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3. NMR Analysis of Pharmaceutically Active Secondary Metabolite of Plant Saponin

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ABSTRACT

Nuclear Magnetic Resonance (NMR) spectroscopy is (arguably) the most powerful tool available for determining the structure of organic compounds. Saponins are a diverse group of compounds commonly found in legumes, e.g. chick peas, soya beans, lentils, peanuts, lentils, Phaseolus beans and alfalfa sprouts; and in some plants commonly used as flavourings, herbs or spices, e.g. ginseng, fenugreek, sage, quillaja bark, thyme, sarsaparilla and nutmeg. Several biological effects have been ascribed to saponins. Extensive research reported that the membrane-permeabilising, immuno-stimulant, hypocholesterolaemic and anti- carcinogenic properties of saponins and they have also been found to significantly affect growth, feed intake and reproduction in animals. Extraction of saponin from plant calotropis gigeantea were done with the help of methanol as a extracting solvent and analyzed the saponin present in the extract with the help of NMR advance analytical techniques. In which the ^{13}C and ^{1}H analysis done in Cacl3 Solvent and successfully obtained the spectrum data for elucidatation the structure of plant saponin.

KEYWORDS:

Saponins, Calotropis gigantea, NMR, Leaching, spectrum.

Introduction:

Most branches of spectroscopy are dedicated to elucidating structures of compounds. Nuclear Magnetic Resonance (NMR) is a very powerful spectroscopic technique not only for structure elucidation of organic, inorganic and biomolecules but has also evolved as a leading medical diagnostic tool (MRI) due to its non- invasive character [1]. Saponin is one of the most important compounds in calotropis gigeantea which exhibit various pharmacological activities. It is the secondary metabolite found in various plants. To date, approximately 70 kinds of saponin have been isolated from various plant sources [2-6]. Most of them are protopanaxdiol and protopanaxtriol, which are aglycones of dammarane-type triterpenoids [3]. Only a few ginsenosides, such as ginsenoside Ro, are aglycones of oleanolic acid.

Identification of ginsenosides is usually performed by nuclear magnetic resonance (NMR) analyses, In the present study, we acquired physicochemical and NMR data from fresh leaves of calotripis gigeantean leaves with the help of methanol

solvent for the extraction of saponin [7-12]. The slow sweep employed yielded 1H spectra in single scan and taking a few seconds to minutes for completion/recording. Each NMR line was scanned individually and hence was time consuming [13].

Chemical shifts and J couplings could not be measured since these are small interactions swamped by 1H dipole-dipole couplings. These structurally diverse compounds have also been observed to kill protozoans and molluscs, to bemantioxidants, to impair the digestion of protein and the uptake of vitamins and minerals in the gut, to cause hypoglycaemia, and to act as antifungal and antiviral agents. These compounds can thus affect animals in a host of different ways both positive and negative. Because of various uses of this saponin compound there is need to advance study in the photochemistry of saponin. So in this study we analysed the spectrum of the saponin compound for the further use in pharmaceutical industry [14-17].

Recently the number of studies investigating saponins has drastically increased due to their diverse and potentially attractive biological activities. Currently the literature contains chemical structures of few hundreds of saponins of plant and animal origin. Saponins consist of a triterpene aglycone with one or more sugar moieties attached to it [18]. However, due to similar physico-chemical properties, isolation and identification of a large diversity of saponins remain challenging. Continuously measured proton NMR data including contents of saponins, types of aglycones and numbers of sugar moieties attached to the aglycone. Saponins consist of a triterpene aglycone, with 30 carbon atoms, with one or more sugar moieties (including hexoses, methylpentoses, and pentoses) attached to the aglycone. Triterpenoid saponins are secondary metabolites synthesized in plant and mammalian cells. Several studies have reported on the role of triperpenoid saponins as natural defence compounds in plants, and some members of triterpenoid saponins have also been found to possess beneficial pharmacological properties. The literature contains structures of several hundred saponins isolated from various sources including plants and animals. Saponins exhibit diverse biological activities, and a number of studies investigating biosynthetic pathways and roles of this class of metabolites in the growth, development and physiology of host organisms has increased [19-20]. Recently triterpenoid saponins also became central target compounds for developing natural pesticides and insecticides for crop management in agriculture. Saponins are amphiphilic in nature, due to the foam forming ability [7-9].

1. Chemicals and Reagent:

All the chemicals used for extraction purpose were of analytical grade. Methanol, Solvent CDCl3. were procured from Sisco Research Laboratories (SRL), India. Distilled water prepared by a Millipore water purification system. Standard Saponin extrapure purchased from SRL India was used as standard for the experimental purpose. The fresh leaves of Calotropis gigantea were obtained from the campus of Savitribai Phule Pune University, Pune (Maharashtra) India.

2. Experimental:

The NMR technique relies on the ability of atomic nuclei to behave like a small magnet and align themselves with an external magnetic field. When irradiated with a radio frequency signal the nuclei in a molecule can change from being aligned with the magnetic field to being opposed to it. Therefore, it is called "nuclear" for the instrument works on stimulating the "nuclei" of the atoms to absorb radio waves. The energy frequency at which this occurs can be measured and is displayed as an NMR spectrum. The leaves of Calotropis gigantea were thoroughly washed for removal of dust and any other contamination. Which was used for all further experimental work. The NMR analysis was done at Central Instrumentation facility (CIF) Dept. of Chemistry Savitribai Phule Pune University Pune. NMR instrument used were Bruker. Acquisition Parameters Time 13.52 h, Solvent used for NMR analysis is CDCl3. In this experiment proton NMR spectra were measured from extracts of plant calotropis gigantean leaves.

3. Results and Discussion:

NMR spectrum appears as a series of vertical peaks/signals distributed along the x-axis of the spectrum (Figures 1). Each of these signals corresponds to an atom within the molecule being observed.

The position of each signal in the spectrum gives information about the local structural environment of the atom producing the signal. The ^{13}C and ^{1}H NMR spectra for saponin are shown in figures 1 and 2, respectively. Saponin, with nine different carbons produces a ^{13}C NMR spectrum with nine individual signals. Again, the positions of the signals indicate the individual structural environments of each carbon. The 1H NMR spectrum of saponin (Figure 1) shows 3 signals, due to three different hydrogen environments. The signals in the 7-8 ppm range are typical for hydrogens attached to an aromatic (benzene) ring. The NMR spectra measured on complex plant extracts represent signals from the most abundant metabolites while signals of low level metabolites remain undetected or largely hidden by dominating metabolites. In addition, due to the complexity of the sample matrix, the NMR signals derived from different molecules overlap and may hamper data interpretation. Thus, it is almost impossible to identify well resolved NMR signals of saponins from such a complex sample mixture. The resulting discriminative spectral regions mostly represent saponin peaks (Figure 2). In addition to aliphatic and anomeric regions, the spectral region from 0–120 ppm was found to be the most powerful region for discrimination.

Table I shows that the proton and carbon chemical shifts of the saponin molecule with different intensities of peak for the saponin compound at three different level. Table II shows that the proton and carbon chemical shifts of the saponin molecule for ^{13}C NMR analysis of saponin with nine peaks at different intensities. This data obtained from spectrum is useful for the further structure elucidation of the saponin.

4. Conclusions:

Present study deals with the NMR analysis of plant saponin form plant source of calotropis gigeantea. In which saponin secondary metabolite compound is analyzed with the help NMR spectrum, here we conclude that successfully we obtained the NMR spectra of

saponin agycone, sugar group and functional group according to the ^{13}C and ^{1}H data from the NMR analysis. Which is further useful for the pharmaceutical application in the industry for the drug development and therapeutics applications.

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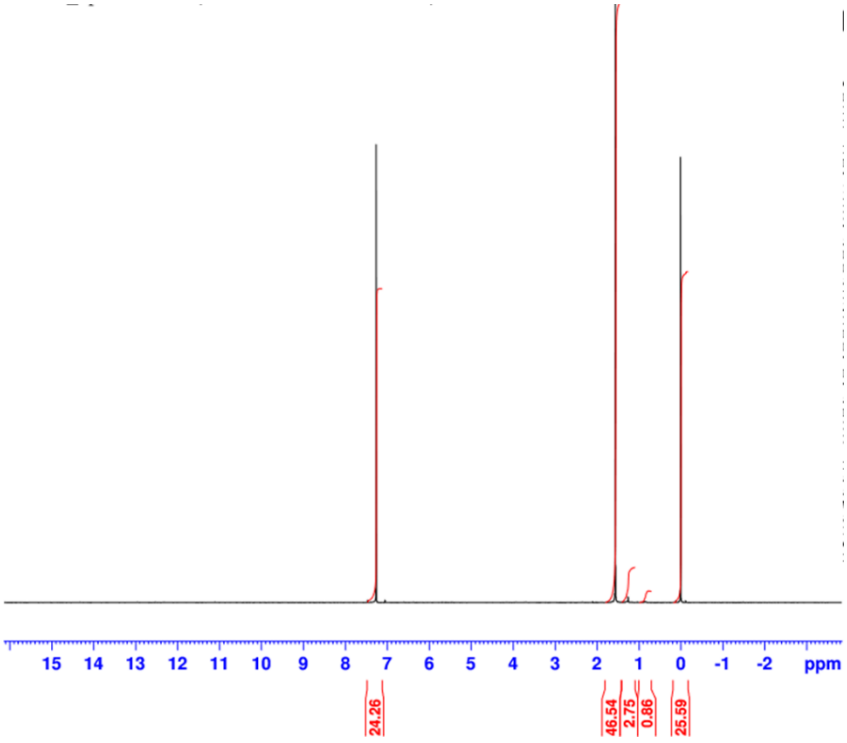

Figure 1: ^{1}H NMR Spectrum of Saponin [Proton spin CDCl3]

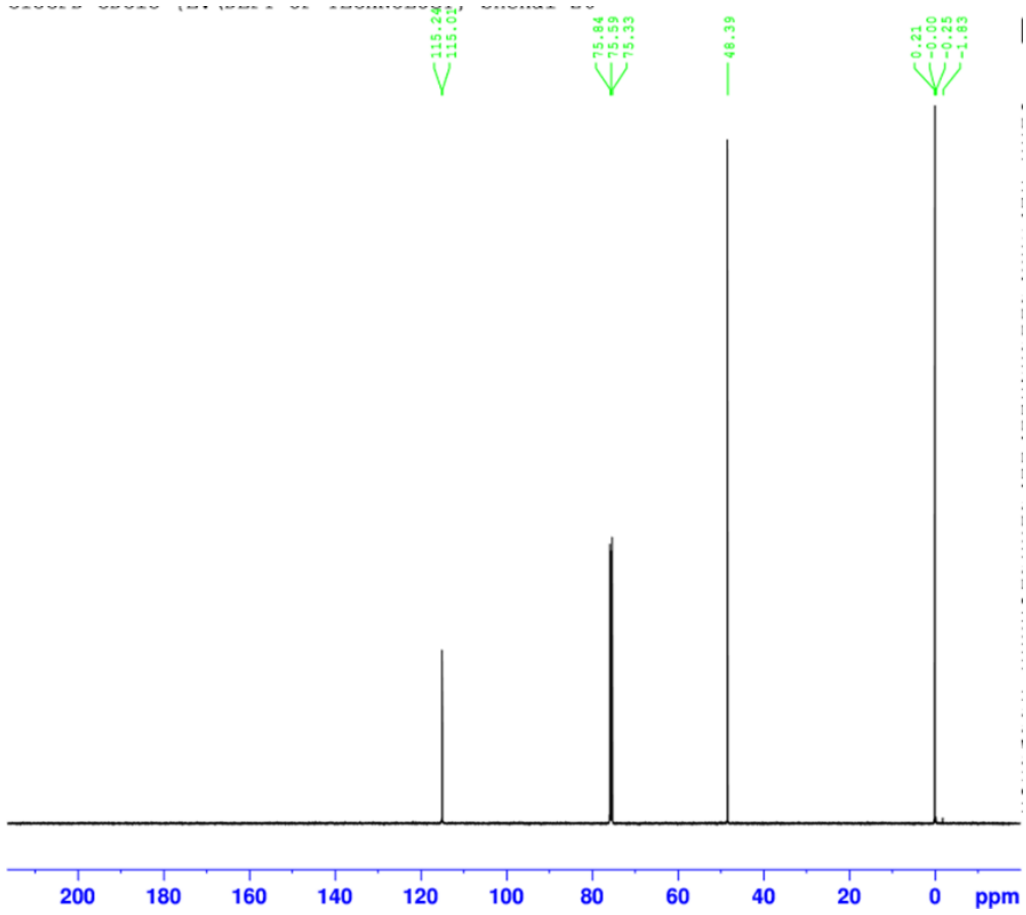

Figure 2: ^{13}C NMR Spectrum of Saponin [Proton spin CDCl3]

Table I. ^{1}H: The proton and carbon chemical shifts of the saponin molecule.

Peak 1 D F1	Intensity
7.2619	*11.40*
1.5565	*15.00*
-0.0001	*11.34*

Table II. ^{13}C: The proton and carbon chemical shifts of the saponin molecule.

Peak 1 D F1	Intensity
115.008957	*3.776678*
75.842155	*6.127605*
75.585831	*6.090614*
75.330444	*6.214734*
48.392456	*1.500000*
0.208088	*9.333590*
-0.003573	*1.767610*
-0.251331	*1.331198*
-1.832407	*1.202270*