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Computational selections of terpenes present in the plant *Calotropis gigantea* as mosquito larvicide's by blocking the sterol carrying protein, AeSCP–2

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Abstract

The present study reports the phytochemical properties of *Calotropis gigantea* (Asclepiadaceae) commonly known as milk weed. In addition, *in silico* docking analysis was also carried out to assess the mosquito larvicidal potential of three terpene compounds isolated from *C. gigantea*. Considerable amount of primary metabolites, essential macro and micro nutrients were documented in the plant. The GC-MS analysis of the chloroform extract revealed the presence of eight terpenes in the plant. From the docking studies it is evident that *a*-amyrin has a great potential against AeSCP-2. The phytochemical screening and docking results gives strong baseline information for the posterity.

Introduction

Calotropis gigantea (Asclepiadaceae), known as milk weed, is a common wasteland weed, drought resistant, salt tolerant, grows wild up throughout India (Sastry and Kavathekar, 1990). It is one of the peculiar plants not consumed by grazing animals (Sharma, 1934).

The identified phytochemicals in this plant are usharin, gigantin, *a* and *β*-calotropeol, *β*-amyrin, fatty acids, hydrocarbons, a mixture of tetracyclic triterpene compounds, sterols, and giganteol (Murti and Seshadri, 1943, 1945a,b). Cardenolide, calotropin (Kupchan et al., 1964), *a*-amyrin, *β*-amyrin, taraxasterol, *β*-sitosterol, *a*-amyrin methylbutazone, *β*-amyrin methylbutazone, *a*-amyrin acetate, *β*-amyrin acetate, taraxasteryl acetate, lupeol acetate B, gigantursenyl acetate A, gigantursenyl acetate B (Sen et al., 1992; Habib et al., 2007), flavonol glycoside, akundarol, uscharidin, calotropin, frugoside, calotroposides A to G were isolated. Thus, the plant has immense potential to cure various diseases and disorders (CSIR, 1992; Duke, 1992; Chitme et al., 2004; Tenpe et al., 2007). The present study reports *in silico* docking analysis carried out to assess the mosquito larvicidal potential of four terpene compounds isolated from *C. gigantea*.

Materials and Methods

Plant material collection and processing

The aerial parts of the plant parts were collected from Tiruchirappalli, Tamil Nadu, India, on September 2008. The collected plant materials were then brought in to the laboratory and washed thoroughly with the distilled water to remove the dirt and other contaminations. Then the washed plant materials were dried carefully under shade, at room temperature so as to retain their fresh green colour, and also to prevent decomposition

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of the 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.

Phytochemical analysis

The drugs were used to determine the organic carbon (Walkley and Black, 1934); carbohydrate (Hedge and Hofreiter, 1962); protein (Lowry et al., 1951); lipid (Folch et al., 1957); ash content (Renaud et al., 1994); total nitrogen (Balasubramanian and Sadasivam, 1987); phosphorous, potassium (Jackson, 1973); calcium, magnesium, zinc, copper, iron, manganese, boron, molybdenum, chromium, nickel, cadmium, lead, cobalt, mercury, arsenic, cyanide, Selenium and silver (Baker and Suhr, 1982; Allen, 1989).

Elemental analysis

Two grams of dried sample was digested in a mixture of nitric acid, sulfuric acid and perchloric acid in the ratio 11:6:3, for 24 hours to remove the organic matters. The digested sample was made up to 100 mL and used for the assay of the trace elements through atomic absorption spectrophotometer (AAS- Varion 200AA) using suitable hollow-cathode lamps. Appropriate working standard was prepared for each element. All elements were determined through this procedure. A blank reading was also obtained.

Extraction and GC-MS analysis of terpenes

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 50 mL of analytical grade chloroform was added and refluxed for 8 hours. After completion of the 8 hours, the round bottom flask was cooled and the extract was filtered through the Buchner funnel. The extract was evaporated to dryness under nitrogen atmosphere using turbo evaporator. The residue obtained was dissolved in 2 mL 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. Analytes 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. The tentative identification of volatile components was achieved by comparing the mass spectra with the data system library (NIST) and other published spectra (Mass Spectrometry Data Centre., 1974), supported by retention index data, which were compared with available literature retention indices. All compounds were quantified as 3-octanol equivalents.

Docking studies

The present biocomputational investigation was carried out to identify the candidate therapeutic terpene compounds having potential for inhibiting the growth of mosquito larvae. The 3-D crystal structure of the protein AeSCP-2 was retrieved from the protein data bank (PDB). AeSCP-2 protein is a low molecular weight with high levels of expression in the midgut of the larvae and high binding affinity to cholesterol in the *Aedes aegypti* mosquito species (Kitamura et al., 1996). Structural and active site enumeration were done by using pymol molecular visualization software and CASTP (Computed Atlas of Surface Topography of Proteins). Four phytochemicals namely *a*-amyrin, oleanolic acid, 5-norbornene-2-carboxylic acid and pyrethrin were screened against the protein AeSCP-2. 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 Chemsketch Software (www.acdlabs.com). The molecular docking analysis was performed using Argus Lab 4.0 which is widely distributed public domain molecular docking software.

Results and Discussion

Leaf sample of *C. gigantea* was analyzed for the composition of ash materials and the organic carbon, primary metabolites, essential macro nutrient, essential micro nutrient and trace elements. The plant contains 1.69% of ash and 27.5% of organic carbon (Table I). The primary metabolites, carbohydrate, protein and lipids were 0.97, 0.55 and 0.29% respectively. The essential macronutrients such as nitrogen (2.16%), phosphorous (0.42%), potassium (2.97%), calcium (4.59%), magnesium (3.16%), and sulfur (0.48%) were found in considerable level. The essential micro nutrients were also found in considerable level. Among micronutrients the amount of iron (156.3 ppm) was the highest and molybdenum (0.1 ppm) was the lowest. The heavy metals except arsenic and cyanide, all other heavy metals were found to be present in considerable level in this plant. Lead was the most dominating heavy metal (0.16 mg/L), whereas nickel (0.02 mg/L) was the least. Compared to previous studies our results are also in considerable level. Similar study on *C. procera* showed varied concentrations of phytochemicals (Altaf, 1997) and similar variations were also found in our study. These differences could be due to ecological, time of collection, collection sites or may be because of increasing pollution or environmental factors. Previous

Table I

Phytochemicals present in the *Calotropis gigantea*

Parameter	Quantity%	Parameter	Quantity	Parameter	Quantity
Ash	1.7	Magnesium	3.2%	Nickel	0.02 ppm
Organic carbon	27.5	Sulfur	0.5%	Cadmium	0.04 ppm
Carbohydrate	1.0	Zinc	3.2 ppm	Lead	0.16 ppm
Proteins	0.6	Copper	1.0 ppm	Cobalt	0.05 ppm
Lipids	0.3	Iron	156.3 ppm	Mercury	0.001 ppm
Nitrogen	2.2	Manganese	22.6 ppm	Arsenic	bdl
Phosphorous	0.4	Boron	0.1 ppm	Cyanide	bdl
Potassium	3.0	Molybdenum	0.1 ppm	Selenium	0.59 ppm
Calcium	4.6	Chromium	0.002 ppm	Silver	0.02 ppm

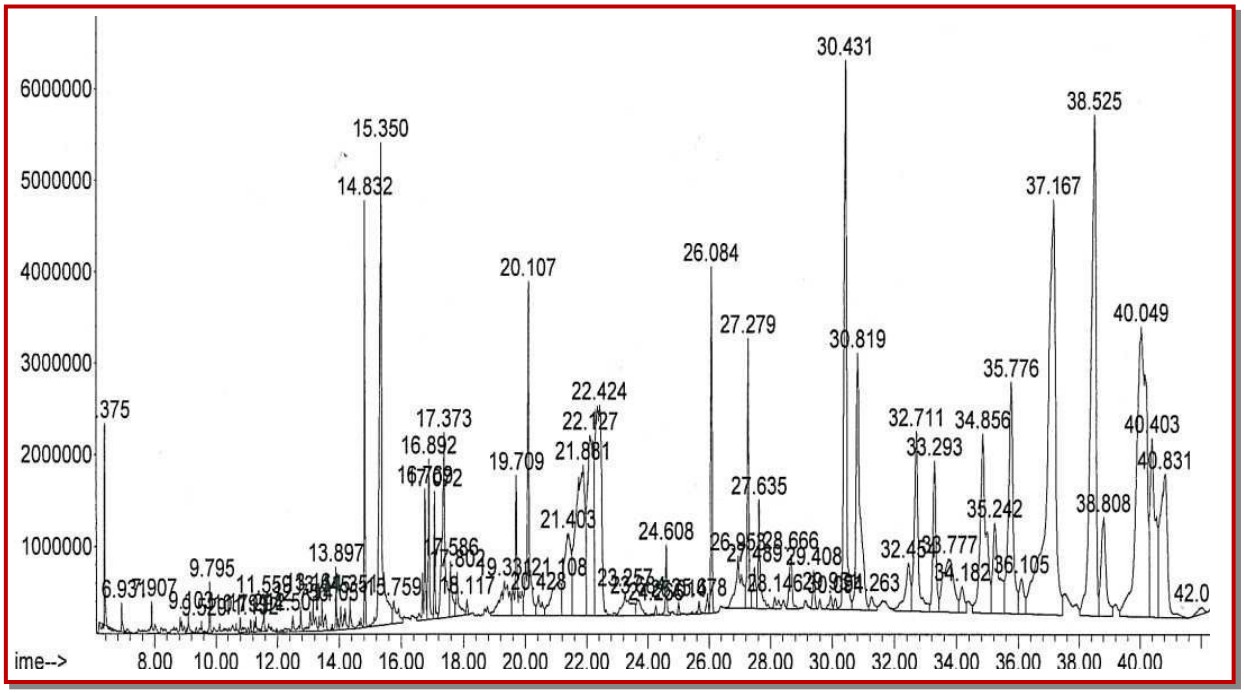


Figure 1: GC mass spectra of *Calotropis gigantea*

studies have also report the presence of phytochemicals like terpenes, cardenolides, flavonoids, pregnanes, non-protein amino acid and cardiac glycoside as major constituents in *Calotropis sp.* and presence of these phytochemicals sturdily acknowledge the medicinal property of this plant (Wang et al., 2008; Ali and Gupta, 1999).

The GC mass spectra (Figure 1) showed the presence of eight terpenes in the plant namely bicyclo (3.1.1) heptane,2,6,6-trimethyl-,(1alpha, 2alpha, 5alpha); phytol; Urs-12-en-24-oic acid, 3-oxo-,methyl ester,(+)-; squalene; taraxasterol; *a*-amyrin; beta-amyrin and 12-oleanen-3-yl acetate, (3alpha) (Table II). The 12-oleanen-3-yl acetate, (3alpha) was the major portion of the terpenes which showed the peak area percentage of 16.9.

Approximately 3 billion people, one half of the world's population, live in at risk regions for malaria infection. This leads to about 250 million malaria cases every year and nearly one million deaths. One of the most crucial obstacles for eradicating malaria is a widespread resistance of malarial parasite to almost all chemotherapeutic agents (Snow et al., 2005).

Considering the dreadful global issue of the health, *in silico* docking analysis was carried out. Four phytochemicals namely *a*-amyrin, oleanolic acid, 5-norbornene-2-carboxylic acid and pyrethrin were selected for screening against the protein AeSCP-2. The compounds

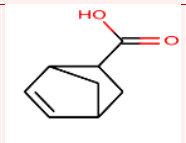
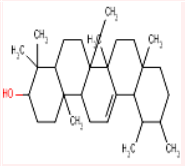
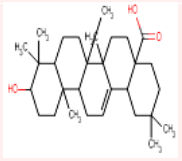
Table II

Terpenes screened from *Calotropis gigantea*

Sl. No.	Retention time	Peak area%	Compound name	CID No.	Molecular formula	M. Wt [g/mol]
1	13.8	0.5	5-Norbornene-2-carboxylic acid	78949	C8H10O2	138.2
2	16.8	1.1	Phytol	5280435	C20H40O	296.5
3	26.0	1.8	Squalene	1105	C30H50	410.7
4	35.7	5.8	alpha-Amyrin	225688	C30H50O	426.7
5	37.0	8.4	beta-Amyrin	73145	C30H50O	426.7
5	38.4	16.9	Oleanolic Acid	10494	C30H48O3	456.7
6	40.2	4.2	Taraxasterol	5270604	C30H50O	426.7
7	-	47.8	Other compounds	-	-	-

Table III

Docking studies

Compound name	Pubchem ID	Compound structure	Molecular weight (g/mol)	Hydrogen donor/ acceptor	Docking energy level (Kcal/mol)
5-Norbornene-2-carboxylic acid	CID: 78949		138.2	1,2	-8.4
Alpha-amyrin	CID: 25688		426.7	1,1	-14.1
Oleanolic acid	CID: 10494		456.7	2,3	-13.8

were docked in Argus lab 4.0. Docking energy was found to be -14.1213 Kcal/mol, -13.7876 Kcal/mol and -8.3558 Kcal/mol in *a*-amyrin, oleanolic acid and 5-norbornene-2-Carboxylic acid respectively (Table III). Hence, from the docking studies it is evident that *a*-amyrin (*C. gigantea* derived terpene) has a great potential against AeSCP-2.

Medicinal plants are being probed as an alternate source to get therapeutic compounds based on their medicinal properties. *C. gigantea* is easily available in most of the agricultural and non-agricultural fields and the usage of this plant for medicinal purpose was reported by several researchers.

Conclusion

C. gigantea represents a rich source of valuable medicinal compounds and leaves of *C. gigantea* contain *a*-amyrin which could be a potential source for inhibiting the AeSCP-2.

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Conflict of Interest

Authors declare no conflict of interest.

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