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Isolation and spectroscopic characterization and molecular modeling of novel compounds obtained from latex of calotropis procera

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ABSTRACT

Calotropis procera R. Br. (Asclepiadaceae) is a well-known medicinal plant with leaves, roots, and bark being exploited by popular medicine to fight many human and animal diseases. This work deals with the fractionation of the crude latex produced by the green parts of the plant and aims to evaluate its toxic effects upon egg hatching and larval development of Aedes aegypti. The whole latex was shown to cause 100% mortality of 3rd instars within 5 min. It was fractionated into water-soluble dialyzable (DF) and non-dialyzable (NDF) rubber-free materials. Both fractions were partially effective to prevent egg hatching and most of individuals growing under experimental conditions died before reaching 2nd instars or stayed in 1st instars. Besides, the fractions were very toxic to 3rd instars causing 100% mortality within 24 h. When both fractions were submitted to heat-treatment the toxic effects were diminished considerably suggesting low thermostability of the toxic compounds. Polyacrylamide gel electrophoresis of both fractions and their newly fractionated peaks obtained through ion exchange chromatography or desalting attested the presence of proteins in both materials. When submitted to protease digestion prior to larvicidal assays NDF lost most of its toxicity but DF was still strongly active. It may be possible that the highly toxic effects of the whole latex from C. procera upon egg hatching and larvae development should be at least in part due to its protein content found in NDF.

Introduction

Calotropis procera is a wild growing plant that belongs to the family Asclepiadaceous. It is known by various names like swallow wort, dead sea apple, and Sodom apple or milk weed. In Indian sub-continent the plant is known by different names like Ark in Hindi, orka in Oriya, Alkarka in Sanskrit and Vellerukku in Tamil. Calotropis procera R. Br. (Asclepiadaceae) is a plant widely distributed in Asia. The plant is popularly known because it produces large quantity of latex, which is easily collected from its green parts when the plant is wounded. The aspect of this natural secretion resembles that of rubber tree Hevea brasiliensis (Willd.ex Adr.Juss.) Müll.Arg. (Euphor-biaceae).Local people use it successfully to combat some skin fungi infections. The abundance of latex in the green parts of the plant reinforces the idea that it is produced and accumulated as a defense strategy against organisms such as virus, fungi, and insects [1] although it is possible to find wounded plants of Calotropis or to see insects visiting their leaves. When injured, leaves or the nearest other green parts exude the latex secretion which has a clingy effect capable of immobilizing insects. Additionally, the presence of plant defense-related proteins such as helve in, alpha-amylase inhibitor, among others, has been described to occur in the latex secretion of other plants [2] and this seems to be also the case of Calotropis latex [3]. Scientific descriptions have mentioned other relevant activities for the latex of C. procera, such as antibacterial, analgesic or possessing in vitro schizonticidal activity, among others [4]. In early 1980s a brief communication pointed out the whole latex of C. procera as a suitable source of active compounds exhibiting larvicidal activity [5-8]. In the traditional Nepali medicinal system, it has been used for a variety of disease conditions like leprosy, ulcers, tumors, piles and diseases of spleen, liver and abdomen[9,10].

The plant is also known for its toxic properties that include iridocyclitis, dermatitis and acts like a poison and produces lethal effects. The aqueous extract of the dried latex produces inflammation when administered by subcutaneous injection(7). Leaves and roots of this plant have been used to relieve pain under different conditions. The aqueous extract of dry latex of this plant also acts as an analgesic and antipyretic [Preliminary studies on the analgesic activity of latex of Calotropis procera. Antipyretic effect of latex of Calotropis procera. ethanol extracts of its. The latex of Calotropis procera contains the active ingredients for use in the treatment and prevention of cancer[11]. The aim of this project is isolation and spectroscopic characterization and molecular modeling of novel compounds.

Materials and methods

All the chemicals used in this study were of analytical grade which obtained from Merck. Solvents used were of analytical grade and were purified by standard procedures. Latex samples - Samples of C. procera were collected from botanical garden. Fresh latex was collected from healthy plants by small incisions near the youngest leaves and left to flow off into distilled water in order to obtain a mixture 1:1 (v/v).The mixture was gently handled to maintain homogeneity during transport to the laboratory. It should be emphasized that the process of latex sampling does not compromise the health of plants. As a matter of fact they regenerate very fast. Samples were centrifuged (5000 g) at room temperature (25°C) in a non-refrigerated bench top centrifuge for 10 min. The precipitated material, showing rubber aspect, was pooled apart while the supernatant was

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submitted to exhaustive dialysis (cut off 8000 Da) against distilled water at 25°C and submitted to centrifugation as aforementioned. The new supernatant, devoid of rubber, was freeze-dried. This fraction was named non-dialyzable fraction (NDF)[12]. The dialyzable fraction (DF) was obtained after the first hour of dialysis when the supernatant was initially submitted to dialysis using a proportion of 1:1sample distilled water. Thus, the DF corresponded to the water used in the first h of dialysis done immediately after the first centrifugation step. All experiments with the DF were performed with both fresh preparations or lyophilized material. The compounds were purified by column chromatography using different concentration of alcoholic solvent and then tested by TLC. The compounds were dried at room temperature and the stoichiometric analyses(C, H and N) of the isolated compounds were performed using Elementar vario EL III (Germany) model. Their IR spectra were recorded on Perkins–Elmer FTIR spectrophotometer in KBr and polyethylene pellets. ^{1}H NMR spectra were recorded in DMSO-d_6 solvent on a Bruker Advance 400 instrument

3d – Molecular Modeling

3D molecular modeling of the proposed structure of the complexes was performed using CsChem3DUlta -11 program package. The correct stereochemistry was assured through the manipulation and modification of the molecular coordinates to obtain reasonable low energy molecular geometries. The optimized structures of the compounds were performed by MM2 programme contained CS chem. Office programme. The potential energy of the molecule was the sum of the following terms: $E = E_{str} + E_{ang} + E_{tor} + E_{vdw} + E_{oop} + E_{ele}$.Where all E's represent the energy values corresponding to the given types of interaction. The subscripts str, ang, tor, vdw, oop and ele denote bond stretching, angle bonding, torsion deformation, van der waals interactions, out of plain bending and electronic interaction, respectively.

Results and discussions

The latex of *C. procera* is milky and resembles the well-characterized latex from *Hevea brasiliensis* (rubber tree). The rubber fraction was successfully separated from the water-soluble material by a single step of dialysis against distilled water. Both water-soluble fractions (NDF and DF) separated by a dialysis membrane (cut off 8000) seem to be toxic against eggs hatch and larvae of Ae.aegypti[13-15]. First of all, it should be emphasized that the most potent larvicidal action was achieved with the whole latex. Hence it is reasonable to consider that rubber fraction shows harmful effects upon larvae. Indeed, assays performed using the rubber fraction instead of the soluble fractions caused larval death in a few min. However, the low water solubility of the rubber drastically diminishes its usefulness in mosquito control programs. The isolated compounds exhibited following spectral characterization and obtained two novel compounds.

Elemental analysis

Satisfactory results of elemental analysis (Table 1) and spectral studies revealed that the compounds were of good purity. From the elemental analysis the empirical formula of isolated compounds to be proposed as in given table.

Vibrational spectroscopy

The IR spectra of isolated compounds exhibited absorptions and have been assigned in table 2.

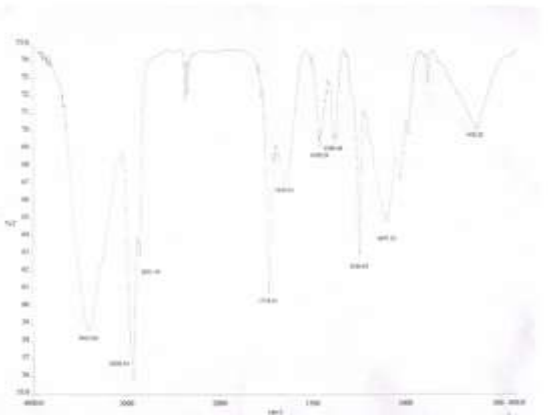

Figure1 FT-IR spectra of isolated compound 1

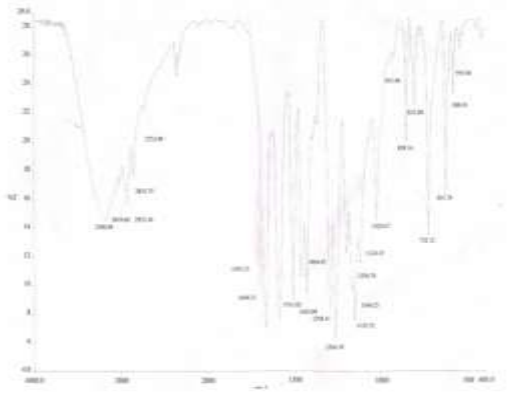

Figure2. FT-IR spectra of isolated compound 2

^{1}HNMR assignment of isolated compounds

^{1}HNMR data were carried out in DMSO-d_6 with 400Mz resolution and have been assigned in table 3.

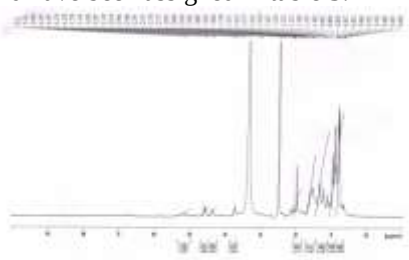

Figure 3. ^{1}HNMR spectra of compond1

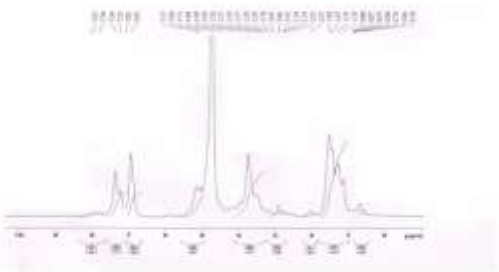

Figure 4. ^{1}HNMR spectra of compound 2

Tof-mass spectral analysis

Mass spectrometry has been successfully used to investigate molecular species$[M]^{+}$ in solution []. The molecular ion peaks of the compound have been used to confirm the proposed formula (Table 4). The pattern of the mass spectrum gives an impression of the successive degradation of the target compound with the series of peaks corresponding to the various fragments. Their intensity gives an idea of stability of fragments. The compound1 starts degradation and finally forms m/z: 851.68 (100.0%), 852.68 (62.2%), 853.69 (19.4%), 854.69 (4.4%), 853.68 (1.1%),

852.69 (1.0%) and compound2 as m/z:732.31(100.0%),733.32(36.8%),734.32(9.8%),735.32(2.0%) ,735.31(1.7%).

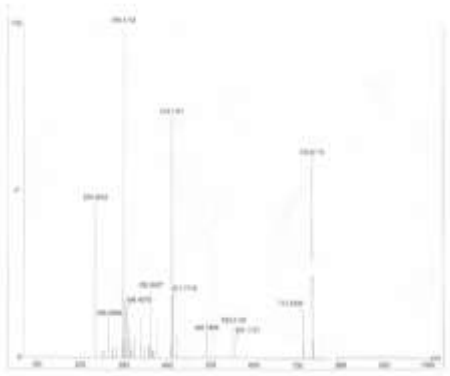

Figure 5.ESI-Mass spectra of compoun1

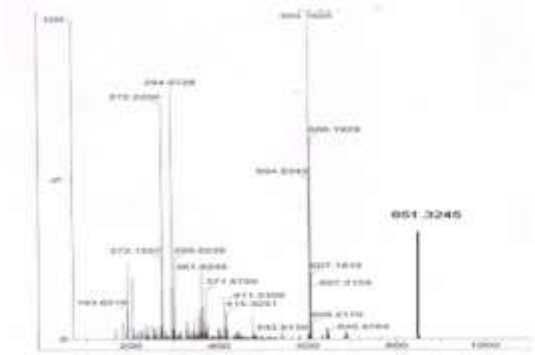

Figure 6. ESI-Mass spectra of compound 2.

From the above results the structure to be proposed the isolated compounds obtained from latex of Calotropis procera

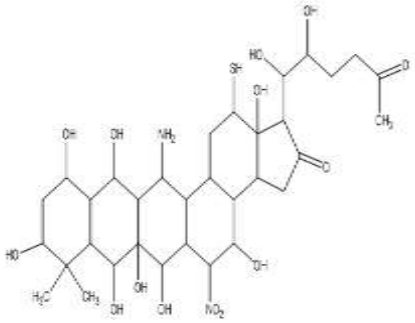

Figure 7.13-amino-1-(1,2-dihydroxy-5-oxohexyl)-4,6,6a,7,9,11,12,15a-octahydroxy-15-mercapto-8,8-dimethyl-5-nitrodocosahydro-1H-indeno[5,4-a]tetracen-2(3H)-one(compound1)

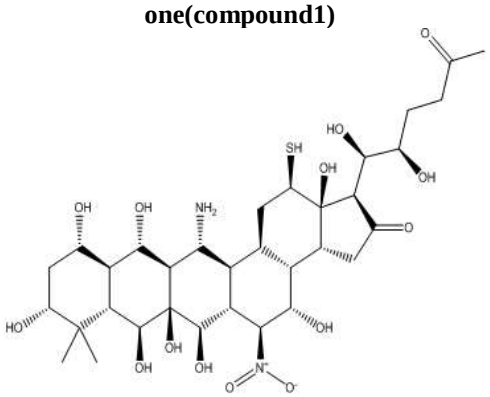

Figure 8. Asymmetry structure of compound1

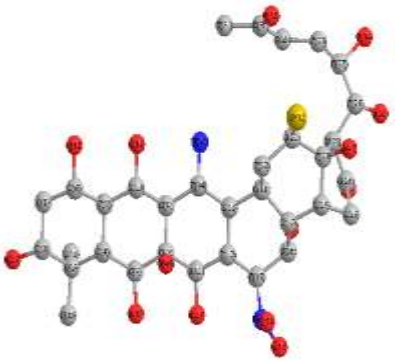

Figure 9.Optimised structure of compound1.and Steric energy 79.49j/mol

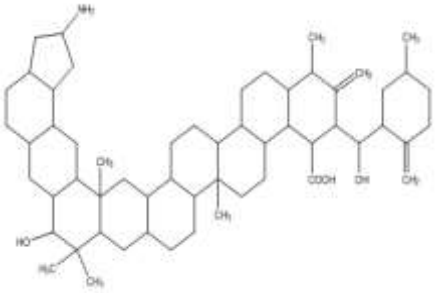

Figure10.16-amino-11-hydroxy-3-(hydroxy(5-methyl-2-methylenecyclohexyl)methyl)-1,6a,10,10,18b-pentamethyl-2-methylenetetracontahydro-1H-chryseno[2,1-a]cyclopenta[o]pentaphene-4-carboxylic acid

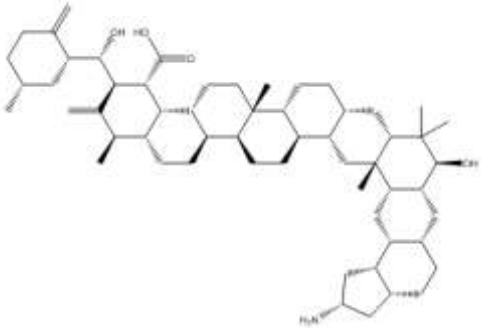

Figure 11. .Asymmetry structure of compound2

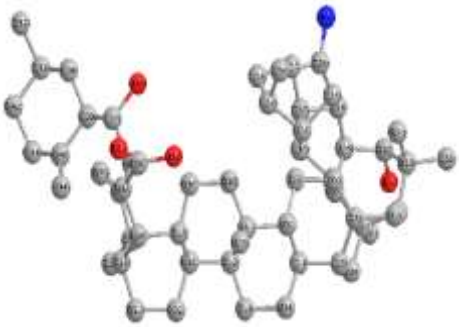

Figure 12. Optimised structure of compound 2 and steric energy 65.754

Conclusions

The latex of C. procera is milky and resembles the well-characterized latex from Hevea brasiliensis (rubbertree). The rubber fraction was successfully separated fromthe water-soluble material by a single step of dialysis against distilled water. Both water-soluble fractions (NDFand DF) separated by a dialysis membrane (cut off 8000)seem to be toxic against eggs hatch and larvae of Ae.aegypti. The latex of C. procera possesses a potent toxic activity against larvae of the yellow fever and dengue mosquitoes. Aeaegypti. The study gave a

weight into the possibility of formulating suitable preparation from the water-soluble fractions (DF or NDF) of C. procera latex for use in mosquito control programs. Nepalese people are using as ethanomedicine and ant mosquito control since long time. The novel compounds have been synthesized and it will be applicable for the ailment of anti-HIV.

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Table-1 Color, reaction yield and elemental analysis of complexes

Complex	Empirical formula	Molecular Weight	Color	Yield (%)	Analysis: found (calculated)(%) C	H	N	O	S	M.P.^{0}C(Expt)
Compound 1	$C_{33}H_{52}N_2O_{14}S$,	732.31	white	90	54.08	7.15	3.12	30.57	4.38	125^{o}C
Compound 2	$C_{57}H_{89}NO_4$	852.32	white	80	80.32	10.52	1.64	7.51	--	135^{0}C

Table 2. IR spectral data (cm^{-1}) of the isolated compounds

Frequency	ν_{N-H}	C=NH	OH	NH_2	NO_2	C=O	S-H	COOH
$C_{33}H_{52}N_2O_{14}S$	2928(s)	2851(s)	3401(s)	1222(m)	983(s)	1734	1380	
$C_{57}H_{89}NO_4$	3180(s,b)	1635(m)	1121 (s)	1340(s)	--------	841	1028	1617

Table 3.^{1} H N M R data of the isolated compounds

Compounds	δ (ppm)
$C_{33}H_{52}N_2O_{14}S$	[1.5(s)1H,SH],3.58-3.65(s)10H,OH,5.11(s),1NH_2,(1.63-3.12)18HCH,(6.79-7.16)(s)!H,NH_2,(0.96-1.72-2.45)2CH_2,3H(0.99-2.13)CH_3
$C_{57}H_{89}NO_4$	[1.58(m),1H,OH],[11(s),1H,COOH,][2.0(s)4H,NH],5.11(s)1H,NH_2,]1.32—2.01(m)19H,CH 1.18(m) 6H,CH_3].