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IN-SILICO DOCKING ANALYSIS OF *Calotropis gigantea* (L.) R.BR DERIVED COMPOUND AGAINST ANTI-CERVICAL CANCER ACTIVITY

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Abstract- The present study reports the phytosterols present in the aerial part of the plant *Calotropis gigantea* (Asclepiadaceae) commonly known as milk weed. In addition to this an *in silico* docking analysis was also carried out to validate the anti-potential of these phytosterol compounds. The GC-MS analysis of the chloroform extract revealed the presence of eight sterols namelys 9,19-Cyclolanost-24-en-3-ol,acetate; Campesterol; Stigmasterol; gamma-Sitosterol; Desmosterol; Stigmasta-5,24(28)-dien-3-ol,(3.beta.,24Z)- ;Ergost-22-en-3-0l, (3.beta., 5.alpha., 22E, 24R)- ;Ergost-8,24(28)-dien-3-ol,4,14-dimethyl,(3.beta.,4.alpha.,5.alpa.,). The docking analysis showed that all the sterol compounds showed the docking energy in the range of -12 to -16 Kcal/mol. The desmosterol exhibits the higher docking energy, showing the maximum potential against the HPV16 E6 cervical oncoprotein.

Keywords- *Calotropis gigantea,* phytochemical, sterols, *in-silico* docking, cervical cancer.

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Introduction

Cancer is a major cause of death and the number of new cases, as well as the number of individuals living with cancer, is expanding continuously. Cervical cancer is one of the most common cancers among women worldwide ,Its mortality exemplifies health inequity, as its rates are higher in low & middle income countries [1], and in low socio-economic groups within countries [2]. Around 80% of global cervical cancer cases are in low & middle income countries [3]. The human papilloma virus (HPV) is the main causative agent for cervical cancer. The viral DNA from specific group of HPV can be detected in 90% of all cervical cancer[4]. High-risk HPV encode two major oncoproteins termed as E6 and E7, and the respective genes are the only viral genes that are generally retained and expressed in cervical cancer tissues.

Calotropis gigantea R.Br. (Asclepiadaceae) commonly known as milkweed or swallowwort is a common wasteland weed in India [5]. A total of 187 plant species, belonging to 102 genera and 61 families have been identified as an active or promising source of phytochemicals with antitumor properties, corresponding to a 41 percent increase during the last five years. Among them, only 15 species (belonging to ten genera and nine families) have been utilized in cancer chemotherapy at clinical level, whereas the rest of the identified species are either active against cancer cell lines or exhibit chemotherapeutic properties on tumor-bearing animals under experimental conditions. Phenylpropanoids are the most widely distributed compounds (18 families), followed by terpenoids (14 families), and alkaloids (13 families) [6].

The present study was undertaken to identify the potential phytosterols to further substantiate the earlier claims by various researchers on its potential use in traditional medicine. The HPV E6 protein is one of the viral oncoprotein that is expressed virtually in all HPV positive cancers. Therefore, E6 is the main anticancer treatment target. Moreover there are almost negligible works on the docking studies of the phytosterols isolated form *Calotropis gigantean* against oncoprotein HPV 16 E6. Therefore, analysis was also carried out to assess the anticancer potential of the sterols isolated from *Calotropis gigantean* against cervical cancer.

Materials and Methods

Plant Material Collection and Processing

The aerial parts of the *Calotropis gigantean* were collected from Tiruchirappalli, India (10°43'42. 43"N, 79°00'44. 60"E). The collected plant materials were washed thoroughly with the distilled water in order to remove the dirt and other contaminations. The thoroughly washed plant materials were dried under shade, at room

temperature so as to retain the fresh green colour of the leaves, and also to prevent the decomposition of the potential active compounds. The dried leaves were powdered using a stone grinder. The powdered materials were stored in airtight, dark, glass container to prevent photochemical reactions.

Extraction and GC-MS Analysis of Sterols

The crude drug was subjected to extraction with analytical grade solvent of chloroform for GC-MS analysis. 25 g of the crude drug was taken in a round bottom flask and 50ml of analytical grade chloroform was added and refluxed for 8 hrs. After completion of the 8hrs the round bottom flask was cooled and the extract was filtered through the Buchner funnel. The extract was evaporated under nitrogen atmosphere using turbo evaporator. The residue obtained was dissolved in 2ml chloroform and transferred into the GC vial and injected into the GC-MS port.

GC-MS analysis was performed on an Agilent gas chromatograph model 6890 N coupled to an Agilent 5973 N mass selective detector. Analysts were separated on an HP-5MS capillary column (30 m X 0.25 mm X 1.0 µl) by applying the following temperature program: 40°C for 5 min, 40-70°C at 2°C/min, 70°C for 2 min, 70-120°C at 3°C/min, 120-150°C at 5°C/min, 150-220°C at 10°C/min and then 220°C for 2 min. Transfer line temperature was 280°C . Mass detector conditions were: electronic impact (EI) mode at 70 eV; source temperature: 230°C; scanning rate 2.88 scan S^{-1}; mass scanning range: m/z 29-540. Carrier gas was helium at 1.0 ml min^{-1}. The tentative identification of volatile components was achieved by comparing the mass spectra with the data system library (NIST 98) and other published spectra [7], supported by retention index data, which were compared with available literature retention indices [8]. All compounds were quantified as 3-octanol equivalents.

Docking Studies

The present bio computational investigation was carried out to identify the potential therapeutic sterols present in the *Calotropis gigantea* against cervical cancer. The 3-D crystal structure of the HPV16 E6 oncoprotein (PDB-ID: 2FK4) responsible for cervical cancer was retrieved from the protein data bank (PDB). Structural and active site enumeration were done by using pymol molecular visualization software and CASTP (Computed Atlas of Surface Topography of Proteins). Six phyto sterols *viz.* Desmosterol; Gamma-sitosterol; 9, 19-Cyclo-9.beta.-lanost-24-en-3.beta.-ol acetate; campesterol; Stigmasterol; and fucosterol were screened against the HPV16 E6 oncoprotein. The details of these phytochemical were obtained from pubchem database and there chemical structures were generated from SMILES notation (Simplified Molecular Input Line Entry Specification) by using the Marvin Sketch software. The molecular docking analysis was performed by widely distributed public domain molecular docking software Argus Lab 4.0.

Results and Discussion

Calotropis gigantea, commonly known as wasteland weeds is a highly potential plant resource. The cytotoxicity of various extracts of its root, leaves and flowers of *Calotropis* has been shown [9-13]. Previous experiments with this plant, extracts of the root and the leaves showed cytotoxic activity against human epidermal nasopharynx carcinoma [14]. Cytostatic activity of *C. procera*, was earlier reported [10]. *In vitro* anti-tumour activity and a high level of *in vivo* tolerance of this plant were recorded by [15].The whole latex of *Calotropis gigantea* possesses anticancer and cytotoxic activity against hepatocellular carcinoma [16]. The water-soluble protein fraction of the latex was evaluated and found to possess a potential cytotoxic activity against different human cancer cell lines. [17].

The GC-MS analysis showed the presence of eight phytosterols in *Calotropis gigantea* [Table-1] and the chromatogram is shown in [Fig-1] Gamma-Sitosterol showed maximum peak area of 3.86%, followed by Campesterol and Stigmasta-5,24(28)-dien-3-ol, (3.beta.,24Z) with 2.57% and 2.04% respectively. Out of the eight identified phytosterols six compounds were subjected to study the anticancer potential through *in silico* docking analysis by using the docking software Argus lab 4.0 and the results are presented in [Table-2]. The phytosterol exhibited the docking energy between -12 to -16.0235 Kcal/mol. Of which, the maximum docking energy was found in Desmosterol (-16.0235 Kcal/mol) and Gamma-sitosterol (-13.5785 Kcal/mol). Higher docking energy shows good binding energy and hence more efficient in blocking the activity of the particular protein. The role of phytosterols againt cervical and breast cancers has been already reported by the *in silico* docking studies [18,19].

Hence, from the above biocomputational studies it is evident that the sterols present in the *Calotropis* is an extremely valuable for the treatment of deadly cervical cancer. Therefore in this context it is therefore alluded to isolate, purify and characterize the potential phytosterols especially Desmosterol in order to supplement the conventional drug development.

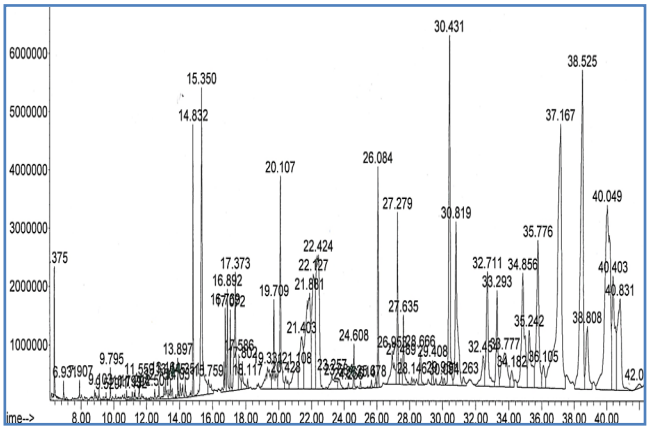
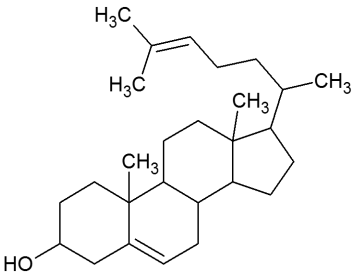
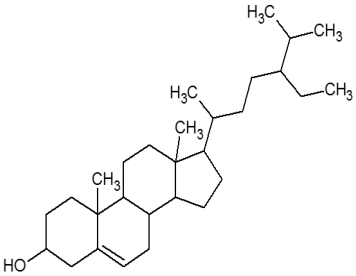
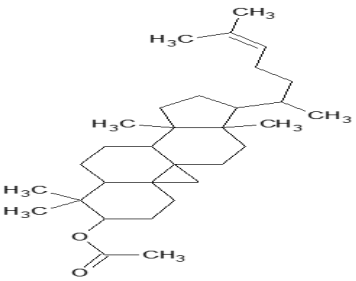
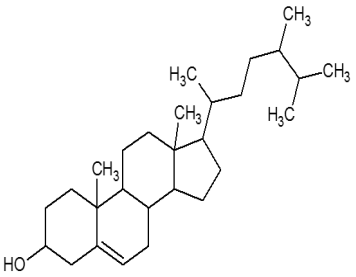
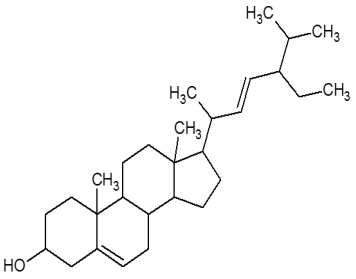
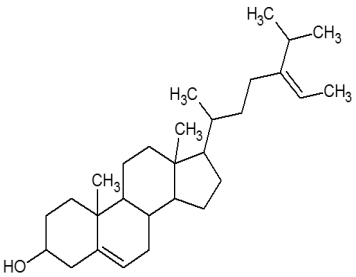


Fig. 1- GC MS chromatogram of *Calotropis gigantea*

Table 1- Sterols present in Calotropis gigantean

S. No.	Retention Time	Peak Area %	Compound Name
1	38.7	1.88	9,19-Cyclolanost-24-en-3-ol,acetate
2	32.6	2.57	Campesterol
3	33.2	1.73	Stigmasterol
4	34.8	3.86	gamma-Sitosterol
5	31.2	0.2	Desmosterol
6	35.2	2.04	Stigmasta-5,24(28)-dien-3-ol,(3.beta.,24Z)-
7	23.6	0.32	Ergost-22-en-3-0l,(3.beta.,5.alpha.,22E,24R)-
8	34.1	0.42	Ergost-8,24(28)-dien-3-ol,4,14-dimethyl-, (3.beta.,4.alpha.,5.alpa.,)-

Table 2- Docking energy of the phytosterols against oncoprotein HPV 16 E6

Compound name	Pubchem ID	Compound structure	Molecular weight (g/mol)	Hydrogen bound donor/acceptor	Docking energy
Desmosterol	CID 439577		384.63766	1,1	-16.0235
Gamma-sitosterol	CID 636741		1,1	414.7067	-13.5785
9,19-Cyclo-9.beta.-lanost-24-en-3.beta.-ol, acetate	CID 518616		468.75408	0,2	-12.881
campesterol	CID 173183		400.68012	1,1	-12.8619
Stigmasterol	CID 5280794		412.69082	1,1	-12.799
fucosterol (or) Stigmasta-5,24(28)-dien-3-ol, Stigmasta-5,24 (28)-dien-3.beta.-ol	CID 5378817		1,1	412.69082	-12.6366

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