILKORASIDE FROM MANILKARA ZAPOTA ON %CELL VIABILITY OF HL-60

Where, Control = RPMI 1640 media, Standard= Cyclophosphamide, EE = Ethanolic extract of stem barks of *Manilkara zapota* Linn., Manilkoraside = Isolated saponin from ethanolic extract of stem barks of *Manilkara zapota* Linn.

TABLE 2: EFFECT OF STANDARD, ETHANOLIC EXTRACT AND MANILKORASIDE ON % CELL VIABILITY OF HT-29

GROUPS	CONCENTRATIONS (µg/ml)			
	10	20	40	80
Control	96.33±1.72	96.33±1.72	96.33±1.72	96.33±1.72
Standard	52.18±6.05**	49.52±5.78**	37.12±4.70**	24.09±3.41**
EE	65.84±4.81**	61.93±4.72**	54.27±3.89**	45.39±5.27**
Manilkoraside	63.25±5.24**	60.40±4.45**	52.44±4.31**	42.48±2.64**

All values are expressed as Mean±SEM. Data were analysed by One-way ANOVA followed by Dunnet's test

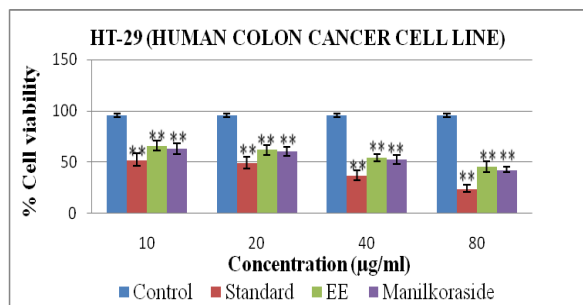


FIG. 3: EFFECT OF EE AND MANILKORASIDE FROM *MANILKARA ZAPOTA* ON %CELL VIABILITY OF HT-29

Where, Control = RPMI 1640 media, Standard= Cyclophosphamide, EE = Ethanolic extract of stem barks of *Manilkara zapota* Linn., Manilkoraside = Isolated saponin from ethanolic extract of stem barks of *Manilkara zapota* Linn.

TABLE 3: EFFECT OF STANDARD, ETHANOLIC EXTRACT AND MANILKORASIDE ON % CELL VIABILITY OF A 549

GROUPS	CONCENTRATIONS (µg/ml)			
	10	20	40	80
Control	95.57±2.23	95.57±2.23	95.57±2.23	95.57±2.23
Standard	55.08±5.54**	51.59±6.06*	41.41±6.30**	31.08±5.59*
EE	67.90±3.20*	64.34±4.54**	62.27±5.51**	53.88±3.41**
Manilkoraside	64.82±5.01**	62.09±5.09**	58.43±4.19**	52.03±6.14**

All values are expressed as Mean±SEM. Data were analysed by One-way ANOVA followed by Dunnet's test

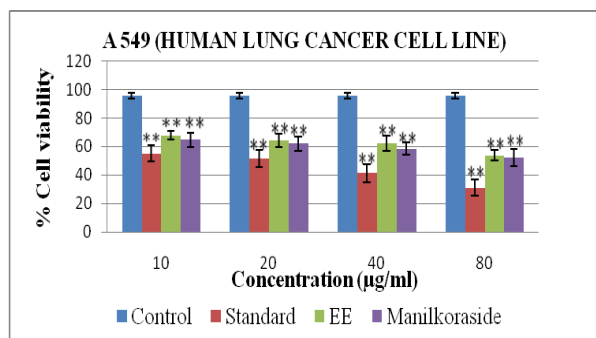


FIG. 4: EFFECT OF EE AND MANILKORASIDE FROM *MANILKARA ZAPOTA* ON %CELL VIABILITY OF A 549

Where, Control = RPMI 1640 media, Standard= Cyclophosphamide, EE = Ethanolic extract of stem barks of *Manilkara zapota* Linn., Manilkoraside = Isolated saponin from ethanolic extract of stem barks of *Manilkara zapota* Linn.

TABLE 4: EFFECT OF STANDARD, ETHANOLIC EXTRACT AND MANILKORASIDE ON % CELL VIABILITY OF A 431

GROUPS	CONCENTRATIONS (µg/ml)			
	10	20	40	80
Control	95.16±2.45	93.16±2.45	93.16±2.45	93.16±2.45
Standard	57.25±5.21**	49.98±3.61**	43.58±4.57**	34.35±4.12**
EE	70.78±3.58**	69.77±2.22**	62.13±3.82**	54.51±3.20**
Manilkoraside	69.46±3.27**	65.50±3.60**	58.76±3.51**	52.30±4.25**

All values are expressed as Mean±SEM. Data were analysed by One-way ANOVA followed by Dunnet's test

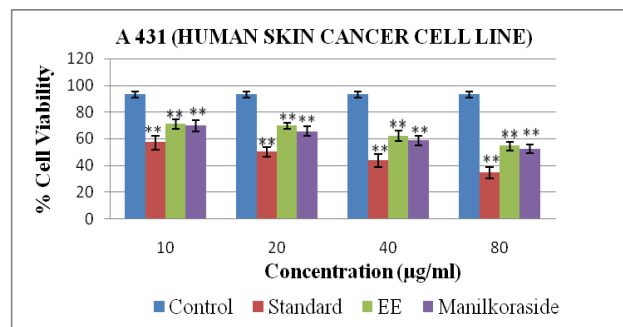


FIG. 5: EFFECT OF EE AND MANILKORASIDE FROM *MANILKARA ZAPOTA* ON %CELL VIABILITY OF A 431

Where, Control = RPMI 1640 media, Standard= Cyclophosphamide, EE = Ethanolic extract of stem barks of *Manilkara zapota* Linn., Manilkoraside = Isolated saponin from ethanolic extract of stem barks of *Manilkara zapota* Linn.

TABLE 5: EFFECT OF STANDARD, ETHANOLIC EXTRACT AND MANILKORASIDE ON % CELL VIABILITY OF MCF-7

GROUPS	CONCENTRATIONS (µg/ml)			
	10	20	40	80
Control	93.61±2.00	93.61±2.00	93.61±2.00	93.61±2.00
Standard	58.50±3.36**	53.90±4.84**	44.57±3.39**	36.43±2.60**
EE	83.78±6.07	81.25±2.74	78.66±2.90*	68.40±2.53**
Manilkoraside	81.19±2.65	80.66±3.35	75.56±3.76**	67.96±3.15**

All values are expressed as Mean±SEM. Data were analysed by One-way ANOVA followed by Dunnet's test

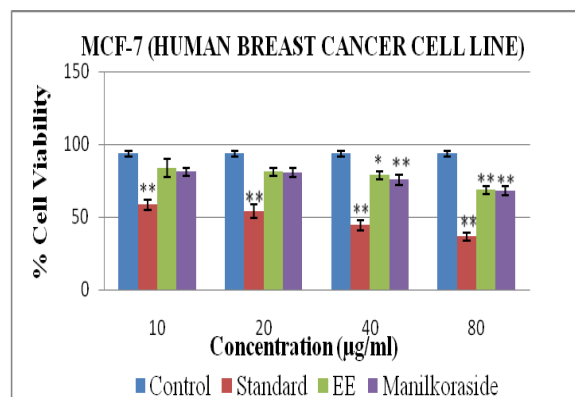


FIG. 6: EFFECT OF EE AND MANILKORASIDE FROM *MANILKARA ZAPOTA* ON %CELL VIABILITY OF MCF-7

Where, Control = RPMI 1640 media, Standard= Cyclophosphamide, EE = Ethanolic extract of stem barks of *Manilkara zapota* Linn., Manilkoraside = Isolated saponin from ethanolic extract of stem barks of *Manilkara zapota* Linn.

Cytotoxicity assay

The effect of RPMI 1640 media (control), cyclophosphamide (standard), ethanolic extract of stem barks of *Manilkara zapota* and manilkoraside on the growth of HL-60, HT-29, A 431, A 549 and MCF-7 cell lines were examined by MTT assay. Dose response curves constructed between the range of 10-80 µg/ml, express decreasing number of viable cells with increasing concentration of cyclophosphamide, ethanolic extract and manilkoraside. Calculation of EC₅₀ was done by using graphs generated from Microsoft Excel 2007 edition. The susceptibility of cells to the cyclophosphamide, ethanolic extract and manilkoraside exposure was characterized by

EC₅₀ values. Results indicate that the antiproliferative effect strengthens with increase in the concentration of ethanolic extract and manilkoraside. Highest cytotoxicity of ethanolic extract and manilkoraside was found against HL-60 and HT-29. However ethanolic extract and manilkoraside was found to have moderate activity on A 549, A 431, and MCF-7. EC₅₀ values showed that manilkoraside have best anticancer activity than ethanolic extract.

MTT colorimetric assay

TABLE 6: EFFECT OF CYCLOPHOSPHAMIDE (STANDARD) ON % CELL SURVIVAL OF CELL LINES

CELL LINES	CONCENTRATIONS (µg/ml)			
	10.00	20.00	40.00	80.00
HL-60	47.83	44.78	26.58	
11.86				
HT-29	50.91	47.27	36.85	23.39
A 549	54.07	49.42	40.55	29.22
A 431	55.99	50.26	40.87	31.77
MCF-7	58.82	52.92	42.71	
37.76				

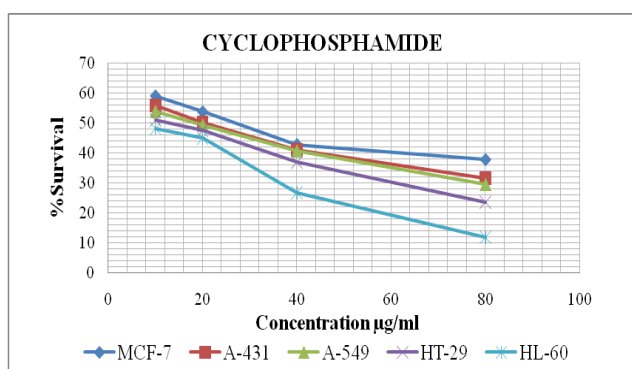


FIG. 7: EFFECT OF CYCLOPHOSPHAMIDE ON % CELL SURVIVAL OF CELL LINES

Where, HL-60=Human leukemia cell line, HT-29= Human colon cancer cell line, A 549= Human lung cancer cell line, A 431=Human skin cancer cell line, MCF-7=Human breast cancer cell line

TABLE 7: EFFECT OF ETHANOLIC EXTRACT ON % CELL SURVIVAL OF CELL LINES

CELL LINES	CONCENTRATIONS (µg/ml)			
	10.00	20.00	40.00	80.00
HL-60	58.14	52.74	36.53	22.32
HT-29	63.63	60.73	54.55	44.60
A 549	65.12	63.95	59.88	52.76
A 431	71.09	68.23	63.67	54.30
MCF-7	81.30	80.53	78.35	67.61

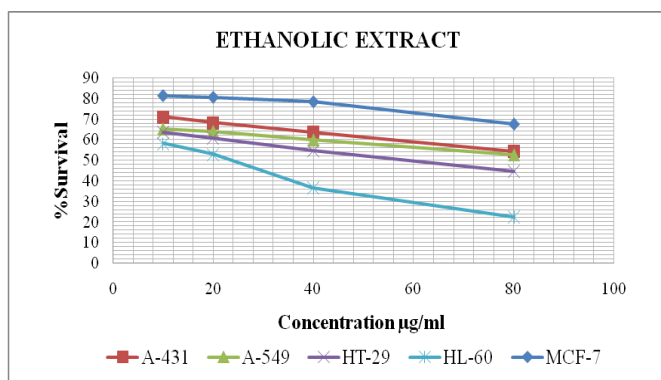


FIG. 8: EFFECT OF ETHANOLIC EXTRACT OF STEM BARKS OF MANILKARA ZAPOTA LINN. ON % CELL SURVIVAL OF CELL LINES

Where, HL-60=Human leukemia cell line, HT-29= Human colon cancer cell line, A 549= Human lung cancer cell line, A 431=Human skin cancer cell line, MCF-7=Human breast cancer cell line

TABLE 8: EFFECT OF MANILKORASIDE FROM ETHANOLIC EXTRACT OF STEM BARKS OF MANILKARA ZAPOTA LINN. ON % CELL SURVIVAL OF CELL LINES.

CELL LINES	CONCENTRATIONS (µg/ml)			
	10.00	20.00	40.00	80.00
HL-60	55.22	49.75	30.85	
18.62				
HT-29	62.54	59.76	51.27	
41.70				
A 549	62.65	60.90	56.10	
50.78				
A 431	68.36	64.71	59.38	
52.73				
MCF-7	78.35	78.29	75.46	
66.67				

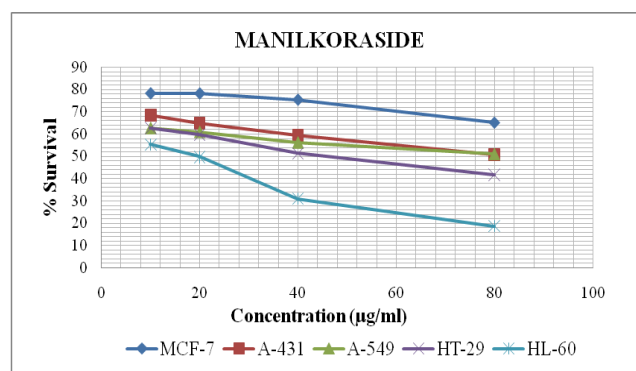


FIG. 9: EFFECT OF MANILKORASIDE FROM ETHANOLIC EXTRACT OF STEM BARKS OF MANILKARA ZAPOTA LINN. ON % CELL SURVIVAL OF CELL LINES.

Where, HL-60=Human leukemia cell line, HT-29= Human colon cancer cell line, A 549= Human lung cancer cell line, A 431=Human skin cancer cell line, MCF-7=Human breast cancer cell line

TABLE 9: EC₅₀ VALUES OF CYCLOPHOSPHAMIDE, ETHANOLIC EXTRACT AND MANILKORASIDE FROM STEM BARKS OF MANILKARA ZAPOTA LINN. AGAINST VARIOUS CELL LINES

EC ₅₀ (µg /ml)	CELL LINES			
	HL-60	HT-29	A 549	A 431
Cyclophosphamide	08.00	16.00	20.00	22.50
28.00				
Ethanolic extract	24.00	64.00	>80.00	>80.00
>80.00				
Manilkoraside	20.00	48.00	80.00	>80.00
>80.00				

Where, HL-60=Human leukemia cell line, HT-29= Human colon cancer cell line, A 549= Human lung cancer cell line, A 431=Human skin cancer cell line, MCF-7=Human breast cancer cell line. ">" signifies greater than.

CONCLUSION

The present work confirms that Manilkoraside, a new pentacyclic triterpenoid saponin was first time isolated and elucidated from the ethanolic extract of stem barks of *Manilkara zapota* Linn. Ethanolic extract and manilkoraside presented potent inhibitive effects on HL-60 and HT-29 and moderate activity on A 549, A 431 and MCF-7 cell lines which was studied by performing MTT based cytotoxic assay and trypan blue dye exclusion assay. These results provide promising baseline information for the potential use of ethanolic extract and manilkoraside from stem barks of *Manilkara zapota* Linn. in the treatment of cancer.

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