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Structural determination of an isolated vasodilatory molecule from *Ludwigia octovalvis* (Jacq.) P.H. Raven (Onagraceae)

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Abstract

The determination of the chemical structure of an isolated vasodilatory molecule from *Ludwigia octovalvis* (Jacq.) P.H. Raven (Onagraceae) was undertaken. This molecule was isolated from n-butanolic extract using bioassay-guided isolation with preparative plate chromatographic techniques and its structure was determined using spectroscopy (UV, 1D- and 2D NMR, MS). The vasodilatory activity of the isolate was investigated on guinea pig isolated aorta. The results show 3 spots from n-butanolic extract with respective retention factor (*Rf*) of 0.14, 0.6 and 0.91. Pharmacological investigations showed that the isolated molecule with *Rf* 0.91 relaxes the isolated aorta pre-contracted by norepinephrine. Data from chemical shifts (δ_H) and spin-spin coupling constants (J_{HH}) were compared with literature to confirm the isolate as rutin. This is the first report of rutin isolated from *Ludwigia octovalvis*.

Keywords: *Ludwigia octovalvis*; Vasodilatory; n-Butanolic; Rutin

1 Introduction

Ludwigia octovalvis is a well-known traditional medicine remedy in Madagascar. It is used as an anti-hypertensive as well as in the management of other diseases. Medicinal plants are thought to be an important source of new chemical substances with therapeutic potential [1, 2]. They continue to be a significant source of structurally novel molecules.

Hypertension (HT) has been identified as the most important risk factor for cardiovascular disease, the leading cause of morbidity and premature death. Chronic hypertension harms the heart, kidneys, blood vessels, and brain, resulting in ischemic heart disease, congestive heart failure, renal failure, and stroke. Hypertension, therefore, is one of the most serious issues in modern medical practice [3].

Owing to the severity and prevalence of the disease, many synthetic drugs have been developed for its management. Diuretics, which remove excess water and sodium from the body; beta blockers, which help the heart beat slower and with less force; and vasodilators such as angiotensin-converting enzyme (ACE) inhibitors, angiotensin 2 receptor blockers (ARBs), calcium channel blockers, and alpha blockers are all used to manage hypertension.

Herbal medicine is regaining popularity in many countries around the world, particularly in low- and middle-income countries in the Sub-Saharan region, due to its ease of access and low cost [3, 4].

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Our group recently reported on research on the aerial part of *Ludwigia octovalvis*. Traditional medicine claims that the leaves of the plant are used as anti-gastric ulcer [5], analgesic [6], anti-hypertensive [7] agents abound. However, there is no scientific evidence elucidating its vasodilatory activity. As a result, the current study was designed to evaluate the vasodilatory activity of the aerial parts of *Ludwigia octovalvis* and the molecule responsible for its observed activity.

2 Material and methods

The plant material was obtained from Ambositra, in the Amoron'i Mania region in the southern highland of Madagascar. It was identified at Tsimbazaza Botanical and Zoological Park in Antananarivo, and a voucher specimen was deposited at the Department of Botany.

2.1 Extraction and isolation procedures

Dried aerial parts of *Ludwigia octovalvis* were reduced to fine powder with an electrical grinder (Brook Crompton Series 2000®). The powdered plant material (200 g) was macerated for 72 hours in 3500 ml of an ethanol-water (60:40) mixture. The macerate was dried using a rotary evaporator under vacuum at 60 °C. The crude extract was dissolved in water and partitioned with hexane, ethyl acetate, and n-butanol, yielding hexane, dichloromethane, ethyl acetate, n-butanol, and aqueous extracts, respectively.

Our preliminary study showed that the n- butanolic extract exhibited the highest vasodilatory activity [8]. Consequently, the present investigation focused on the n-butanolic extract. Precoated preparative silica gel (GF_{254}) glass base plates (Merck, 107747) of 1 mm thickness were used. Five hundred microliters (1 g/ml) of the butanolic fraction were deposited on preparative plates and eluted with a mobile phase ethyl acetate - methanol (70:30) (v/v). After drying the plates, the separated compounds were detected under a UV lamp (254/365 nm), the retention factor (Rf) was calculated, and the compounds were grated.

The purity of this molecule was assured using 2 dimension- TLC. Aluminium base precoated silica gel GF_{254} (Merck), was used, eluted with a mixture of ethyl acetate - methanol (5/5) (v/v)

The structure of the isolated compound was determined using NMR spectral data (UV, 1D-, and 2D-NMR) at the University of Ghana, School of Pharmacy's Department of Pharmacognosy and Herbal Medicine.

The ^{1}H NMR spectrum was obtained using a Bruker FT-NMR Avance 500 spectrometer (Ettlingen, Germany). The experiment was conducted at a temperature of 300 K to ensure consistency and accuracy throughout the analysis. The ^{1}H NMR spectrum were recorded at a frequency of 500.0330877 MHz, employing the "zg30" pulse sequence optimized for proton spectroscopy. The acquisition parameters employed were as follows: a time domain with 65,000 data points, an acquisition time of 3.2767999 s, a relaxation time of 1.00 s, and 64 scans in total. The spectral width was set at 10,000 Hz, resulting in a sweep width of 19.9987 ppm. Overall, the total acquisition time required to obtain the ^{1}H NMR spectrum was approximately 1 minute and 7 seconds. Following that, 2D NMR data was processed using Bruker TopSpin software (version 4.3.0).

The sample was subjected to NMR analysis. Deuterated methanol (MeOD) was obtained from Sigma-Aldrich Inc. (St. Louis, MO, USA). A standard 5 mm, 7′′ Norell Inc. (Landisville, NJ, USA) NMR tube (XR-55 series) was used. Measurements were taken on a 500 MHz Bruker spectrometer equipped with an AVANCE console and 5 mm TXI and TCI triple resonance inverse detection cryoprobes with z-axis pulse field gradient.

^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) data for compound K18 (MeOD, δ in ppm, J in Hz) is shown in Table 1.

Pro-analytic methanol, ethyl acetate, butanol for the extraction and ingredients for making Kreb's Henseleit solution, deuterated methanol (MeOD) and deuterated chloroform (CDCl3) were obtained from Sigma-Aldrich Inc. (St. Louis, MO, USA). Norell Inc. (Landisville, NJ, USA) standard 5 mm, 7′′ NMR tubes (XR-55 series) were used. Carbogen gas was purchased from gas supplier SOAM in Antananarivo, Madagascar.

2.2 Vasodilatory investigation

Vasodilatory activity of *Ludwigia octovalvis* n-butanolic extract was investigated on isolated aorta contracted with norepinephrine.

2.3 Experimental animals

Guinea pigs weighing 300-350 g, 8–12-month-old of either sex were selected for the experiments. Animals were obtained from the animal house of the Pharmacology Department, Faculty of Sciences, University of Antananarivo, Madagascar. Animal experiments were carried out in accordance with the guidelines for the care of laboratory animals of the Faculty of Sciences Animal Ethics Committee, and the study was approved under (Reg. no.23-2020). The animals were housed in light and darkness (12:12 hr) alternation and at 22±2 °C temperature. They were fed with standard laboratory diet and had water *ad libitum* and were fasted 12 hours prior to the test.

2.4 Evaluation of the vasodilatory effect of the isolated molecule

2.4.1 Aorta preparation

The animals were sedated before being euthanized via cervical dislocation and thoracotomy. Following pulmonary and cardiac resection, thoracic aortic dissection from the inferior diaphragm to the aortic base was performed.

The aorta was carefully cleansed of any attached connective tissue so as not to damage the endothelium, and then placed at room temperature in a petri dish containing carbogenated Kreb's Henseleit solution. The aorta was cut on the ring with a 3mm length. The aortic ring was then placed in a 3 ml organ bath containing Kreb's-Henseleit solution and aerated with carbogen gas at 37 °C [9].

Aortic ring was mounted on an isometric transducer Statham Gould© under a tension of 2 g, connected to a digital recorder amplifier SIGMA Monitor developed by IOGA (Faculty of Sciences, University of Antananarivo). Subsequently, changes in the aortic dilatation tone were recorded in grams.

The isolated organ was equilibrated in Kreb's-Henseleit solution for 90 minutes. Every 15 minutes, the bath was refilled. Once stabilized, it was tested for viability and sensitization with 10^{-3} M norepinephrine. To assure that the endothelium is intact, 10^{-3} M of acetylcholine was injected in the bath [10]. Then it was rinsed, and left to stabilize for 30 minutes, during which the bath was renewed twice.

2.4.2 Vasodilation activity

Following previous tests, the aortic ring was contracted with 10^{-3} M norepinephrine until maximal contraction was achieved. Following that, the isolated molecule was added to it in increasing concentrations. The test was repeated six times, and the difference in smooth muscle tone was expressed as a percentage of aortic tone each time [11].

The percentage of aortic contractile tone is calculated by dividing the aortic tone after extract administration by the aortic tone after norepinephrine administration, which is assumed to be 100 percent. A decrease in vascular contractile tone indicates vasodilation activity in the blood vessels.

2.5 Statistical analysis

Microsoft Excel 2019 and GraphPad Prism software were used to analyze the results (version 7.0). Data collected at various locations was compared. The statistical significance of differences was determined with one-way ANOVA and the Student t test. Differences with a p value of < 0.05 were statistically significant. The data was presented as mean ± sem.

3 Results and discussion

3.1 Results of extraction

After elution of the plate containing the n-butanolic extract, 3 spots were obtained with *Rf* respectively equal to 0.14, 0.6 and 0.91 with respective weights of 6, 15 and 23 mg. The most well resolved peak (0.91) which incidentally provided the best yield was coded as Rand K18 and investigated further. It is a yellow powder and readily soluble in methanol.

3.2 Vasodilation activity of the isolated molecule

Norepinephrine was injected into the bath containing the isolated aorta. Rand K18 relaxed the organ in a concentration dependent manner. It reduced 8.15 ± 0.36% of the contraction at the concentration of 2.5 µg/ml in the bath. It completely relaxed (100%) the contracted aorta at the concentration of 25 µg/ml in the bath (fig 1). These results

indicate that Rand K18 possesses vasodilatory activity. These results conform to the literature after structural determination, which shows that this molecule is rutin [12].

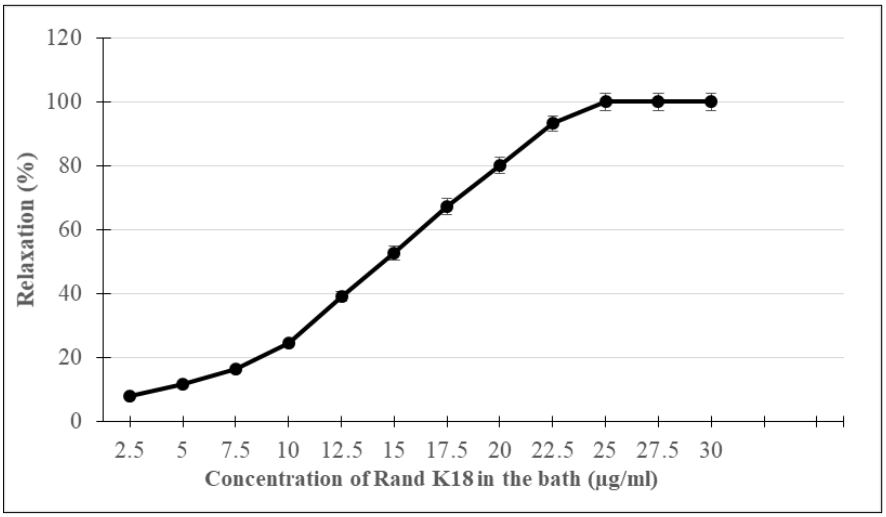

Figure 1 Relaxation of aorta pre contracted with norepinephrine in the presence of Rand K18, injected in the bath, in a cumulative manner (m ± sem; n=6, $p<0.05$)

3.3 Structure of the isolated molecule Rand K18

The NMR data of the isolated molecule is presented in Table I.

On the left are measured values and on the right are literature values [13].

Table 1 ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) data for compound Rand K18 (MeOD, δ in ppm, J in Hz)

No.	^{13}C-NMR	^{1}H-NMR	No.	^{13}C-NMR	^{1}H-NMR
1					
2	158.4		2	156.9	
3	137.4		3	133.9	
4	177.5		4	178.0	
5	162.6		5	161.4	
6	99.4	6.08, d	6	99.7	6.19, d
7	165.7		7	166.3	
8	94.6	6.29, d	8	94.8	6.38, d
4a	148.9		9	156.7	
8a	104.7		10	104.9	
1’	124.3		1’	122.0	
2’	116.1	7.54, dd	2’	116.0	7.53, ps-dd/d
3’	146.4		3’	145.2	
4’	148.1		4’	148.9	
5’	116.4	6.80, d	5’	117.1	6.84, ps-dd/d
6’	121.8	7.63, d	6’	122.5	7.54, ps-dd/d

Based on spectral techniques, the structural elucidation of compound Rand K18 indicates that it is a flavonoid, and after comparison with previous spectral data in the literature [13], it is identified as rutin (Figure 2).

The ^{1}H NMR assignments were determined by inspecting the 1D and 2D NMR data. Subsequently, $^{1}H,^{1}H$ coupling constants in the aromatic rings were computed. Additionally, meta-coupling of $3J$(H-5′, H-6′) and para-coupling of $5J$(H-2′, H-5′) were determined, yielding a complete map of *J*-couplings for the AA′BB′ and AA′XX′ spin systems, as well as the AMX spin systems in the 3′,4′-disubstituted flavonols. Moreover, HMBC and HSQC results showed correlation between H-5′ and H-6′ (Supplementary data).

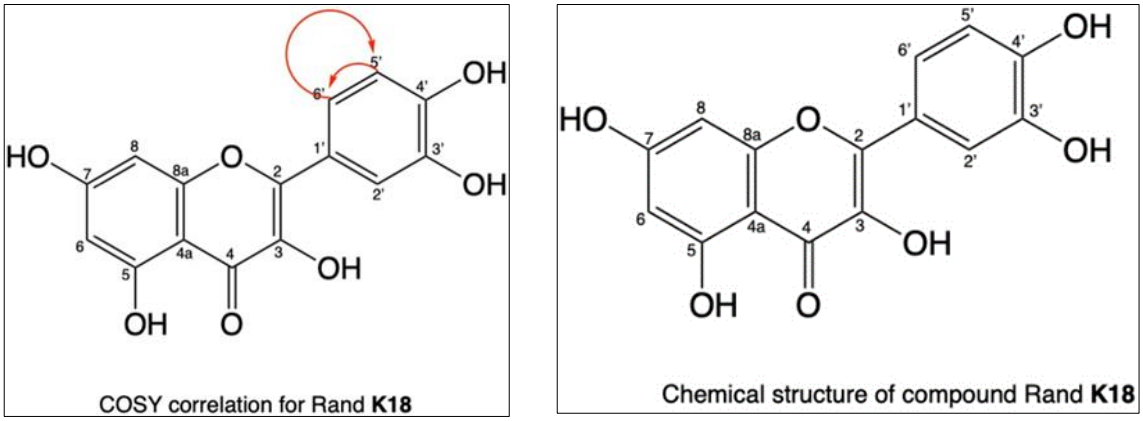

Figure 2 COSY correlation for Rand K18 and chemical structure of compound Rand K18

4 Conclusion

This work has shown that rutin present in n-butanolic extract of *Ludwigia octovalvis* is responsible for its vasodilation action which explains the anti-hypertensive activity of this plant. These results provide scientific validation for the traditional use of *L. octovalvis* in the management of hypertension.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The experiments were conducted following the guidelines of the ethic committee of the Sciences Faculty, University of Antananarivo, Madagascar (Ref: 17/2021).

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Supplementary data

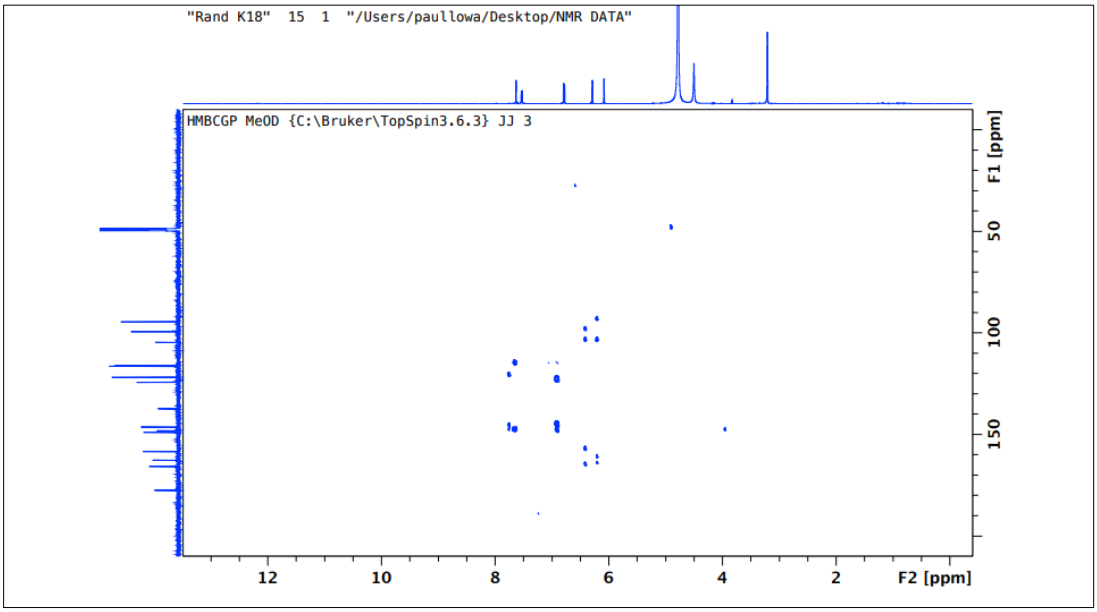

Figure 3 HMBC spectrum for K18

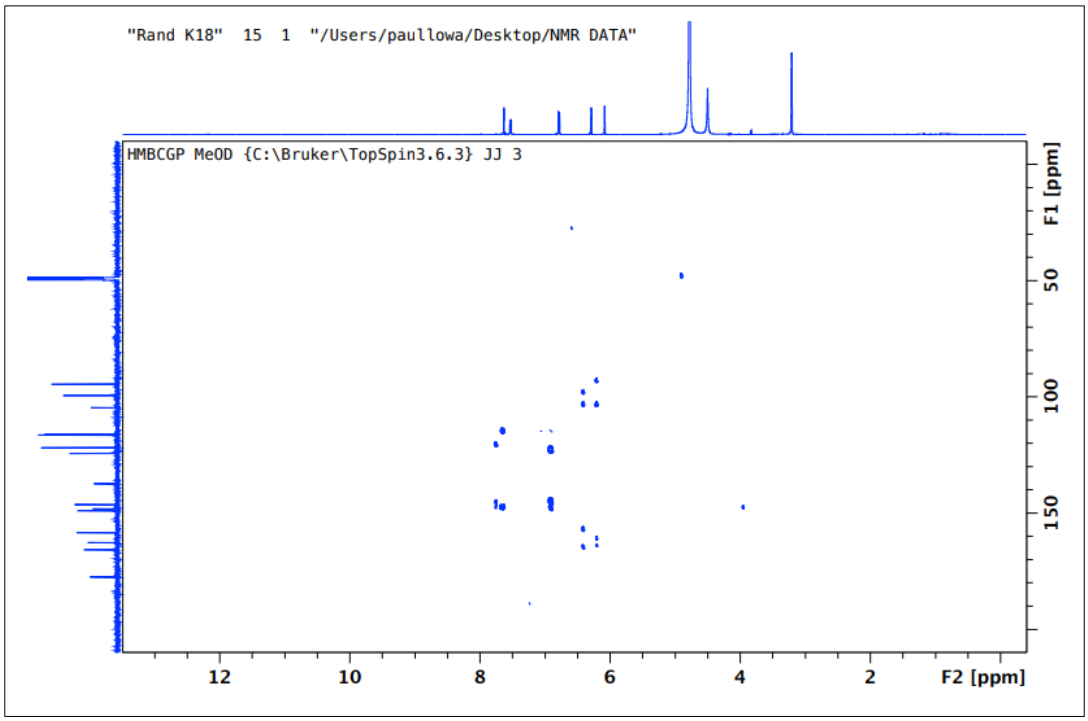

Figure 4 HMBC 1 spectrum for Rand K18

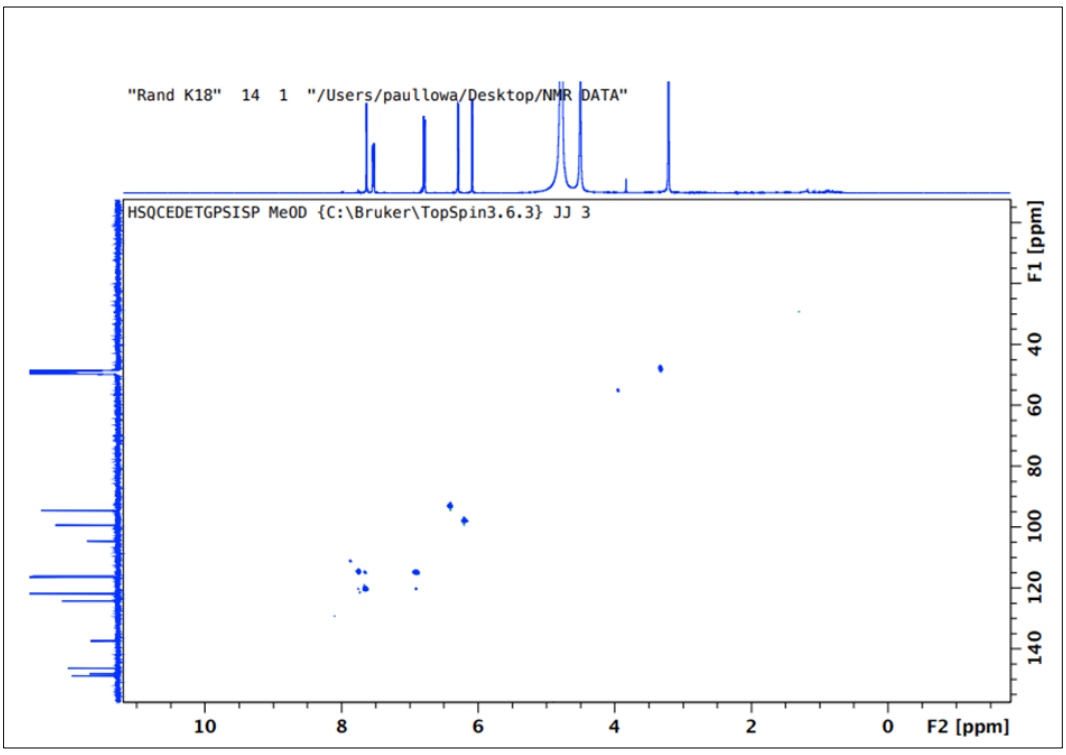

Figure 5 HSQC 1spectrum for Rand K18

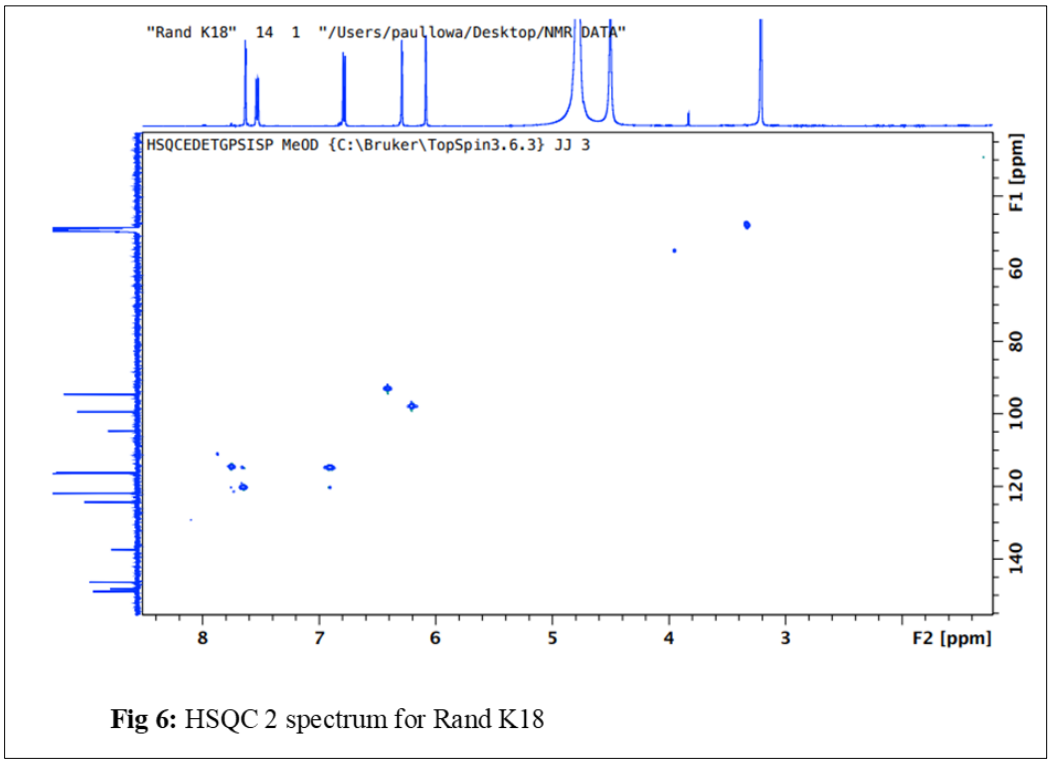

Fig 6: HSQC 2 spectrum for Rand K18

Figure 6 HSQC 2 spectrum for Rand K18