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Note

Rutin Inhibits F, G, N and O gonorrhea strains, 2008 WHO *N-gonorrhea* Reference strains, *in vitro*

Mutendela Tabize Olivier, PhD (cand.)[1]; Freddy Munyololo Muganza, PhD[1,2,*]; Leshweni Jeremia Shai, PhD[3] and Stanley Sechene Gololo, PhD[4]

[1]Department of Chemistry, Sefako Makgatho Health Sciences University, Molotlegi Street, Ga-Rankuwa 0204, Pretoria, South Africa
[2]Department of Health Sciences, Providence University College and Theological Seminary, 10 College Crescent Otterburne, Manitoba, Canada, R0A 1G0 (**Current address**)
[3]Department of Biomedical Sciences, Tshwane University of Technology, P/Bag X680, Pretoria 0001, South Africa
[4]Department of Biochemistry, Sefako Makgatho Health Sciences University, Molotlegi Street, Ga-Rankuwa 0204, Pretoria, South Africa

***Correspondence:** freddy.muganza@prov.ca

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Abstract: Rutin was isolated from methanol extract of the aerial part of *Asparagus suaveolens* using precipitation method. South Africans use *Asparagus suaveolens* to treat gonorrhea infections. The obtained Nuclear Magnetic Resonance (NMR) and Liquid Chromatography-Mass Spectroscopy (LC-MS) data and visiting the published data on the isolation of rutin confirmed the structure. The 2008 WHO *Neisseria gonorrhea* reference strains were used to evaluate microbial activity of rutin against the gonorrhea strains. Rutin found to be bacteriostatic against WHO 2008 *Neisseria gonorrhoea* F, G, N and O strains with the minimum inhibition concentration of 0.40, 0.65, 0.22 and 0.65 mg/ml, respectively. In addition, rutin fare better than the reference drugs and bactericidal against K, L, M, and P strains. These results support the traditional use of *Asparagus suaveolens* against gonorrhea infections by South African indigenous people. To our knowledge, this is the first study indicating the activity of rutin against *N.gonorrhea* strains.

Résumé: La rutine a été isolée à partir d'un extrait au méthanol de la partie aérienne *d'Asparagus suaveolens* en utilisant la méthode de précipitation. Les Sud-Africains utilisent *Asparagus suaveolens* pour traiter les infections gonorrhées. Les données obtenues par résonance magnétique nucléaire (RMN) et par chromatographie liquide-spectroscopie de masse (LC-MS) et la consultation des données publiées sur l'isolement de la rutine ont confirmé la structure. Les souches de référence OMS de *Neisseria gonorrhea* de 2008 ont été utilisées pour évaluer l'activité microbienne de la rutine contre les souches de gonorrhée. La rutine s'est révélée bactériostatique contre les souches de *Neisseria gonorrhea* F, G, N et O de l'OMS 2008 avec une concentration minimale d'inhibition de 0,40, 0,65, 0,22 et 0,65 mg/ml, respectivement. De plus, la rutine se porte mieux que les médicaments de référence et bactéricide contre les souches K, L, M et P. Ces résultats soutiennent l'utilisation traditionnelle d'*Asparagus suaveolens* contre les infections gonorrhées par les populations autochtones sud-africaines. À notre connaissance, il s'agit de la première étude indiquant l'activité de la rutine contre les souches de N. gonorrhée.

Keywords: *Asparagus suaveolens*, rutin, *Neisseria gonorrhoea*, Flavonoid, MIC

1. Introduction

The effect of antimicrobial resistance (AMR) in *Neisseria gonorrhea* (*N. gonorrhea*) on the active treatment and consequently the control of gonorrhea is of long-lasting unease. Acknowledgement of clinical treatment failures of penicillins, in widely dispersed jurisdictions, soon after their introduction incited the World Health Organization (WHO) to change methods for surveillance of *in vitro* AMR in *N. gonorrhea*. 2008 WHO *N.gonorrhea* reference strains is proposed for the internal and external quality assurance and quality control components of gonococcal AMR testing protocols, and is a criterion for any global WHO AMR surveillance programme for *N. gonorrhea*[1,2].

Asparagus suaveolens (*A. suaveolens*) grows up to 1.5 m in the rocky grassland[3]. *A. suaveolens* Burch is a thorny woody shrub with shining pale brown stems, white flowers and red fruit or black berries[4]. Medicinal properties attributed to this plant include its use in the treatment of epilepsy[3.5], veterinary medicine[6-8] and recently by our research group against 2008 WHO *N.gonorrhoea* strains[9]. In our earlier efforts to isolate phytochemicals present in *A. suaveolens* which are responsible for its antigonorrhoea activities[9], palmitone was isolated from *n*-hexane extract and was found to have good activity against the WHO 2008 *N.gonorrhoea* strains[10]. In this current work we are reporting another phytochemical(quercetin-3-*O*-rutinosid rutin **1**) isolated from methanol extract of *A.suaveolens* aerial part. The precipitation technique managed to isolate the product. The product was characterized and evaluated for antigonorrhoea activity against eight 2008 WHO *N.gonorrhoea* strains as part of our ongoing contribution to the list of bioactive phytochemicals against gonococcal disease. *N.gonorrhea* bacteria is still showing resistance against commonly used medications such as Azithromycin and Cephalosporin,[11] therefore, there is still a need for new treatment regimens.

2. Material and methods

The collections of the aerial parts of *A. suaveolens* were on 6 October 2017 as guided by Liu [12] at Bolahlakgomo village of Limpopo Province, South Africa. The South African National Biodiversity Institute (SANBI) under the specimen number PREART 0001903 in the Pretoria's Herbarium, Gauteng Province, South Africa, identified the plant.

Simple precipitation technique isolated rutin as follows: After fractionation from ethanol extract of *A. suaveolens* aerial part crude extract (500g) in the following respective solvents order: *n*-hexane, chloroform and butan-1-ol. By dissolving butan-1-ol fraction (8.678 g) in the mixture of Ethyl Acetate: Methanol (1:1), the fraction was further processed. A yellow precipitate observed upon increasing the amount of Ethyl Acetate in the flask, was collected (50 mg), rinsed with Ethyl Acetate, dried and characterized. The isolated yellow powdery compound was identified to be quercetin-3-O-rutinoside (rutin) (Figure 1) by comparing its NMR spectra, both 1D and 2D (^{1}H, ^{13}C, Cosy, HSQC and HMBC), with those reported in literature[13].

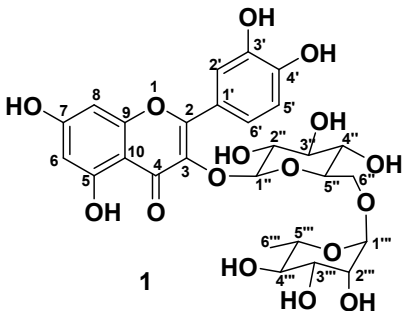

Figure 1: Chemical structure of rutin(**1**)

The National Health Laboratories Services (NHLS) in Johannesburg-South Africa donated the microorganisms used for antigonorrhea assay. The strains used were eight 2008 WHO *N.gonorrhoea* strains comprising the 2008 WHO reference strain panel[14]. The 2008 WHO panel

strains represent the important susceptible and resistant phenotypes and the range of resistances currently seen for the antimicrobials recommended in different guidelines and/or used in the gonorrhoea treatment globally[14]. The characterized compound was subjected to antigonorrhea assay following the procedure described by Olivier *et al.*[9,10] without modification to determine its MIC.

3. Results and Discussion

The ^{1}H and ^{13}CNMR spectra of the isolated compound were similar to that isolated from Aerial Parts of *Polygonatum odoratum* (Mill.) Druce by Ganbaataar *et al.*[13](Table 2).

The characteristics signals of quercetin framework were visible: δ_H = 6.06 (s, H6), 6.45 (s, H8), 7.48 (dd, *J* = 8.4, 2.0 Hz, H6'), 6.74 (d, *J* = 8.4 Hz, H5'), and 7.53 (s, H2'). In addition, the HMBC spectrum proved the rhamnosyl C1'''hydrogen atom (δ_H = 4.38) in correlation with the glucosyl C6'' atom at δ_C = 68.52 indicating a rutinosyl skeleton. Also the glucosyl C1''-hydrogen atom (δ_H = 4.96) correlated with C3 (δ_C = 135.57) of the flavonoid unit in the HMBC spectrum. The results of HSQC established the direct C-H correlation of the isolated compound (Table 1). The (H6) peak at δ_H 6.06 coupled with δ_C at 99.9 (C6) ppm. The eighth proton δ_H at 6.25 show cross peak correlation with δ_C at 94.8 (C8) ppm. Equally, H2's at δ_H 7.53 was coordinated with δ_C at 117.6 (C2'); H5' at 6.74 coupled with δ_C at 116.0 (C5') and H6' at δ_H 7.48 coupled with δ_C at 123.57 (C6') ppm. Additionally, results of HSQC spectral study of glucose and rhamnose unit confirmed that the proton corresponding carbon coupling. The mass of the isolated compound was measured using LC-MS and it was observed as a fragment of m/z = 303 (base peak). This mass fragment represents the flavonoid unit and the remaining mass is of the glycoside unit (glucosyl and rhamnosyl). After confirming the structure of the isolated compound, the material evaluated for antigonorrhea activities (Table 1).

The results showed that the isolated compound is more active against F, G and N strains than both standards (Gentamicin and Amoxicillin) used with MIC values of 0.40, 0.65 and 0.22 mg/ml respectively. Only more active than amoxicillin for O strain (Table 1).

Table 1: Antigonorrhea activity (MIC and MBC) of rutin isolated from the aerial parts of *A. suaveolens* methanol extract.

WHO reference strain characteristics	MIC (mg/ml)			MBC (mg/ml)	MBC/MIC
	Rutin	Standards		Rutin	Rutin
		Gentamicin	Amoxicillin		
F	0.40	1.58	1.84	2.63	6.57
G	0.65	2.84	0.95	3.59	5.52
K	18.2	5.46	0.52	24.68	1.35
L	20.8	0.92	1.45	20.21	0.97
M	10.0	0.89	2.0	11.92	1.19
N	0.22	0.25	4.65	8.45	38.40
O	0.65	0.24	0.79	5.16	7.93
P	15.66	3.45	1.92	15.48	0.98

MIC: Minimum inhibitory concentration;
MBC: Minimum bactericidal concentration

Rutin was also found to be bactericidal (MBC/MIC<4) against K, L, M, P strains and bacteriostatic against the rest of WHO 2008 *N.gonorrhoea* strains according to criteria published by French[15].

Rutin pharmacological action has been well studied and the details well explained by Ganeshpurkar and Saluja[16]. Rutin also found to be a strong antioxidant compound even more than ascorbic acid, in some cases by Srinivasan *et al.*[17]. Antioxidant property is been proven to have an impact on clinical infectious disease[18].

This could explain the reason why the indigenous people in South Africa use *A. suaveolens* to treat gonorrhea infections.

Due to the presence of rutin in *A.suaveolens*, one could speculate that *A.suaveolens* could be used against different other illnesses. For example, the central nervous system sicknesses (i.e.: Neuroinflam mation, neural cells survival, sedative, Anti-Alzheimer, Hyperkinetic movement disorder, stroke, antidepressant); Analgesic and anthritic sicknesses (i.e. Analgesic and antinociceptive, antiarthritis); Endocrine system sicknesses (i.e.: antidiabetics, Antihypercholesterol, Thyroid uptake promoter); the list is very long if referring to the pharmacological potential of Rutin as given by Ganeshpurkar and Saluja[16].

The discovery of rutin in *A. suaveolens* plant has opened a broad spectrum of research topics in connection with *A. suaveolens*. In addition, this discovery has come to add up to the list of pharmacological potential of rutin against sexually transmitted diseases (STDs) which was missing in the review published by Ganeshpurkar and Saluja[16].

However, other pharmacological benefits of *A.suaveolens* must be scientifically proved due to the presence of rutin in the plant; the people of Ga-Sekgopa community in Limpopo Province, South Africa had already given the plants an important societal position in their traditional health care[19].

In summary, in our endeavour to find phytochemicals present in *A. suaveolens* responsible for its antigonorrhoea activities, Rutin is another phytochemical present in *A.suaveolens*, which also showed antigonorrhea *A.suaveolens*reference strains in addition to palmitone that we previously published[10].

Table 2: Chemical shifts of Rutin in $CDOD_3$ compared to ref.[13] for ^1H (400 MHz & 600 MHz) and ^{13}C (100 MHz & 150 MHz) NMR.

Position	δ_C		δ_H(*J* in Hz)		HMBC (H-C)	HSQC (H-C)
	PW	Ref.13	PW	Ref.13		
2	159.3	159.0	-	-	-	-
3	135.5	135.62	-	-	-	-
4	179.3	179.43	-	-	-	-
5	162.8	163.5	-	-	-	-
6	99.9	99.98	6.06	6.26 d(2.1)	C5,C7,C8 ,C10	H6 & C6
7	165.9	167.0	-	-	-	-
8	94.8	94.8	6.25	6.45 d(2.1)	C6,C7,C9,C10	H8 & C8
9	158.4	158.5	-	-	-	-
10	105.5	106.0	-	-	-	-
1′	123.07	123.2	-	-	-	-
2′	117.6	117.6	7.53	7.71 d(2.2)	C1′, C2′, C3′, C4′, C2	H2′ & C2′
3′	145.7	146.0	-	-	-	-
4′	149.7	150.0	-	-	C3′, C4′, C6′	-
5′	116.0	116.0	6.74 d(8.4)	6.92 d(8.4)	C4′, C6′	H5′ & C5′
6′	123.57	123.54	7.48 dd(8.4,2.0)	7.68 dd(8.4,2.2)	-	H6′ & C6′

			3-*O*-C(6)-Glucosyl			
1′′	104.6	104.71	5.15 d(7.8)	5.15 d (7.8)	-	H1′′ & C1′′
2′′	75.6	75.72		3.38–3.53 m	-	H2′′ & C2′′
3′′	78.1	78.20		3.38-3.53 m	-	H3′′ & C3′′
4′′	71.3	71.40	3.68–3.12(m)	3.29-3.33 m	-	H4′′ & H4′′
5′′	77.1	77.24		3.38-3.53 m	-	H5′′ & C5′′
6′′a	68.5	68.5		3.84 dd(11.1, 1.5)	-	H6′′ & C6′′
6′′b				3.38–3.53 m	-	
			6′′-*O*-Rhamnosyl			
1′′′	102.4	102.43	4.38	4.56 d(1.5)	C1′′′, C2′′′, C6′′	H1′′′ & C1′′′
2′′′	72.05	71.40		3.67 dd(3.4, 1.6)	-	H2′′′ & C2′′′
3′′′	72.19	72.11	3.68–3.12(m)	3.58 dd(9.5, 3.5)	-	H3′′′ & C3′′′
4′′′	73.8	73.93		3.29–3.33 m	-	H4′′′& H4′′′
5′′′	69.69	69.71		3.38–3.53 m	-	H5′′′ & C5′′′
6′′′	17.86	17.88	0.98 d(6.4)	1.16 d(6.2)	C4′′′, C5′′′, C6′′′	H6′′′ & C6′′′

PW: Present Work

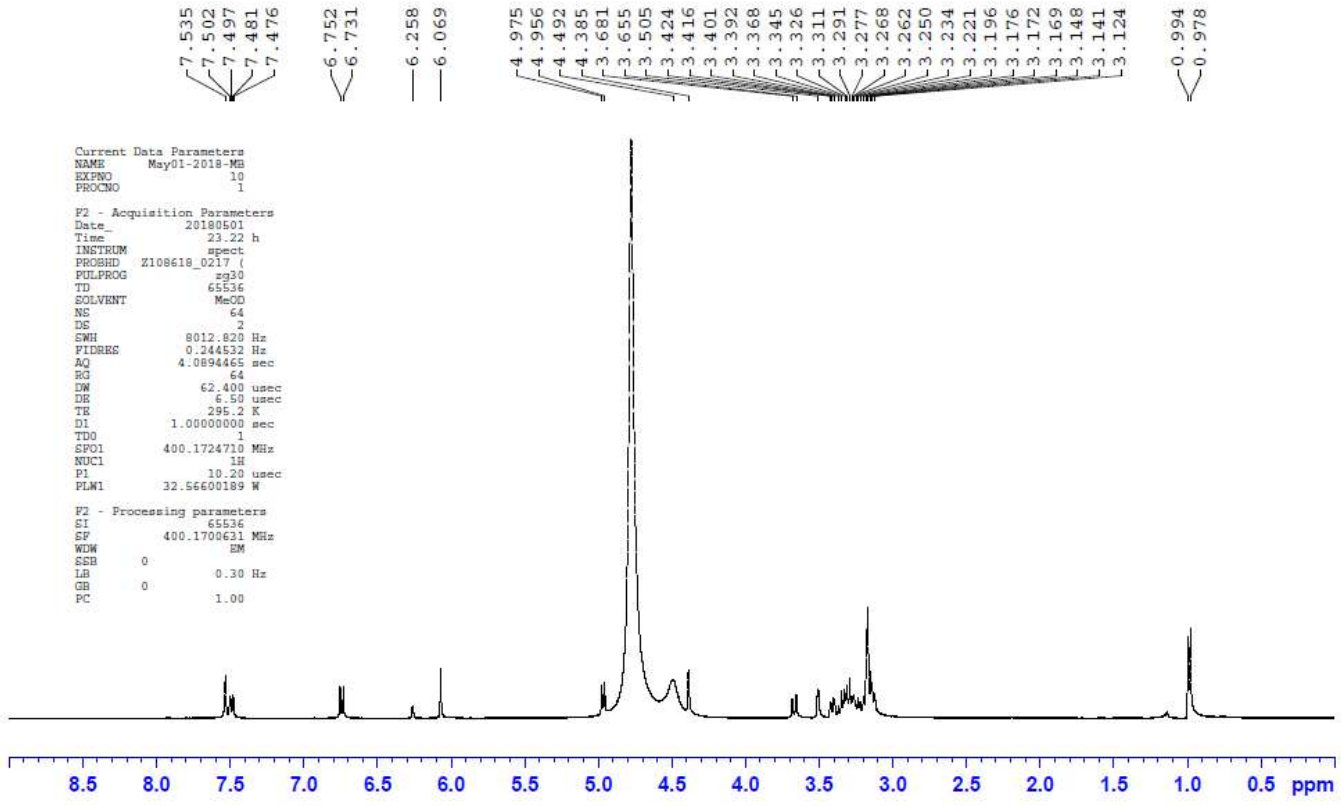

Figure 1: Proton NMR

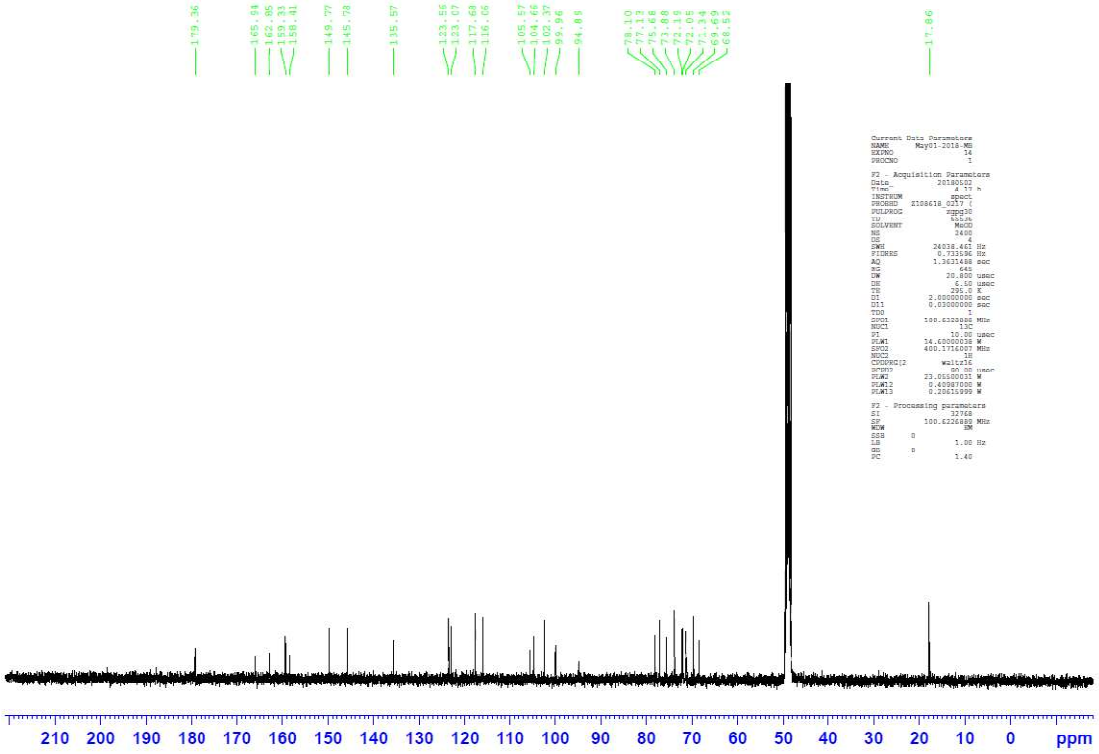

Figure 2: Carbon NMR

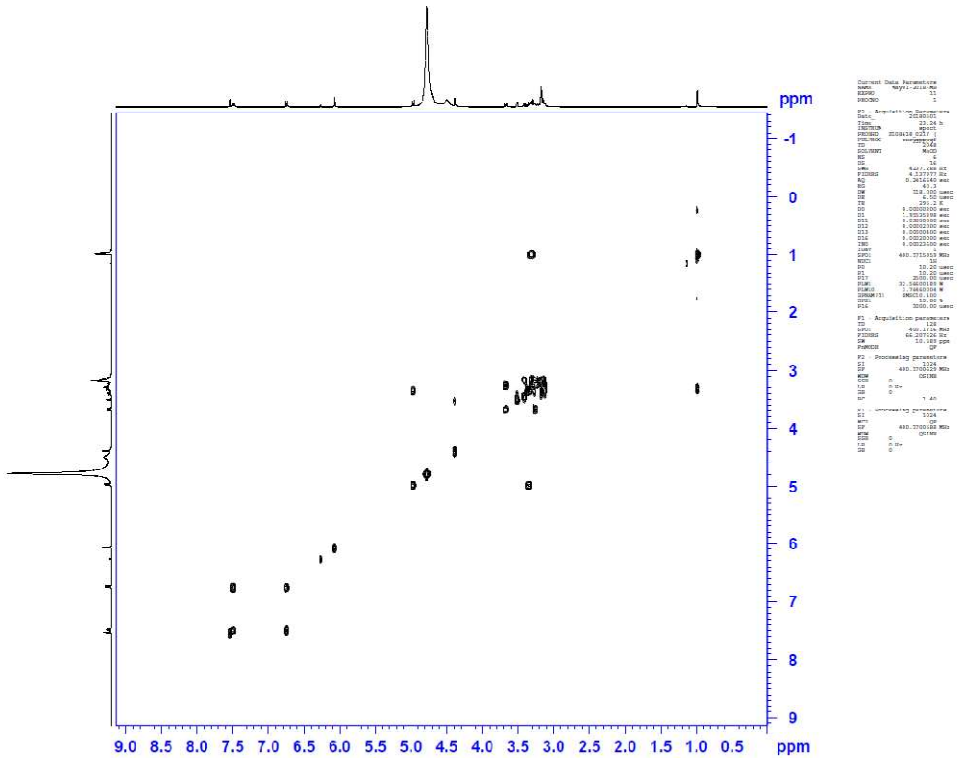

Figure 3: Cosy NMR

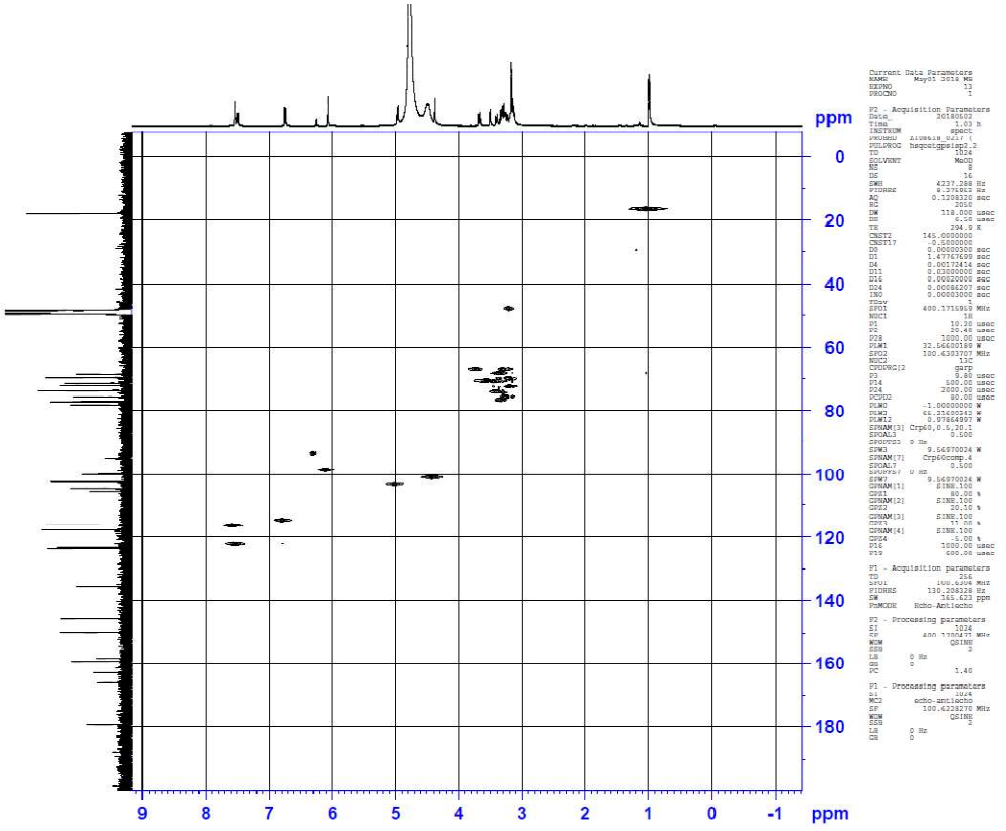

Figure 4: HSQC NMR

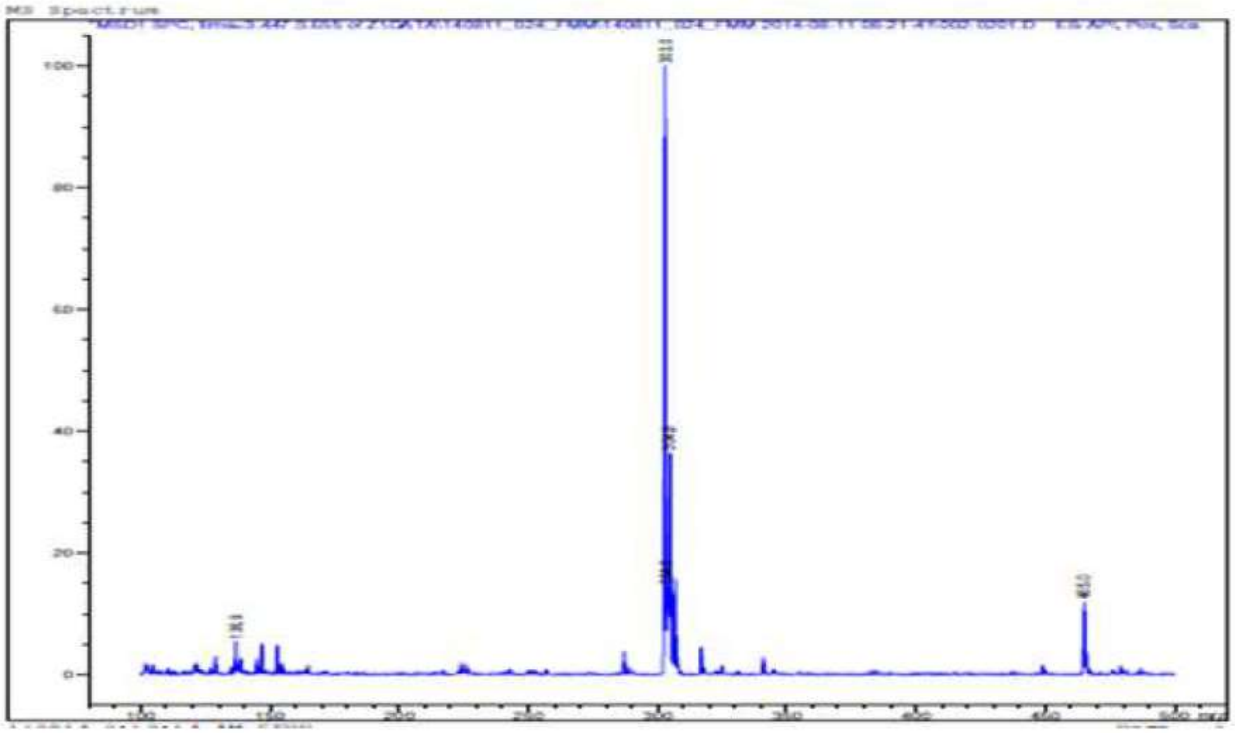

Figure 5: MS-spectrum

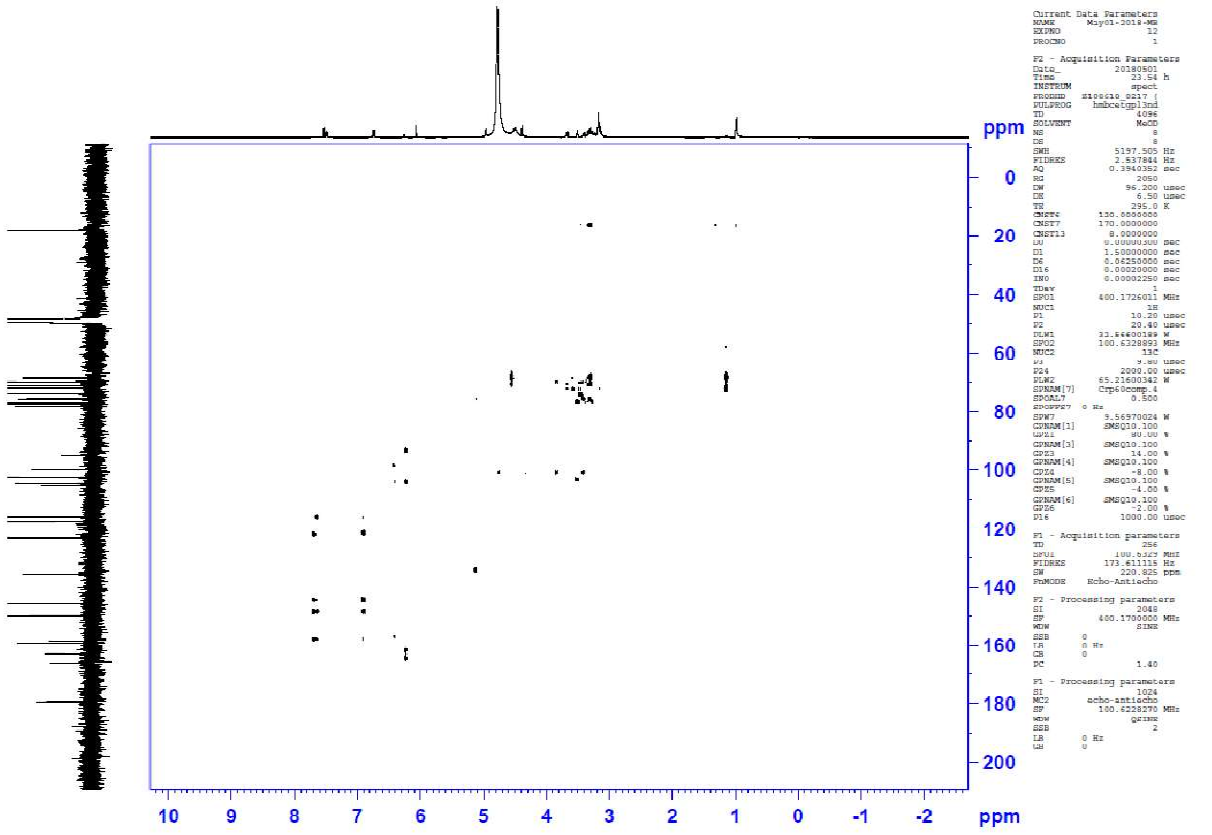

Figure 6: HMBC NMR spectrum

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