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Isolation, spectroscopic characterization and computational modeling of chemical compounds obtained from fruits of Thevetia peruviana

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ABSTRACT

The aim of this work was to investigate the most effective extraction conditions for the production of thevetia seed protein concentrate of reduced cardiac glycoside content. Alcoholic extraction of the glycosides was studied as a function of time, solvent to meal ratio and solvent composition. Thevetia seed meal was extracted with 10:1, 15:1 and 20:1 solvent to meal ratios, for 45 min, 12, 24 and 48 h. the isolated compounds were characterized by spectral techniques and structure of the compounds were proposed.

Introduction

Phytomedicines are a major component of traditional system of healing in developing countries, which have been an integral part of their history and culture. Besides widespread use of botanicals as medicinal products in developing countries, such products are becoming part of the integrative healthcare system of industrialized nations, known as complementary and alternative system of medicines [1]. Existing costly therapy is not affordable well for the millions of individuals particularly in the developing world. Plant extracts are the cheap and easily available source to poor people. Plants are great source of thousands of new useful phytochemicals of great diversity, which have inhibitory effects on all types of microorganisms in vitro. The current pharmaceutical armory of antifungal is a clear cause for satisfaction, not from gloom. However, we still do not have agents that fulfill every one of the criteria that a physician would set as desiderata for antifungal drugs [2]. They need to be active against those fungi causing infections which we cannot yet depend on eradicating. They need to be formulated for both oral and parenteral administrations; they need to be extremely safe and as cheap as possible. The search for new antifungal agents therefore must go on. Identification of new chemo types for drug development remains an urgent need in antifungal therapeutics. Simultaneously, a number of antifungal compounds reported till date is tested for their in vitro activities and not for in vivo activities. In vivo and in vitro activities of a compound may be different and a very small number of plants' extracts or components have been studied for their in vivo activity [3]. Therefore, these should be subjected to animal and human studies to determine their effectiveness in whole organism systems. Also in vitro testing and method of extraction should be standardized so that the search could be more systematic. The current set of clinically available antifungal agents includes three classes of natural products and four classes of synthetic chemicals. We therefore cannot abandon interest in biodiversity as a source of natural antifungal products. Furthermore, the inactive plant extracts may be subjected to chemical diversification of their components to increase the activity. The transformation of chemical groups in natural products into rare chemical groups is possible which is rarely produced by secondary metabolism. Therefore, biosynthesis machinery can be complemented to produce a whole range of new semi synthetic compounds in one step which may become an alternative source of compounds to feed the discovery process for new interesting compound and metal ions. Over the centuries, human beings gathered information by trial and error. In ancient times humans uses various plants and herbs that grew in their environment to treat various illness. Medicinal plants have emerged as some of the most widely studies plants and significant interest has been shown in their chemistry because of their potential application in medicine. Many of these medicine plants contain chemical constituents that could cause harmful effects to human if taken in large quantities .Alkaloids occurring in a large amount make these plants poisonous [4]. Demand on medicinal plant products such as pharmaceuticals, phytochemicals, nutraciuticals, cosmetics and other products on worldwide are increasing day by day. Herbal medicinal products may vary in composition and properties unlike conventional pharmaceutical products. Correct identification and quality assurance of the starting materials is therefore an essential prerequisite to ensure reproducible quality of herbal medicine which contributes to its safety and efficiencies [5].Thevetia peruviana is an ornamental small tree of the Apocynaceae family. It is widespread on the Indian sub-continent its seeds, leaves, fruit and roots are being used in traditional medicine as a local anesthetic, purgative, emetic, abortive, antineuralgic and antiartralgic, for the treatment of hemorrhoids, intermittent fever, acne and to reduce body weight [6,7]. However formal pharmacological studies to establish such therapeutic uses don't exist. Death has been reported from the accidental or intentional ingestion of Thevetia seed. Clinical reports indicate that the toxic effect is similar to the one that is presented with dioxin at high dose. The search for lesser known crops which have valuable potential as animal and human food, has continued to

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receive greater attention in recent times. It is an ornamental shrub, which grow in both temperate and tropical climate throughout the world. There are many varieties of Thevetia found in different locations all over the world. It is an evergreen plant that fruits all year round. The unripe fruit is green, but pale green, brown or black when ripe. Each fruit contains a nut that is longitudinally and transversely divided. All parts of the plant contain the milky juice, which is poisonous. However, oleander extract is used in folk medicines and there are reports that long-term use of oleander may have positive effects in patients with prostrate or breast cancer [8]. The aim of this work is chemical isolation of thevetia peruviana fruits from matured plant characterized by spectral techniques.

Materials and methods

Thevetia peruviana fruits were obtained from various locations in Biratnagar metropolis in Nepal. The seeds were removed from the endocarp by cracking and later in the absence of sun-light. The fruits were cracked to remove the hard pericarp and mesocarp and the soft seeds crushed into a paste. The paste was defatted first by mechanical pressing, followed by solvent extraction using redistilled n-hexane. The methods employed in processing are: heat treatment (autoclaving) and soaking (in water). The seed were autoclaved for 30 minutes at 125^0C at 1.2kgcm -2 pressures and some portion of dry seed were soaked in water for 18hrs. And then sun-dried for 48hrs. The endocarp of the fresh seed of T.peruviana was eliminated and seeds underwent a milling process at 3000 rpm during 30 seconds. The extracts were obtained by a maceration process rest 100 g of seed with 200 ml of each solvent: (ethyl acetate, methanol and water) for 48 hr. For each one of the solvents maceration was repeated twice. Following maceration, the supernatant was obtained, which was centrifuged to 3000 rpm to 10^oC, for 10 min). All organic solvents were eliminated by a rot evaporator vacuum system and the water extract was lyophilized (Virtis model 10-155). The dry extracts were weighed, the yield calculated and samples were maintained at 4^oC [10, 11].The isolated compounds were characterized by spectral techniques.stoichiometric analyses(C, H and N) of the complexes 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

Elemental Analysis

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

Figure1. IR-spectra of compound-1($C_{56}H_{75}N_5O_{19}S$)

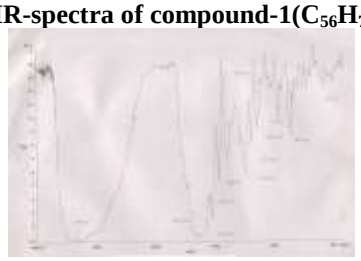

Figure2. IR-spectra of compound-2 ($C_{57}H_{86}N2O_{22}S_2$)

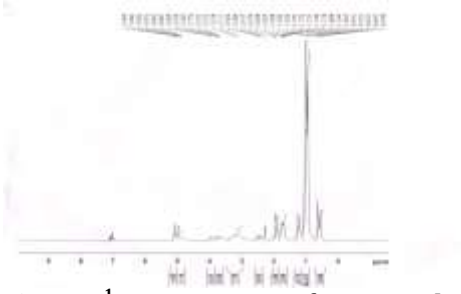

Figure 3.^{1}HNMR spectra of compound 1

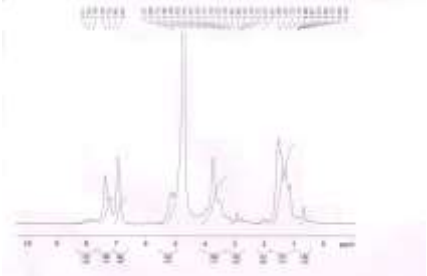

Figure 4.^{1}HNMR spectra of compound

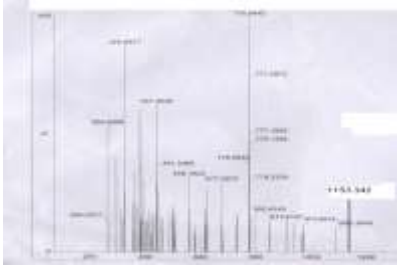

Figure 5.TOF-Mass spectra of compound-1

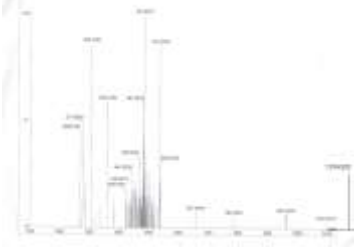

Figure6. TOF-Mass spectra of compound-2

TOF–MS spectra

Mass spectrometry has been successfully used to investigate molecular species $[M]^+$ ions in solution [13,14]. The molecular ion peaks of the compounds have been used to confirm the proposed formula. 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

compounds start degradation and finally as form both isolated compounds as m/z: 1153.48 (100.0%), 1154.48 (63.0%), 1155.48 (23.6%), 1156.49 (6.2%), 1155.47 (4.5%), 1156.48 (3.4%), 1154.47 (1.8%), 1157.49 (1.4%), 1157.48 (1.2%) for compound1 and fragmentation of compound 2 as m/z: 1214.51 (100.0%), 1215.51 (64.0%), 1216.52 (24.3%), 1216.51 (10.5%), 1217.52 (7.4%), 1217.51 (5.9%), 1218.51 (2.3%), 1215.52 (1.8%), 1218.52 (1.7%).

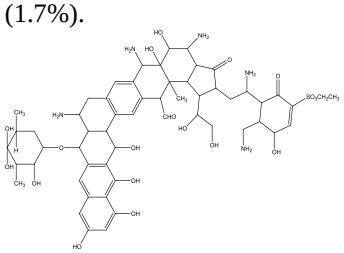

Figure7..Ethyl5-(1-amino-2-(4,6,9-triamino-1-(1,2-dihydroxyethyl)-19-formyl-5,5a,13,15,16,17-hexahydroxy-19a-methyl-3-oxo-10-((2R,3S,5S)-2,4,4-trihydroxy-3,5-dimethylcyclohexyloxy)-2,3,3a,4,5,5a,6,8,9,9a,10,17,17a,19,19a,19b-hexadecahydro-1H-cyclopenta[a]heptaphen-2-yl)ethyl)-4-(aminomethyl)-3-hydroxy-6-oxocyclohex-1-ene-1-sulfonate (Compound-1)

Molecular modeling of isolated compounds

To examine the structural properties, various traditional research techniques were used, but in this article, we were trying to assess observed data at molecular level with the help of molecular modeling. This modeling program was commonly known as computer assisted molecular design (CAMD). Molecular modeling had been successfully used to detect three dimensional arrangements of atoms in compounds. Their utilization in the demonstration of molecular structure of the studied compounds was presented in the article. Molecular mechanics was a mathematical formalism, which attempted to reproduce molecular geometries, bond energies and other related features

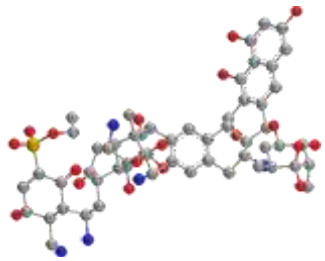

Figure8. Optimized structure of compound-1.and steric energy is 59.13 cal/mol

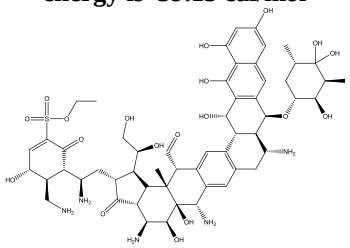

Figure-9.Asymmetry structure of compound 1

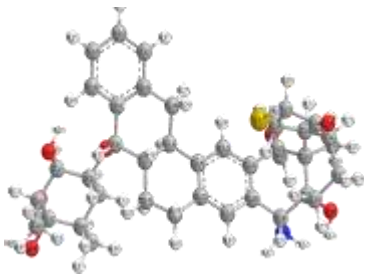

Figure 10. Optimized structure of compound 1

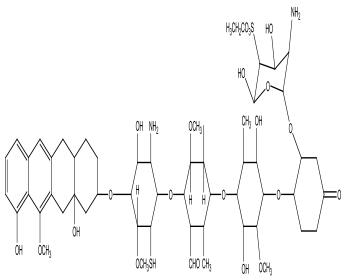

Figure 11. (2R, 3S, 4S, 5R,6R)-ethyl 5-amino-6-(2-((2R,3S,5R,6R)-4-((2R,3S,5S,6S)-4-((2R,3S,5R,6S)-2-amino-4-(10,12a-dihydroxy-11-methoxy-1,2,3,4,4a,5,12,12a-octahydrotetracen-2-yloxy)-3-hydroxy-6-mercapto-5-methoxycyclohexyloxy)-3-formyl-5-methoxy-2,6-dimethylcyclohexyloxy)-2,5-dihydroxy-6-methoxy-3-methylcyclohexyloxy)-5-oxocyclohexyloxy)-2,4-dihydroxytetrahydro-2H-pyran-3-sulfonate

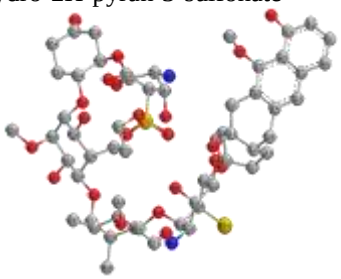

Figure 12. Optimized structure of compound 2

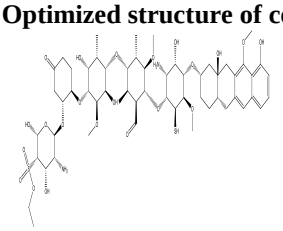

Figure 13. Asymmetry structure of compound2

Conclusions

Thevetia peruviana (Apocynaceae), native to tropical Nepal and Indian subcontinent is a small tree or bush that grows 5 to 9 feet high. Chemical and pharmacological studies demonstrate that the whole plant, particularly the seeds, contains potentially harmful cardiac glycosides .Inges-tion of the seeds produces a clinical picture very similar to that of digoxin poisoning: vomiting, dizziness changes EKG, bradycardia, and AV block. Less common signs and symptoms include diarrhoea, abdominal pain, ectopic beats and palpitations. The high oil content makes the seed a potential source of commercial vegetable oil. The greater proportion of oil in *Thevetia* seeds composed of unsaturated fatty acids. Also, two major groups of compounds were identified to contain anti-fungal photo-activity in *Thevetia*. The compounds are thevealdehyde and fatty acid.

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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 S M.P.^{0}C(Expt)
Compound 1	$C_{56}H_{75}N_5O_{19}S$,	1153.48	Pale yellow	80	58.27 6. 55 6.07 26.34; 2.78 75^{o}C
Compound 2	$C_{57}H_{86}N2O_{22}S_2$	1214.51	white	70	56.33 7.13 2.30 28.96 5.28 95^{o}C

Vibrational Spectra

The IR spectra of free ligands and their complexes have been assigned in table 2.In both compounds and assigned in given table.

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

Frequency	ν_{N-H}	C=NH	OH	NH_2	$-SO_3CH_2CH_3$	C=O	S-H	NO_2
$C_{56}H_{75}N_5O_{19}S$	3007(s,b)	2925(S)	1514(s)	1222(m)	1377(s)	1746	2625	
$C_{57}H_{86}N2O_{22}S_2$	3329(s,b)	1635(m)	1521 (s)	1340(s)	1385(s)	841	2527	1617

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

Compounds	δ (ppm)
$C_{56}H_{75}N_5O_{19}S$	[1.5(s)5H,OH],1.65(s)1H,OH,5.11(s),1NH,(1.63-3.12)5H,(6.79- 7.16)(s)!H,NH_2,(0.96-1.04)3CH_3,1H(1.77)
$C_{57}H_{86}N2O_{22}S_2$	[1.5(m),1H,SH],[2.80-5.35(s),5H,OH,][2.0(s)4H,NH],8.56(s)1H,NH_2,]3.51—4.24(m)4H,CH],[5.03(d)1H,CH],3.76—3.81(m),6H CH],8.19(s)1H ,CH] 8.89(m) 1H CH(Ar)],[7.62(s) 1H,CH],[9.72(s) 1H,CHO],4.73(m)1H CH,3.79 2H,CH2,3.17(m)6H,CH_3,1.18(m) 3H,CH_3].