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Cytotoxic Activity from *Clematis* Species-A Review

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Abstract:- Cancer is one of the major causes of human catastrophe next to cardiovascular diseases. With the advent of modern techniques like chemotherapy, radiotherapy and surgical tools to treat cancer cells, the cost factor and the risk of damage of healthy cells in the surrounding tissue is the major concern. The anticancer compounds derived from natural sources have little side effects [1]. Out of 121 medicines used for cancer treatment 90 have the plants origin [2]. The cytotoxic activity from the genus *Clematis* was studied on the species *C. argentilucida, C. tangutica, C. apifolia, C. ganpiniana, C. montana, C. unicata, C. lasiandra* and *C. mandshurica*. More than 70 saponins have been isolated from these species. The cytotoxic activities attributed to these isolated compounds (Saponins) were recorded against different human cancer cell lines- HL-60, Hep-G2, SGC-7901, etc. in vitro by the MTT colorimetric assay through pharmacological methods. The study revealed that presence of –OH, free-COOH groups, and type, position of sugar chain linked to the aglycone influence the cytotoxic potential. However, comprehensive study on structure based activity consistent to para-clinical and clinical aspects is necessary to establish these species as potential natural substitute to cure cancer. The review will help for future study.

Keywords:- *Cytotoxic Activity, Saponins, C. Argentilucida, C. Tangutica, C. Montana.*

I. INTRODUCTION

Cell division in controlled fashion is the basic process of cell growth and proliferation. The DNA replicates and transmitted to daughter cells which are controlled by hundreds of proteins. The genetic combination affected by defects or environmental factors leads to uncontrolled cell division produces a tumour. The cancer management by physical methods need to be cell specific. The anticancer treatment must destroy the carcinogenic cell without harming the healthy cells. The success rate of cancer treatment by present methods is far low compared to full achievement. Many secondary metabolites isolated from plants have been tested and were effective against the proliferating cell division. The anticancer drugs interfere with the cell–cycle kinetics. The cytotoxic effect is due to damage of DNA in S-phase or by blocking mitotic spindle formation in M-phase. The anti-tumour drugs induce apoptosis in cancer cell by blocking mitosis while interacting with microtubules and tubulin. Many chemotherapeutic agents have been synthesized but have more side effects. There is need of efficient anticancer drug with less side effects [3]. The use of medicinal herbs has been relied by world population since centuries. The extracts or isolates derived from plants are used in traditional medicines to treat cancer. According to a report among the 65 new drugs that have been recorded between 1981 and 2002, 48 are derived from natural products [4]. *Clematis* L. genus (Ranunculaceae) consists of 295 species indigenous in north and south temperate, oceania and tropical African mountains [5]. In India, it is represented by thirty-two species including four sub species and five varieties [6]. Till date more than 120 new saponins are isolated from Clematis, including 70 oleanane, 50 hederagenin and 2 gypsogenin type [7]. In the present study the cytotoxic activity from the genus *Clematis* was studied on the species *C. apifolia, C.argentilucida, C. tangutica, C. ganpiniana, C. montana, C. unicata, C. lasiandra* and *C. mandshurica*. More than 70 saponins have been isolated from these species and 48 were found active for their cytotoxic activities against human cancer cell lines such as- SGC-7901, Hep-G2, BGC-823, HL-60, U251MG, A-2780, HCT-8, Bel-7402, A-549, and SK-OV-3 in vitro by the MTT colorimetric assay. The compounds with free –COOH group at C-28 exhibited stronger cytotoxic activity. The 3-O-β-D-Rib-(1→3)-α-L-Rha-(1→2)-α-L-Ara saccharide chain attached to C-3 of the aglycone showed better activities. Moreover, the studies inferred that activity was type of aglycone and sugar part influenced. However, as demonstrated by the results the activities on the criteria of specific cancer cell lines and structure related activities in clinical experiments is needed. The data has been extracted from Scopus-Elsevier, Google Scholar, Pub Med and Shodhganga. The review will help the researchers to select the species for future investigations.

- *Cytotoxic Compounds from Clematis Species:*

The genus *Clematis* is distributed with chemical compounds like triterpenoid saponins, alkaloids, coumarins, flavonoids, essential oils, macromolecules and steroids. The triterpenoid saponins constitute the major class of constituents. The aglycone of *Clematis* species is oleanane (A), 23-OH hederagenin (B) five-ring structure (Fig-1). These saponins are both monodesmodic and bidesmodic with glycosylation at Agl C←3 and Agl C←28 except in few cases at Agl C←23. The sugar moieties attached are D-Glucose (Glc), L-Arabinose (Ara), D-xylose (Xyl), L-Rhamnose (Rha), D-Ribose (Rib), and rarely D-Allose. The cytotoxic active saponins have been tabulated (Table-1). From the species *C. argentilucida* 34 new and known saponins were identified in different studies. The saponins were mainly of the class oleanolic and hederagenin.

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However, rare class saponins ursane and teraxerane were also isolated. The compounds.

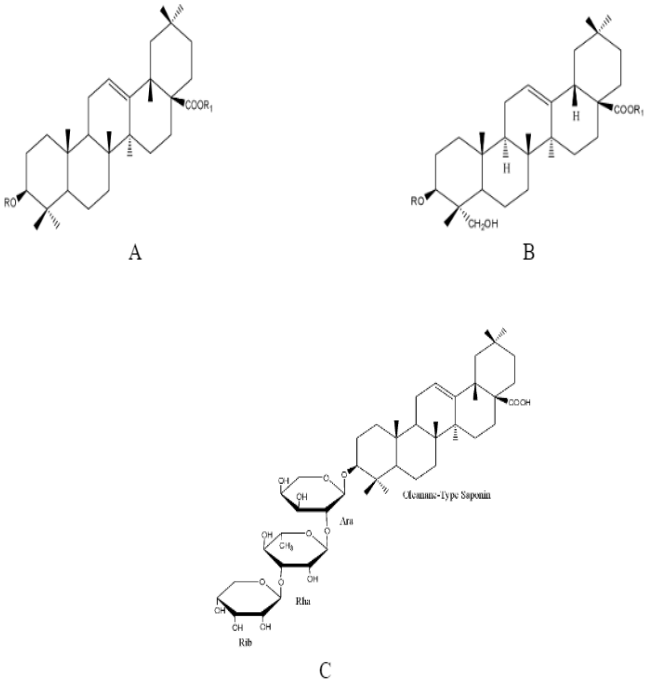

Fig 1 The Aglycones from *Clematis*: A-oleanane, B-hederagenin and C- oleanane type- Saponin.

Were from both bidesmodic and monodesmodic categories. The whole plant of *C. tangutica* was subjected for isolation to afford 27 saponins, out of which 10 found positive on cytotoxic activity experiment. The active saponins were both from monodesmodic and bidesmodic categories up to 5 sugars length of branched and straight chain. The aglycones were oleanane and hederagenin type (Table-1). The roots of *C. unicata* were extracted in 70% ethanol to isolate 19 new and known saponins. The saponins were identified to be bidesmodic with oleanane type aglycones. From the ethanolic extract of *C. lasiandra* 7 triterpenoid saponins were isolated. The identified saponins were based on both oleanane and hederagenin type aglycones. Sugars hydrolysed were mainly ribose, rhamnose, xylose and arabinose. The roots of *C. mandshurica* were extracted in 50% ethanol to isolate 9 cytotoxic potential saponins. The identified saponins were both bidesmodic and with free –COOH group at C-28 position. In hydrolysis sugars confirmed were mainly arabinose, rhamnose, ribose and glucose. Rarely isoferuloyl moity was also encountered in the sugar chain. The structures of 4 saponins identified from *C. lasiandra* were monodesmodic with free –COOH group linked to C-28 position of aglycone. The saponins were both oleanane and hederagenin type and all were active to cytotoxic experiment with promising cytotoxic potential (Table-2).

➢ *Cytotoxic Activity from Clematis Species.*

- *C Argentilucida*

a) Roots of *C. argentilucida* were extracted with 70% ethanol and partitioned in n-butanol to isolate 1-3 pure saponins. The cytotoxic activities of 1-3 were evaluated against three cultured human cancer cells-HL-60, Hep-G2 and U251MG. The experiment was performed in vitro using MTT assay (Table-2). Doxorubicin and nimustine were used as positive control. The results demonstrated that saponins 1 and 2 had strong activity with IC_{50} value from 2.74 to 6.61µM. Whereas, 3 with IC_{50} value 10.35 to 25.40 µM was inactive against all tested cell lines (8).

b) Ethanol extract(70%) of roots of *C. argentilucida* was subjected for isolation of 1- 13 saponins through coloumn chromatography. The cytotoxic activities of all the saponins in vitro experiment was recorded against human glioblastoma cells – U251MG by MTT colorimetric assay using nimustine as positive control. The oleanolic acid expressed highest absorbance with IC_{50} value of 6.95µM (Table-2). The IC_{50} value of compounds 1, 2, 5, 6, 7, 8 was between the ranges 10.46 to 38.51µM. The compound 1 and 9-13 were inactive to the activity test (9).

Table 1 Structure of Cytotoxic Saponins from *Clematis*

Species	Structure of Compounds	Ref.
C. argentilucida a)	(1) 3-O-(β-D-Rib-α-L-Rha-(1→2)-α-L-Ara) Hed-11,13-dien-28-oic acid, (2) 3-O-(-β-D-Rib-(1→3)-α-L-Rha-(1→2)-{-α-D-Glc-(1→4)-β-D-(Xyl) oleanolic acid. (3) Cussonside B.	8
b)	(1) 3-O-[β-D-Xyl-(1→3)-α-L-Rha-(1→2)-β-D-Glc]-28-OH-18H-ursan-20-en., (2) 3-O-[β-D-Xyl-(1→3)- α-L-Rha-(1→2)-β-D-Glc]-28-OH-taraxeran-14-en., (4) 3-O-[-β-D-Rib-(1→3)-α-L-Rha-(1→2)-β-D-Xyl] oleanolic acid. (5) β-hederin, (6) 3-O-α-L-Rha-(1→2)-O-β-D-Glc-Hederagenin., (7) 3-O-[- β-D Xyl (1→3)- α-L-Rha-(1→2)-β-D-Glc]-28-OH-olean-12-en. (8) 3-O-[-β-D-Rib-(1→3)-α-L-Rha-(1→2)-α-D-Ara]-23-OH-olean-11,13-dien-28-oic acid.	9
c)	(4) 3-O-[-β-D-Rib-(1→3)-α-L-Rha-(1→2)-[β-D-Glc-(1→4)]-α-L-Ara oleanolic acid., (7) hemslonin A., (8) 3-O-[-β-D-Rib-(1→3)-α-L-Rha-(1→2)-β-D-Xyl] hederagenin., (14) Clematoside S., (15) cussonside B., (16) saponinCP4, (17) 3-O-[-β-D-Rib-(1→3)-α-L-Rha-(1→2)-[-β-D-Glc-(1→4)]-β-D-Xyl oleanolic acid., (18)saponinCP7.	10
C. tangutica a)	(4) clematoside S, (6) sapindoside B, (12) Kalopanax saponin A, (13) koelreuteria saponin A, (14) clematangoticoside D, (15) clematangoticoside F.	11
b)	(4) 3-O-β-D-Allo-(1→2)-α-L-Rha-(1→2)-α-L-Ara-Hed-11,13-dien-28-O-α-L-Rha-(1→4)-β-D-Glc-(1→6)-β-D-Glc ester., (1) hederacholichiside F3, (3) Tauroside St-H1, (7) hederasaponin B.	12

C. apifolia	(1) oleanolic acid, (2) ursolic acid., (3) Hederagenic acid.	13
C. ganpiniana	(1) 3-O-α-L-Ara-oleanolic acid., (2) 3-O-α-L-Ara-hederagenin., (3) 3-O-α-L-Rha-(1→2)- α-L-Ara-oleanolic acid., (4) α-hederin.	14
C. montana	(1) montanoside A., (2) montanoside B.	15
C. parviloba	(1) clematiside S., (2) α- hederin.	16
C. unicata	(1-8) clematiunicinosied A-H., (9) clematernoside E., (10) huzangoside D., (11) clematigoside A., (12) huzangoside B., (13) huzangoside C., (14) clematichinenoside C., (15) clemastanoside D., (16) hederasaponinB., (17) CP7., (18) CP6., (19) CP4.	17
C. lasiandra	(1) 3-O-β-D-Rib-(1→3)-α-L-Rha-(1→2)-[-β-D-Glc-(1→4)]-β-D-Xyl- hederagenin., (2) 3-O-β-D-Rib-(1→3)-α-L-Rha-(1→2)-β-D-Xyl-olean-28-O- β-D-Glc ester., (3) 3-O-β-D-Rib-(1→3)-α-L-Rha-(1→2)-β-D-Xyl- hederagenin., (4) 3-O-β-D-Rib-(1→3)-α-L-Rha-(1→2)-[-β-D-Glc-(1→4)]- α-L-Ara-hederagenin., (5) 3-O-β-D-Rib-(1→3)-α-L-Rha-(1→2)- β-D-Xyl -oleanolic acid., (6) 3-O-β-D-Rib-(1→3)-α-L-Rha-(1→2)- α-L-Ara-olean-28-O- β-D-Glc ester., (7) 3-O-β-D-Rib-(1→3)-α-L-Rha-(1→2)- α-L-Ara-oleanolic acid.	18
C. mandshurica a)	(1) mandshunoside C., (2) mandshunoside D., (3) mandshunoside E., (4) clematochinenoside C., (5) clematochinenoside A., (6) huzangoside B., (7) clematiganoside A., (8) clematiganoside B.	19
b)	(1) mandshunoside A., (2) mandshunoside B.	20

c) The saponins 1-18 isolated from the roots of *C. argentilucida* were tested for cytotoxic potential against the cells-HL-60, Hep-62 and SGC-7901 in MTT assay using adriamycin as positive control. The IC_{50} value of each compound was measured after 72 h treatment. The monodesmodic saponins 4, 7, 8 and 14-18 were strongly active with IC_{50} value from 0.87 to 19.48 µM (Table-2). The saponin namely 3-O-β-D-Rib-(1→3)-α-L-Rha-(1→2)-[β-D-Glc-(1→4)]-α-L-Ara oleanolic acid showed maximum cytotoxic potential against all the cells. However, all the bidesmodic saponins 1, 2, 5, 6 and 13 were inactive (10).

- *C. Tangutica*

a) The 75% methanol extract of whole plant of *C. tangutica* was partitioned between n-butanol yielded 1-16 saponins. The cytotoxic activities were recorded against the cancer cells- Hep-G2, U251MG, HL-60 and SGC-7901. The experiment was performed using MTT method and adriamycin and nimustine as positive control. The tested dose of saponins was 0.5, 2, 10, 50 µM. The saponins 4, 6 and 12-15 showed positive cytotoxic activity (Table-2). The saponins 12-15 were most effective with IC_{50} values between 8.18 to 10.32µM against SGC-7901 cells, 1.88 to 7.26 µM against HepG2 cells, 5.21 to 8.72 µM against HL-60 cells and 15.37 to 27.20 µM against U251 MG cell lines. Saponins 4 and 6 were selectively active against SGC-7901 cells only and all other saponins were inactive to the experiment (11).

b) The triterpenoid saponins isolated from *C. tangutica* were evaluated for cytotoxic activity against SK-OV-3, Hela and HGC-27 cell lines. The saponin 4 and 1 showed positive results with IC_{50} value 20.17 and 66.18µM against HGC-27 respectively (Table-2). Whereas, saponin 7 was active against all the cell lines with IC_{50} value ranges between 16.47 to 71.36µM. Saponin 3 was selective with IC_{50} value 48.70 µM against SK-OV-3 only. All other saponins were inactive to experiment (12).

- *C. Ganpiniana*

The 70% ethanol extract of whole plant of *C. ganpiniana* was subjected to chromatography to afford 1-4 saponins. The isolated saponins were subjected to cytotoxic activity against breast cancer cell lines-MDA-MB-231, MCF-7 in MTT assay using adriamycin as positive control. The tested dose of the compounds 1-4 was 0.08, 0.4, 2, 10, 50 g/ml for the span of 24 h. The results of the experiment showed compounds 1-4 were positive with IC_{50} values between the range of 0.7 to 12.3 g/ml against MCF-7 cells and 0.9 to 16.5 g/ml against MDA-MB-231 cultured breast cancer cell lines in comparison to adriamycin with IC_{50} values of 2.4 g/ml against MCF-7 and 2.7 g/ml against MDA-MB-231 cell lines (Table-2). The compounds 1 and 2 expressed promising growth inhibition and compound 4 (α-hederin) showed strongest activities attributed to the structure. It was suggested that synergic effect of presence of 23-OH and 3-O-L-Rha-(1→2)-L-Ara improved the cytotoxic effect. (14).

- *C. Parviloba*

The stem part of *C. parviloba* was extracted with 70% ethanol to afford 1-16 triterpenoid saponins. The cytotoxic activity of saponins was evaluated against A-2780, A-549, Bel-7402, BGC-823 and HCT-8 cancer cell lines in MTT experimental method. The compounds 2, 4, 13- 15 were tested. Compounds 2 and 4 showed moderate activity with IC_{50} value to the range between 1.44 to 6.86 mg/ml against the cell lines- A-2780, Bel-7402, BGC-823 and HCT-8 whereas compounds 13-15 were inactive to all cancer lines (Table 2). The results indicated that number and position of sugar moieties was related to activities (16).

- *C. Unicata*

The 70% ethanol extract of roots of *C. unicata was* subjected for isolation to afford 1-19 triterpenoid saponins. The cytotoxic activities of all the saponins including aglycone was recorded in MTT method against cultured Caski cells using cisplatin as control. The compounds 13, 17

and 19 showed cytotoxic effects with IC_{50} value of 9.05±3.61µM, 26.07±1.47µM and 2.97±1.07µM. All other compounds were inactive with IC_{50} value>80 (Table 2). Moreover, it was observed that saponins having glycosylated aglycones were more active than aglycones (17).

- *C. Lasiandra*

The whole plant of *C. lasiandra* was subjected to extraction with 70% ethanol and partitioned in n-butanol to afford 1-7 saponins. The cytotoxicities of isolated saponins were measured by MTT method against HepG2, HL-60 and SGC-7901 cultured human tumor cells. The adriamycin was used as positive control. The results showed that compounds 1, 3, 4, 5 and 7 exhibited potential activity with IC_{50} values between the ranges of 1.40-19.50 µmol/L whereas saponins 2 and 6 were inactive (Table-2). It was suggested that activity related to type, position and number of sugar moieties attached to the aglycone (18).

Table 2 Cytotoxic Activities of Clematis Species.

Species	Bioactive dose	Experimental models	Ref.
C. argenti-lucida a)	IC_{50} (µg/ml) Comp. 1 2 3 Control HL 60 6.61 3.82 >100 0.35± 0.03 Hep-G 2.30 2.74 >100 0.52± 0.05 U251MG 25.40 10.35 >100 0.92± 0.04	MTT colorimetric assay	8
b)	Comp. (IC_{50}, µM) U251MG cells 1 38.51 ± 5.09 6 25.16 ± 3.78 2 29.08 ± 4.33 7 28.38 ± 3.16 4 6.95 ± 0.87 8 21.07 ± 2.05 5 10.46 ± 1.15 CNU 1.02 ± 0.09	MTT colorimetric assay	9
c)	(IC_{50}, µM). Comp. HL-60 Hep-G2 SGC-7901 4 2.91 ± 0.24 2.26 ± 0.18 0.87 ± 0.09 7 6.43 ± 0.17 17.86 ± 0.31 19.48 ± 0.25 8 12.74 ± 0.29 9.97 ± 0.40 11.50 ± 0.37 14 8.46 ± 0.35 7.82 ± 0.25 6.19 ± 0.18 15 7.34 ± 0.17 19.16 ± 0.39 18.22 ± 0.18 16 4.13 ± 0.15 6.52 ± 0.53 5.13 ± 0.32 17 3.29 ± 0.21 3.79 ± 0.29 1.94 ± 0.40 18 4.99 ± 0.37 7.15 ± 0.26 9.36 ± 1.35 C. 0.32 ± 0.04 0.17 ± 0.02 0.16 ± 0.05 C. = Adriamycin as positive control.	MTT colorimetric assay	10
C. tangutica a)	(IC_{50}, mM) Comp. SGC-7901 HepG2 HL-60 U251MG 4 24.14 ± 2.10 >50 >50 >50 6 21.35 ± 1.88 >50 >50 >50 12 8.18 ± 0.53 1.88 ± 0.41 5.21 ± 0.52 17.32 ± 1.25 13 10.32 ± 0.48 4.24 ± 0.58 6.58 ± 0.42 15.37 ± 1.18 14 9.08 ± 0.29 7.26 ± 0.78 8.72 ± 0.91 27.20 ± 2.78 15 9.01 ± 0.21 6.01 ± 0.52 5.34 ± 0.38 21.12 ± 3.16 C. 0.33 ± 0.05[A] 0.18 ± 0.02[A] 0.36 ± 0.05[A] 0.95 ± 0.06[B] C= Adriamycin[A] & Nimustine[B] as positive control.	MTT colorimetric assay	11
b)	IC_{50} (µM) **1** HGC-27 20.17 & 66.18 **2** HGC-27 16.47-71.36 **3** SK-OV-3- 48.70	in vitro	12
C. apifolia	IC_{50} (µg/ml) 7.7 to 25.6	MTT assay/ in vitro	13
C. ganpiniana	IC_{50} (µg/ml) MCF-7 MBA-MA 231 MCF-7 MBA-MA 231 1 1 2.1 3 12.3 16.5 2 2.3 3.7 4 0.7 0.9	MTT assay on breast cancer cells/ in vitro	14
C. montana	50 or 100 µg/ml	Cytotoxicity activity/ in vitro	15
C. parviloba	IC_{50} (µg/ml)	MTT assay/ in vitro	16

	1.44 to 6.86		
C. unicata	Comp. IC_{50}(μM). 1 >80 6 >80 11 >80 16 >80 2 >80 7 >80 12 >80 ------- 3 >80 8 >80 ----- 18 >80 4 >80 9 >80 14 >80 ------ 5 >80 10 >80 15 >80 ------ 13 9.05 ± 3.61 17 26.07 ± 1.47 19 2.97 ± 1.07 Cisplatin 3.37 ± 0.77	MTT assay/ in vitro	17
C. lasiandra	(IC_{50}, μmol/L) Comp. HL-60 Hep-G2 SGC-7901 1 9.23 ± 0.25 5.62 ± 0.29 3.75 ± 0.39 2 N 100 N 100 N 100 3 12.74 ± 0.29 9.97 ± 0.40 11.50 ± 0.37 4 7.02 ± 0.24 3.35 ± 0.17 1.96 ± 0.34 5 1.40 ± 0.52 19.50 ± 5.20 8.37 ± 0.17 6 N 100 N 100 N 100 7 3.29 ± 0.21 6.52 ± 0.53 5.13 ± 0.32 C. 0.27 ± 0.04 0.34 ± 0.05 0.29 ± 0.03 C. = Adriamycin as positive control.		18
C. mandshurica a)	IC_{50} value (mM C. HCT-116 HT-29 C. HCT-116 HT-29 1 3.0 2.8 5 8.4 7.3 2 0.6 0.9 6 12.9 13.5 3 2.7 4.2 7 16.1 12.5 4 12.3 10.3 Paclitaxel 0.0035 0.0034		19
b)	IC_{50} value (μM) C. HCT-116 HT-29 1 2.1 3.7 2 2.5 3.3		20

II. CONCLUSION

➢ *The cytotoxic activity evaluated from the Clematis species has been summed up for the following generalizations that-*

- The compounds with free –COOH group attached at C-28 of the aglycone displayed increase in cytotoxic potential.
- The monodesmodic saponins without free–COOH group at C-28 were inactive for test.
- Saponins having same trisaccharide chain with oleanane and iso-oleanane type aglycone were more potent than hederagenin type (-OH group at C-23) and ursane type (-OH group at C-28) aglycone.
- The aglycone with substitution of -OH group at C-23 have negative effect on cytotoxic activity.
- Compared to the oligosaccharide chain attached at C-3 for the active saponins, compounds with common 3-O-β-D-Rib-(1→3)-α-L-Rha-(1→2)-α-L-Ara chain displayed relatively stronger cytotoxicities [21]. The observation was altogether different for the replacement of xylose sugar with arabinose at C-23. The xylose displayed stronger cytotoxic effect against HL-60 than with Hep-G2 cancer cell lines. Therefore, it was suggested that the cytotoxic effects were related simultaneously to the structure of saponins and type of cancer cell lines.
- The chemical structure comparison of saponins further inferred that the presence of both the groups 23-OH and 3-O-L-Rha-(1→2)-L-Ara attached to the aglycone have synergetic effect and further enhance the cytotoxicity of the compounds.

The above observations were in agreement with those reported in literature that the –OH group at C-23 of the aglycone of oleanane had negative effect. [22,23], saponins possessing a disaccharide chain exhibited weaker cytotoxicity than with a trisaccharide chain, saponin with the oleanolic acid aglycone was the most cytotoxic suggesting that the position, number and type of the sugar units also affected the resultant cytotoxicity [24,25]. Moreover, the studies inferred that the cytotoxic activity of such saponins was influenced by the structures of both the aglycones and the sugar parts.

Hence, as demonstrated by the results the activities on the criteria of specific cancer cell lines and structure be evaluated through clinical experiments to explore these species as potential natural substitute for cancer treatment.

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