ISOLATION AND STRUCTURAL IDENTIFICATION OF COMPOUNDS WITH BOTH NEMATICIDAL AND FUNGICIDAL ACTIVITIES FROM *Acacia saligna* LEAVES

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ABSTRACT

Pure compounds have been isolated from *Acacia saligna* leaves and tested for nematicidal and fungicidal activities against three nematode species and three phytopathogenic fungi. The structures of the isolated compounds were elucidated by UV, ^{1}H and ^{13}C – NMR spectroscopic techniques. The nematicidal and fungicidal activities of the isolated compounds were performed *in vitro*.

Key words: *Acacia saligna* - Nematicidal activities - Fungicidal Activities – Myricetin - Benzoic Acid Derivative - Flavonol Glycosides.

INTRODUCTION

Using synthetic pesticides over several decades in agricultural fields has disrupted control by natural enemies and has led to outbreaks and resurgences of pests, the development of widespread resistance to pesticides, undesirable effects on non-target organisms,

environmental problems, and human health hazards. Besides these problems synthetic pesticides are expensive and usually purchased with scarce hard currency. In addition, the growing preference for organic products plants has brought about the need for the development of new types of selective control with reduced use of synthetic pesticides.

One source of potential new pesticides is the natural products produced by plants. Plants are known to produce a very diverse range of bioactive compounds to protect themselves from injurious pests. These natural plant products (botanical pesticides) are often active against a limited number of species and are biodegradable to non-toxic products .Therefore much attention has been focused on plant materials looking for compounds potentially useful as commercial pesticides in agricultural fields.

Acacia saligna (Mimosaceae) is an extremely rugged tree, adaptable to derelict land and exceptionally arid conditions in Australia and North Africa (El-Lakany, 1987). It grows rapidly and is used for fixing sand dunes, stabilizing drift sands (El-Bagoury *et al.*, 1995 and Abou–Deya, 1999) and beautification projects. Its leaves are platable and can be used either fresh or dried as supplementary feed for sheep and goats (Abou El-Nasr *et al.*, 1996 and Howard *et al.*, 2002). The water and ethanolic extracts of the aerial part of this plant exhibited potent molluscicidal activity against *Biomphalria alexandrina* sanils (Ahmed *et al.*, 1999), also the ethyl acetate extract of leaves showed free radical scavenging activity (Moussa *et al.* 2005). Previous phytochemical investigation of this plant has led to the isolation of coumarin and steroidal components from the ethanol extract (Ahmed *et al.*, 1999), hydrocarbons, fatty alcohols, aldehydes, triterpenes and sterols from the hexane extract (El-Sawi *et al.*, 2003) and flavonoids from the ethyl acetate extract (Mousa *et al.*, 2005).

The objective of this study was to evaluate *In vitro* both the nematicidal activity of this plant against three nematode species, *Meloidegyne javanica, Rotylenculus semipentrans* and *Tylenculus semipentrans*, causing root knot, and die back in many economically important crops such as vegetables,cotton,citrus....etc ; and the fungicidal activity against three phytopathogenic fungi, *Fusarium oxysporum, Sclerotium rolfsii* and *Verticillium dahliae*, causing root rot and wilt in many economically important crops such as tomato,

beans ......etc; along with the isolation and structural elucidation of the active constituents responsible for these activities.

MATERIAL AND METHODS

1. Plant material

The leaves of *Acacia saligna* were collected in May 2005 from the Faculty of Agriculture garden, Fayoum, Egypt and identified by Flora and Phyto – taxonomy Research, Agriculture Research Center, Herbarium (CAIM).

A voucher specimen (A. S. 10) was deposited in the Herbarium of the Environmental Chemistry and Natural Resources Center, Faculty of Agriculture, Cairo University.

2. Assessment of biological activities

2.1. Nematicidal activity

2.1.1. Test organisms

The three nematode species, Root–Knot nematode *Meloidagyne javanica* (*M.javanica*), Reniform nematode *Rotylenulus semipentrans* (*R. semipentrans*) and Citrus nematodes *Tylenculus semipentrans* (*T. semipentrans*) were isolated and propagated locally in Agricultural Research Center (ARC), Plant Disease Institute, Department of Nematology.

2.1.2. Determination of nematicidal activity:

The nematicidal activity of extracts and the isolated compounds was determined *In vitro* as follows:

One ml of each nematode *M. javanica*, *R. semipentrans* and *T. semipentrans* containing approximately 100 fresh newly hatched juveniles were separately transferred to Petri dishes (5 cm diameter) containing 1 ml of aqueous solution of each concentration tested (25, 50, 100 mg ℓ^{-1}) and then incubated at 25° C. Dead and surviving nematodes were counted after 24 hours and percentages of mortality were determined (Al-Sayed *el al.*, 1996). Three replicates of each concentration were prepared and three petri dishes containing 1 ml distilled water were used as control.

2.2. Fungicidal activity:

2.2.1. Tester strains

Pure strains of the three phytopathogenic fungi, *Fusarium oxysporum* (*F. oxysporum*, *Sclerotium rolfsii* (*S. rolfsii*) and *Verticillium dahliae* (*V. dahliae*) were isolated from tomato plants in

the Agricultural Research Center, (ARC), Plant Pathology Institute, Department of Integrated Control Management.

2.2.2. Determination of fungicidal activity

The fungicidal activity of the extracts and isolated compounds was determined *In vitro* by two different techniques as follows:

a. Inhibition zone:

The filter paper disc technique was modified as follows: approximately 20 ml of nutrient glucose agar medium (N.G.A) were poured into 8.5 cm Petri dishes (culture plate). At least 3 replicates were used for each treatment. After medium solidification equal disks 4 mm of different test fungal growth were cut from $10 - 13$ days of (N.G.A) plates using a corkborer and transferred into the side of each test plates and equal sterilized filter paper disc were plated in the other side of the plate. Then 1 ml of each concentration tested (25, 50, 100 mg ℓ^{-1}) were poured on the filter paper disks. The plates were incubated at 25° C for 7 days .Inhibition zone was determined by measuring the diameter of mycelial growth intervals until the growth in one plate covered the medium surface.

$$\% \text{ inhibition index} = \frac{\text{mean diameter of control} - \text{mean diameter of treatment}}{\text{mean diameter of control}} \times 100$$

b. The percents (%) Inhibition of spores germination:

Spores of tested fungi germination was determined by collecting the spores after 18 days, by gently brushing the surface of the growth in the presence of 10 ml distilled water, the slide – germination fungicide bioassay technique described by Sharvrelle (1979) was adapted by Ashmawy (1997), droplet of the tested concentration compound was mounted on the surface of the slide, using an adjustable micropipette then the droplets were allowed to dry. Once the tested concentration droplet on the slide dried, a one μ L of spore suspension of the tested isolated fungi was placed on the slide, and then the slides were incubated in a humidity chamber. On the next day and after 48 h. the slides were microscopically examined and the percents (%) of spore germination were calculated for every replicate treatment.

3- Extraction:

One kg of the air dried powdered leaves was consecutively extracted with the following solvents in order of increasing polarity: petroleum ether (5 ℓ), chloroform (5 ℓ), ethylacetate (6 ℓ) and methanol (6 ℓ) at room temperature (30° C ± 2). The extracts were evaporated to dryness under reduced pressure to give the following residues; pet. ether (8.1 g), chloroform (17.5 g), ethylacetate (11.8 g) and methanol (55.2 g). Then the four extracts were tested for their nematicidal activity against three nematode species and fungicidal activity against three fungi species.

A portion of the bioactive methanolic extract (45 g) was suspended in water (150 ml), and extracted with n -Butanol (3×100 ml) to give BuOH soluble components (Fr. A. 11.2 g). The aqueous layer was freeze dried (33.5 g) and was then extracted with ethanol (3×100 ml). After centrifugation both the supernatant and the precipitate were dried under reduced pressure to afford 6.9 g (Fr.B) and 26.4g (Fr.C) respectively. The three fractions i.e A, B and C were tested for their nematicidal and fungicidal activities.

4- Analytical thin layer chromatography (TLC):

TLC analysis was carried out on precoated silica gel plates (F_{254} 0.25 mm and F_{254} 2.0 mm Merck) using the following solvent systems:

1. Chloroform- Methanol – Water (50:50:5)
2. Chloroform- Methanol (50:50)
3. Ethylacetate – Acetic acid – Formic acid – Water (100:11:11:27)
4. n-Butanol – Acetic acid – Water (4:1:5) upper layer
5. Dichloromethane – Methanol – Water (50:25:5)
6. Chloroform – Acetone (50:6)

Zones were detected under UV light (254 nm and 365 nm) and by spraying with concentrated H_2SO_4 followed by heating at 105° C for 5 min. or with 5 % $AlCl_3$. Sugars were detected by spraying with napthoresorcinol phosphoric acid followed by heating at 105° C for 10 min.

5- Isolation of the bioactive components:

The most potent bioactive fraction (Fr. B, 6.9 g) was subjected to the isolation of the bioactive components as follows:-

Fraction B (6.5 g) was chromatographed over a Sephadex LH-20 column (40 g) and eluted with methanol. Sixteen fractions of eluent were collected. The eluates were combined on the basis of similar

TLC profiles to afford four fractions and then tested for nematicidal and fungicidal activities.

The bioactive fraction No. 4 (1.5 g. between 200-300 mℓ) was further purified over a silica gel column (100 g, 230-400 mesh, Merck) and eluted with a mixture of $CHCl_3$: MeOH: H_2O (50:50:5: 400 ml).

According to differences in composition monitored by TLC, 7 fractions (1$^\backslash$ - 7$^\backslash$) were obtained and then tested for nematicidal and fungicidal activities. The bioactive fractions No. 2 (150 mg between 50 - 100 ml) and No. 4 (135 mg between 130 – 180 mℓ) further rechromatographed several times over sephadex LH-20 and preparative TLC (PTLC) as shown in Fig (1) yielded active compounds I and II. The purity of these two compounds was established by the resolution of each one as a single spot in four different TLC systems.
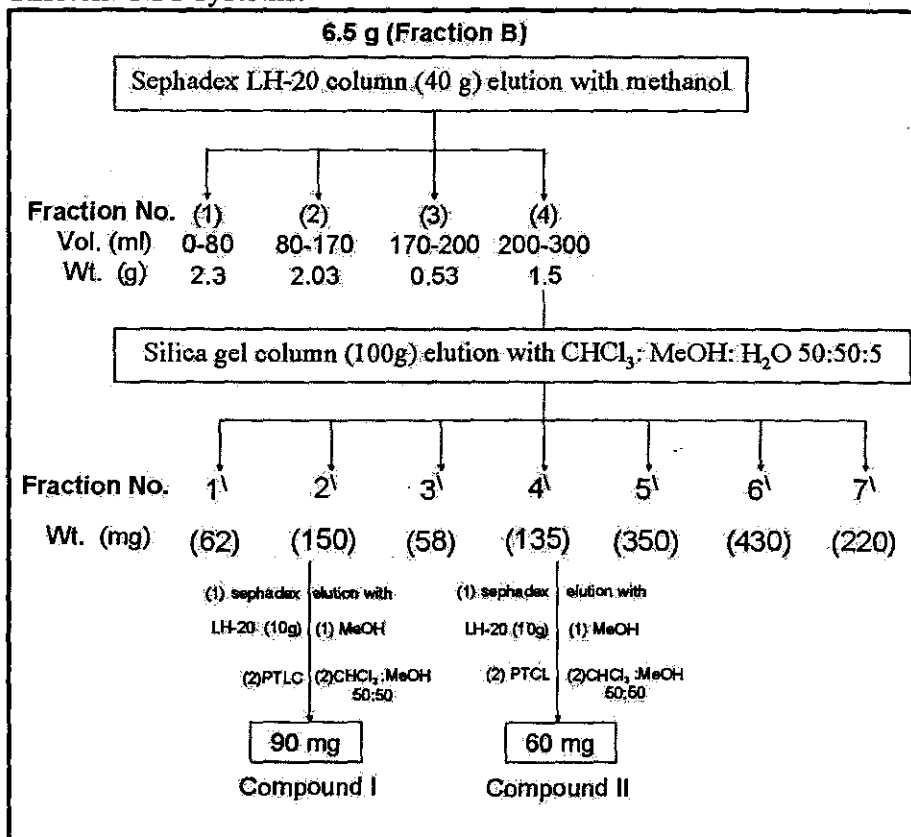

Fig. (1): Flow diagram for the isolation of active Compounds I and II.

6. Structure identification of the purified compounds:

The purified compounds were characterized by detection, tests, acid hydrolysis and spectroscopic methods.

6.1. Detection Tests

The preliminary screening of the isolated compounds for the classes of phytoconstituents, saponins, flavonoids, alkaloids, glycosides and phenolic compounds, was performed according to the methods described by Farnsworth (1966).

6.2. Acid hydrolysis

The purified compound to be hydrolysed (2 mg) was heated with aqueous 10% HCl (2 mg) in a sealed tube in a 100° C water–bath for 4 hours. The aglycone was extracted with diethylether and analysed by TLC with chloroform: acetone (50: 6). The aqueous layer was neutralized with N, N dioctylamine (10% in $CHCl_3$). After evaporation to dryness, the sugars were identified by TLC with $CHCl_3$: MeOH: H_2O (50: 25: 5) by comparison with authentic samples.

6.3. Spectroscopic methods

6.3.1. Nuclear Magnetic Resonance (NMR) spectroscopy

^1H and ^{13}C – NMR Spectra were recorded in CD_3OD and DMSO-d_6 on a Varion Mercury VXR 300 (300 MHz for ^1H and 75 Hz for ^{13}C at the Central Laboratory, Faculty of Science, Cairo University. Chemical shifts (ppm) were related to that of the solvent.

6.3.2. UV spectrometry

The UV spectrum was recorded on a ceal 3000 series spectrophotometer according to Mabry *el al.* (1970).

RESULTS AND DISCUSSION

Biological evaluation

The air dried leaves of *Acacia saligna* were successively extracted by Pet. ether, $CHCl_3$, EtOAc and MeOH, then the nematicidal and fungicidal activites of each extract were tested. Only the methanol extract showed nematicidal and fungicidal activity against the six pests tested [Table (1)]. The results showed that the % mortality of the three nematodes tested increased with increasing concentration of MeOH extract, as the % mortality value increased from 39 to 55, 36 to 50 and 44 to 52% respectively by increasing the concentration from 25 to 100 mg ℓ^{-1}. Also the data showed that the MeOH extract

exhibited fungicidal activity against the three fungi tested. The % of spore germination inhibition of *F. oxysporum* and *V. dahliae* increased with increasing concentration (from 45 to 62 and 42 to 60 %, respectively) whereas the % of spore germination inhibition of *S.rolfsii* was 100 % at the three concentrations tested. The bioactive methanolic extract was fractionated into three fractions (see materials and methods) which were tested for the nematicidal and fungicidal activities.

The results [Table (1)] showed that the three fractions tested varied in their nematicidal and fungicidal activites. The ethanol fraction (fr. B) showed the highest nematicidal and fungicidal activites. The percents of mortality of the three species at 100 mg ℓ^{-1} were 70.6, 69.3 and 70 %, respectively; also the percents of fungi spore germination inhibition at 100 mg l^{-1} were 80, 100 and 78 against the three fungi tested respectively. The data obtained revealed that the methanolic extract and and its fractions were more effective against the fungi *S. rolfsii* than the other fungi tested, as the % of spore germination inhibition was 100 % at all concentrations tested.

Bioactivity guided separation of the most potent fraction (Fr. B) resulted in the isolation of two chromatographically pure compounds I and II .The data pertaining to the biological potential of the isolated compounds are presented in Table (1), which reveals that both compounds I and II had nematicidal and fungicidal effects on the six pests tested. Therefore compounds I and II were partly responsible for the nematicidal and fungicidal properties of *Acacia saligna* leaves.

The data obtained revealed variability in the % of nematode mortality fdr each compound for given pests. It is clear that compound I had stronger effects towards the three nematode species than compound II at the three concentrations tested, whereas the two compounds exhibited the same strong fungicidal activity against the three fungi tested.

It is interesting to note that this is the first study to provide data on compounds isolated from the leaves of *Acacia saligna* evaluated against the three nematodes, *Meloidagyne javanica, Rotylenulus semipentrans* and *Tylenculus semipentrans* as well as the three phytopathogenic fungi, *Fusarium oxysporum, Sclerotium rolfsii* and *Verticillium dahliae*.

Table (1): Nematicidal and Fungicidal activities (methanolic extract, fractions and its isolated compounds) of *Acacia saligna*:

Sample	Concentration mgl⁻¹	Nematode Mortality %			Fungi % Inhibition of spore germination		
		M. Javanica	*T. semipentran*	*R.semipentrans*	*F. oxysporum*	*S. rolfsii*	*V. dahliae*
MeOH	100	55.2	50.6	52.3	62.4	100	60.3
extract	50	42.3	46.2	49.2	56.0	100	56.2
	25	39.2	36.2	44.6	45.3	100	42.4
EtOH	100	70.6	69.3	70.5	80.3	100	78.0
extract	50	65.3	62.4	66.3	72.3	100	70.3
Fraction (B)	25	52.4	50.9	51.9	71.3	100	69.3
H₂O	100	56.6	51.6	53.2	64.3	100	62.5
extract	50	43.3	47.5	50.0	60.2	100	58.0
Fraction (C)	25	37.9	35.2	36.3	45.1	100	44.1
BuOH	100	50.3	48.9	49.0	54.2	100	55.3
extract	50	42.3	40.6	42.5	49.2	100	47.0
Fraction (A)	25	31.6	32.3	32.0	41.2	100	39.6
	100	98.9	79.8	100	100	100	100
Compound I	50	66.9	35.3	66.2	100	100	100
	25	52.3	19.3	48.2	100	100	100
	100	50.2	43.2	76.5	100	100	100
Compound II	50	20.0	19.2	52.3	97.5	100	100
	25	10.3	5.6	36.8	94.9	100	80.0

Structural identification of the active isolated compounds:

Two pure compounds I and II were isolated from the most potent fraction, the ethanolic fraction (Fr. B) of Acacia *saligna* leaf extract through column chromatography and preparative TLC as described in the meterials and methods.

These compounds were identified as follows ; myricetin 3-O (2''-O-galloyl)- α- rhamnopyranoside- 7- methyl ether (I) and 3-hydroxy-5- methoxy benzoyl-O-β-D- glucopyranosyl (1-2) β- D-glucopyranoside) (II).

Compound I

It was obtained as a fine yellow powder, which gave a positive colour test for flavonoids on TLC. This compound also showed two distinct bands at λ 265 and 352 nm in the UV spectrum suggesting it is a flavonol derivative (Mabry *et al.*, 1970). On acidic hydrolysis L-rhamnose was identified on TLC by direct comparison with an authentic sample as the sole sugar moiety in this compound. The presence of α-L rhamnose was confirmed by the appearance of an anomeric proton at δ 5.32 (1H, d, J= 1.5Hz) and methyl group at δ 0.97 (3H, d, J= 6.0Hz) in the ^{1}H-NMR spectrum [Fig (2)] as well as the carbon signals in the ^{13}C-NMR spectrum [Fig (3) and Table (2)] at δ103.59 and δ17.70 ppm for C-1 and C-6 respectively.

The flavonol moiety was established as Myricetin by the appearance of a pair of meta coupled doublets of one proton each at δ 6.20 (J= 2.1 Hz) and δ 6.36 (J= 1.8 Hz) assigned to H-6 and H-8 of ring A and an aromatic singlet at δ 6.95 (2H, s) ascribed to H-2' and the H-6' of ring B.

The presence of the Myricetin moiety was confirmed by comparing the carbon chemical shifts [Fig (3) and Table (2)] with previously reported work (Markham *et al.*, 1978).

The glycosylation site at C-3 hydroxy was confirmed by the ^{13}C-NMR spectrum through the down field resonance of C-2 at δ 158.6 ppm and the up field signals of C-3 at δ 136.32 ppm in comparison with the Myricetin data (Markham *et al.*, 1978). The presence of a methoxyl group was established by the appearance of three proton signals at δ 3.77 ppm in the ^{1}H-NMR spectrum and carbon signal at δ 55.96 ppm in the ^{13}C-NMR spectrum. The lack of a bathochromic shift with the addition of NaOAc in the UV spectrum indicated the absence of a free hydroxyl group at the C-7 position and the presence of the methoxyl group in this position.

The ^{1}H- and ^{13}C-NMR spectra also showed the presence of a galloyl group due to the appearance of an aromatic singlet at δ 8.42 ppm (2H,S) for protons H-2‴ and H-6‴ along with the carbon chemical shifts of this moiety [Fig (2) and Table (2)]. As well as by comparing the spectral data with previously reported (Khalid et al., 1989) The significant enhancement of the ^{13}C-NMR chemical shift of the C-2 position of rhamnose [Table (2)] as compared with the same position previously reported (Markham et al., 1978 and Lee et al., 2000) suggested that the galloyl group was located at the rhamnose C-2″ position. Thus the structure of compound I was characterized as myricetin 3-O (2″-O-galloyl) - α- rhamnopyranoside- 7- methyl ether [Fig. (4)].

This compound has previously been isolated from the leaves of *Acacia confusa* (Lee et al., 2000) but this is the first report of this compound in *Acacia saligna*.

The isolated compound I exerted a nematicidal activity against the three nematode species *M. javanica*, *R. semipentrans* and *T. semipentrans* and fungicidal activity against the three fungi species, *Fusarium oxysporum*, *Sclerotium rolfsii* and *Verticillium dahliae* [Table (1)].

The literature survey reveals that both the myricetin and galloyl ingredients of the isolated compound are highly potent compounds, which show variety of biological activities such as antibacterial activity against pathogenic strains of human diseases (Nishino et al., 1987, Saxena et al., 1994 and Simin et al., 2002) and antiviral activity (Ono et al., 1990 and Chaubel et al., 2005). However, there is no previous study on the fungicidal activity of the isolated flavonol glycosides i.e myricetin 3-O (2″-O galloyl) - α- rhamnopyranoside -7- methyl ether against the three phytopathogenic fungi ,or on the nematicidal activity against the three nematode species .

Table (2): ^{1}H NMR and ^{13}C NMR Spectral Data of Compound [I].

Carbon atom No.	^{13}C NMR (ppm)	^{1}H NMR	Carbon atom No.	^{13}C NMR (ppm)	^{1}H NMR
2	158.6		5$^\backslash$	146.89	
3	136.32		6$^\backslash$	109.99	δ 6.96(2H,s)
4	179.65		1$^{\backslash\backslash}$	103.59	δ 5.32 (dj=1.5Hz)
5	163.21		2$^{\backslash\backslash}$	72.22	δ 4.22 t (1.6Hz)
6	100.04	δ 6.36 (dj=1.8Hz)	3$^{\backslash\backslash}$	71.93	δ 3.59 d (5.1Hz)
7	166.31		4$^{\backslash\backslash}$	72.07	δ 3.31-3.38 m
8	94.9	δ 6.20 (dj=2.1Hz)	5$^{\backslash\backslash}$	69.21	δ 3.31-3.38 m
9	159.33		6$^{\backslash\backslash}$	17.07	δ 0.97 (dj=6.0Hz)
10	105.86		1$^{\backslash\backslash\backslash}$	120.92	
OCH3	55.96	δ 3.77 (3H,s)	2$^{\backslash\backslash\backslash}$	110.69	δ 8.43 (2H,s)
1$^\backslash$	122.02		3$^{\backslash\backslash\backslash}$	147.14	
2$^\backslash$	109.99	δ 6.96 (2H,s)	4$^{\backslash\backslash\backslash}$	139.58	
3$^\backslash$	146.89		5$^{\backslash\backslash\backslash}$	147.14	
4$^\backslash$	137.97		6$^{\backslash\backslash\backslash}$	110.69	δ 8.43 (2H,s)
			7$^{\backslash\backslash\backslash}$	169.30	

Compound II

It was obtained as a white amorphous powder, which gave a positive reaction with Fe Cl$_3$ on TLC, suggesting it is a phenolic compound. Acid hydrolysis of this compound yielded D-glucose as sugar moiety (TLC). The presence of two β-D- glucopyranose was indicated by the ^{1}H- and ^{13}C-NMR spectra [Fig (5), Table (3)]. It showed two anomeric protons at δ 4.90 (1H, d, J= 7.2 Hz) and 5.12

(1H, d, J= 7.5 Hz) in the ^{1}H-NMR spectrum and two anomeric carbon atoms at δ 101.98 and 104.04 ppm in the ^{13}C-NMR spectrum.

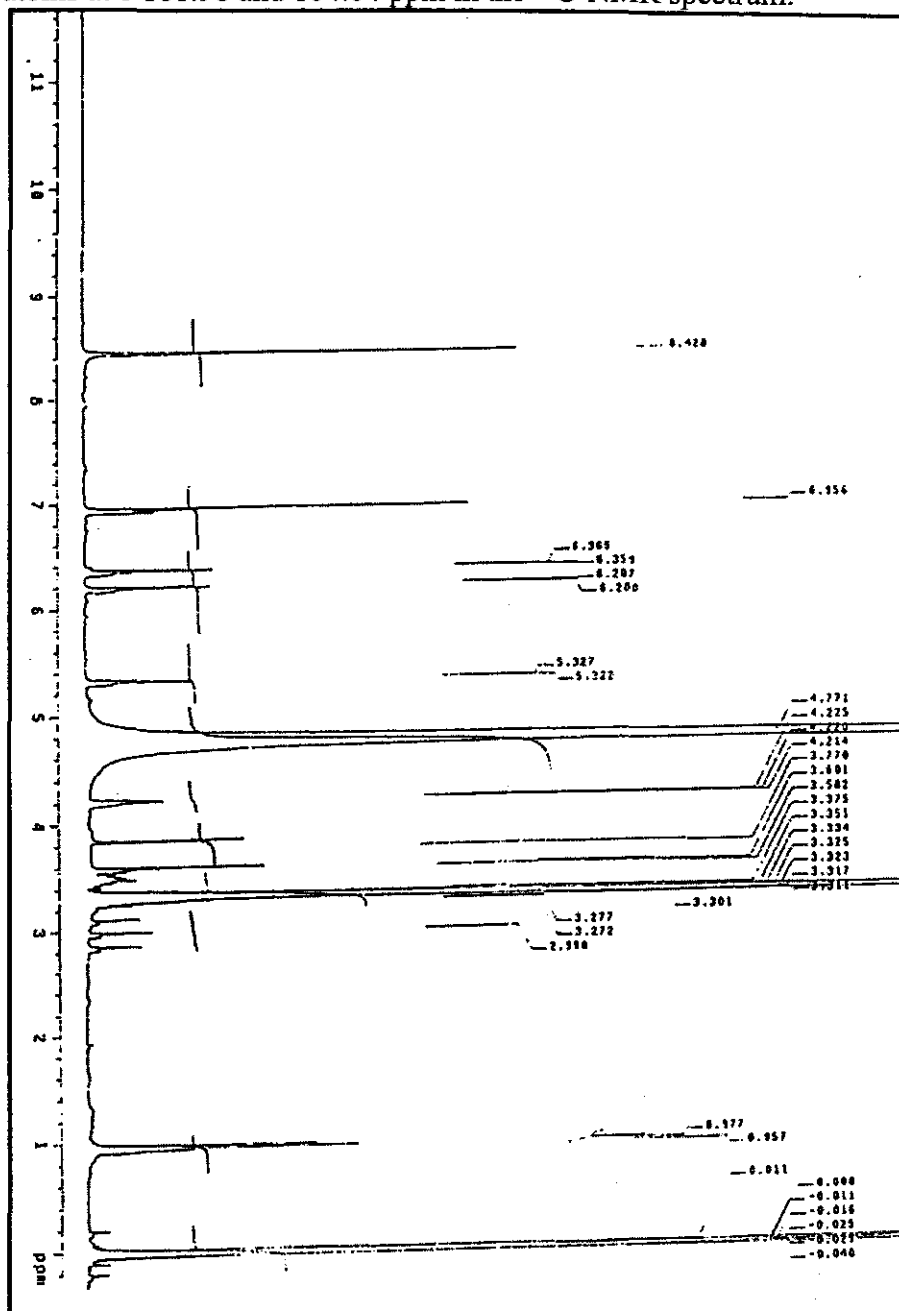**Fig (2):** ^{1}H-NMR spectrum of compound (I) in CD$_3$OD
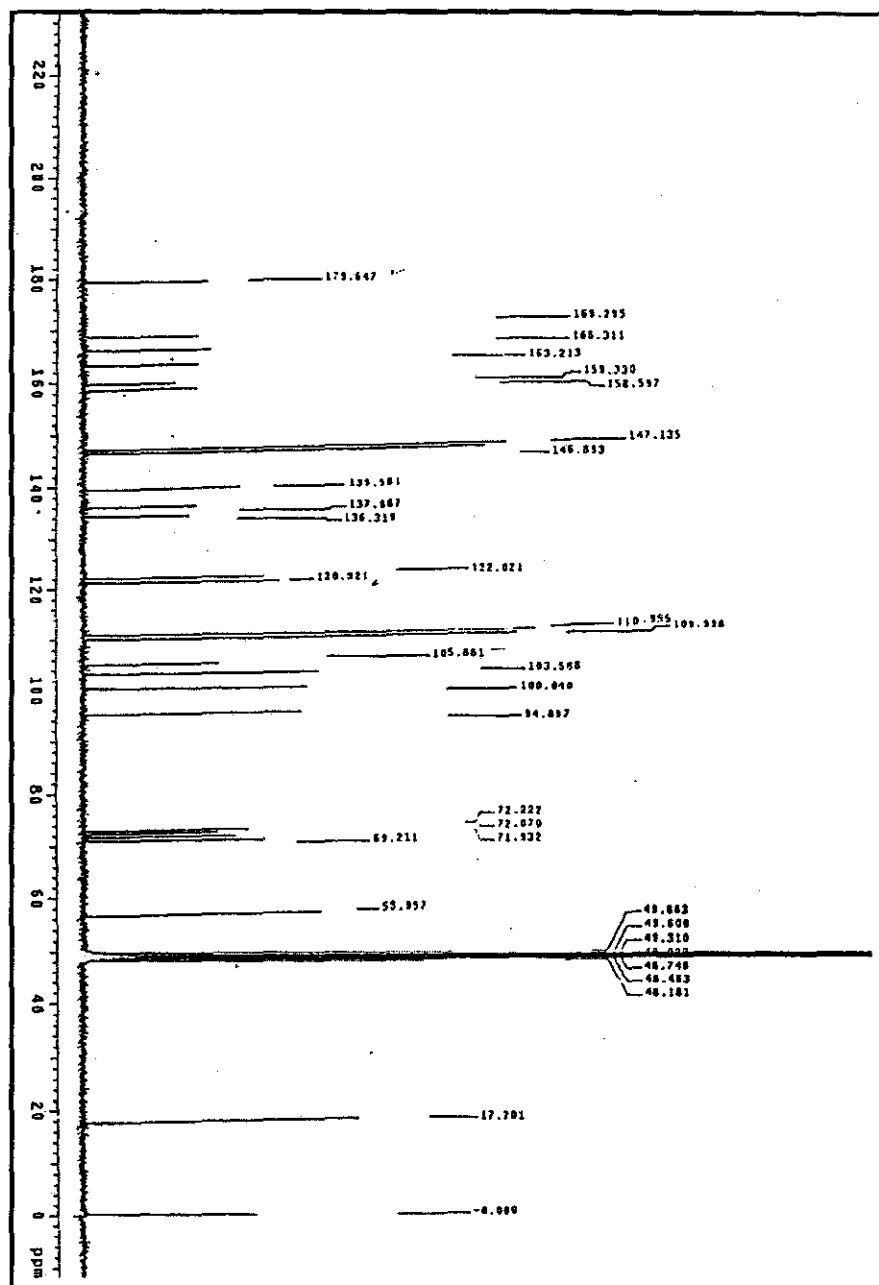
Fig (3): ^{13}C-NMR spectrum of compound (I) in CD$_3$OD

Table (3): ^{1}H NMR and ^{13}C NMR Spectral Data of Compound [II]

Carbon atom No.	^{13}C NMR (ppm)	^{1}H NMR	Carbon atom No.	^{13}C NMR (ppm)	^{1}H NMR
1	124.4		4$^\backslash$	70.33	
2	108.18	δ7.47 (dj=3.3 Hz)	5$^\backslash$	77.13	
3	162.14		6$^\backslash$	62.13	
4	96.89	δ 9.53 (dj=1.5 Hz)	1$^{\backslash\backslash}$	104.04	δ 5.12 dj=7.5Hz)
5	151.73		2$^{\backslash\backslash}$	74.35	
6	109.69	δ 6.47 (dd, j=1.0 -3.6 Hz)	3$^{\backslash\backslash}$	76.77	
7	177.96		4$^{\backslash\backslash}$	70.62	
1$^\backslash$	101.98	δ 4.9 (dj=7.2Hz)	5$^{\backslash\backslash}$	76.77	
2$^\backslash$	81.14		6$^{\backslash\backslash}$	61.25	
3$^\backslash$	78.18		OCH$_3$	57.49	δ 3.86,s

The presence of a phenyl group was established by the appearance of carbon atom signals between δ 96.89 to δ 162.14 ppm in the ^{13}C-NMR spectral data [Fig (6), Table (3)]. The ^{13}C-NMR spectrum also showed the presence of a methoxyl group (OCH$_3$) and carbonyl group (CO) due to carbon atom signals at δ 57.49 and 177.96 ppm respectively. The presence of a methoxyl group (OCH$_3$) was confirmed by the signal at δ 3.86 (3H, S) in the ^{1}H NMR spectrum [Fig (5), Table (3)]. The three aromatic proton signals only at δ 6.47 (1H, d, J= 3.6 Hz), δ 7.47 (1H, d, J= 3.3Hz) and δ 9.5 (1H,d, J= 1.5Hz) are assignable to the protons of C-2, C-6 and C-4 respectively in the ^{1}H NMR spectrum indicating that the other positions on the phenyl group were substituted. The presences of the substituted

groups (OH, OCH$_3$ and COO) were established by the spectral data (^{1}H NMR and ^{13}C -NMR).

The down field shift of C-2$^\backslash$ and C-2$^{\backslash\backslash}$ of glucose (about 6.8 ppm) in comparison with previously reported data (Markham *et al.*, 1978) indicated that the two glucose linked to each other through a (1-2) linkage. The linkage site of the disaccharide at the carboxyl group was confirmed through the down field resonance of this carbon.

Thus the structure of compound II [Fig (7)], was characterized as 3-hydroxy-5-methoxy benzoyl- O- β- D- glucopyranosyl (1-2) β- D-glucopyranoside). This compound was isolated for the first time from this plant.
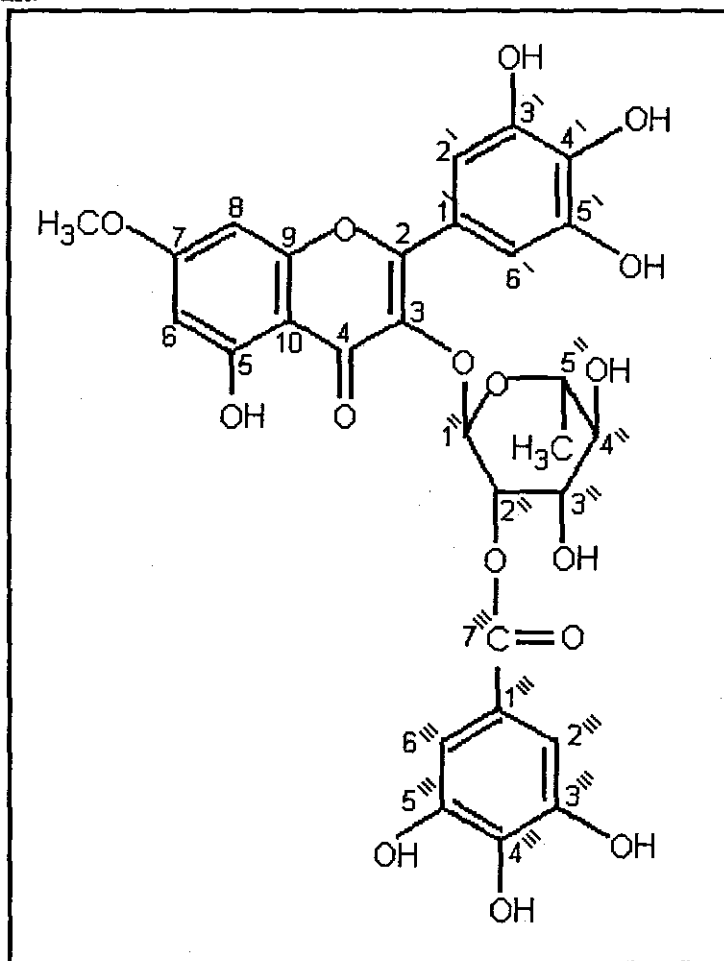
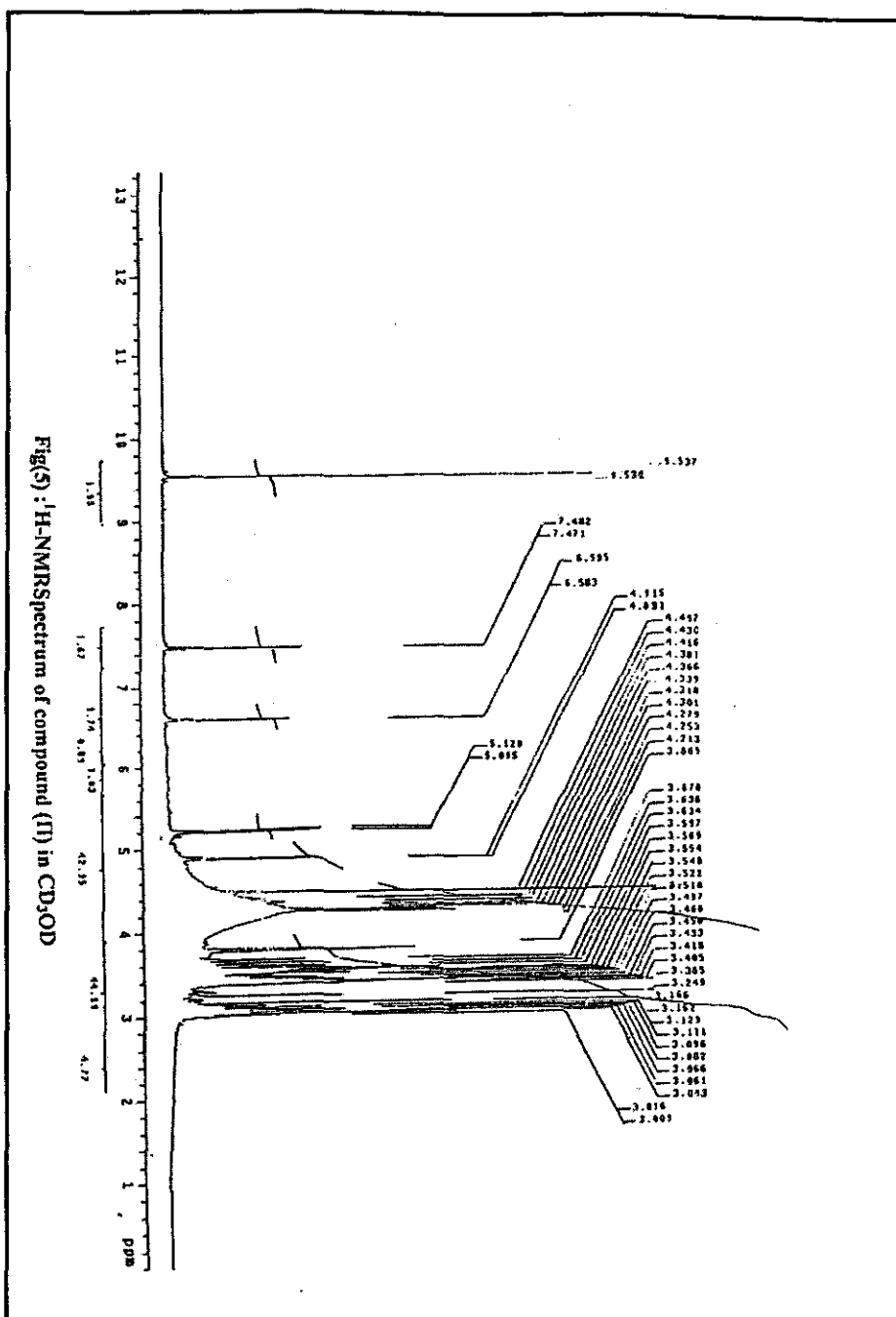

Fig (4): Structural Formula of compound (I).
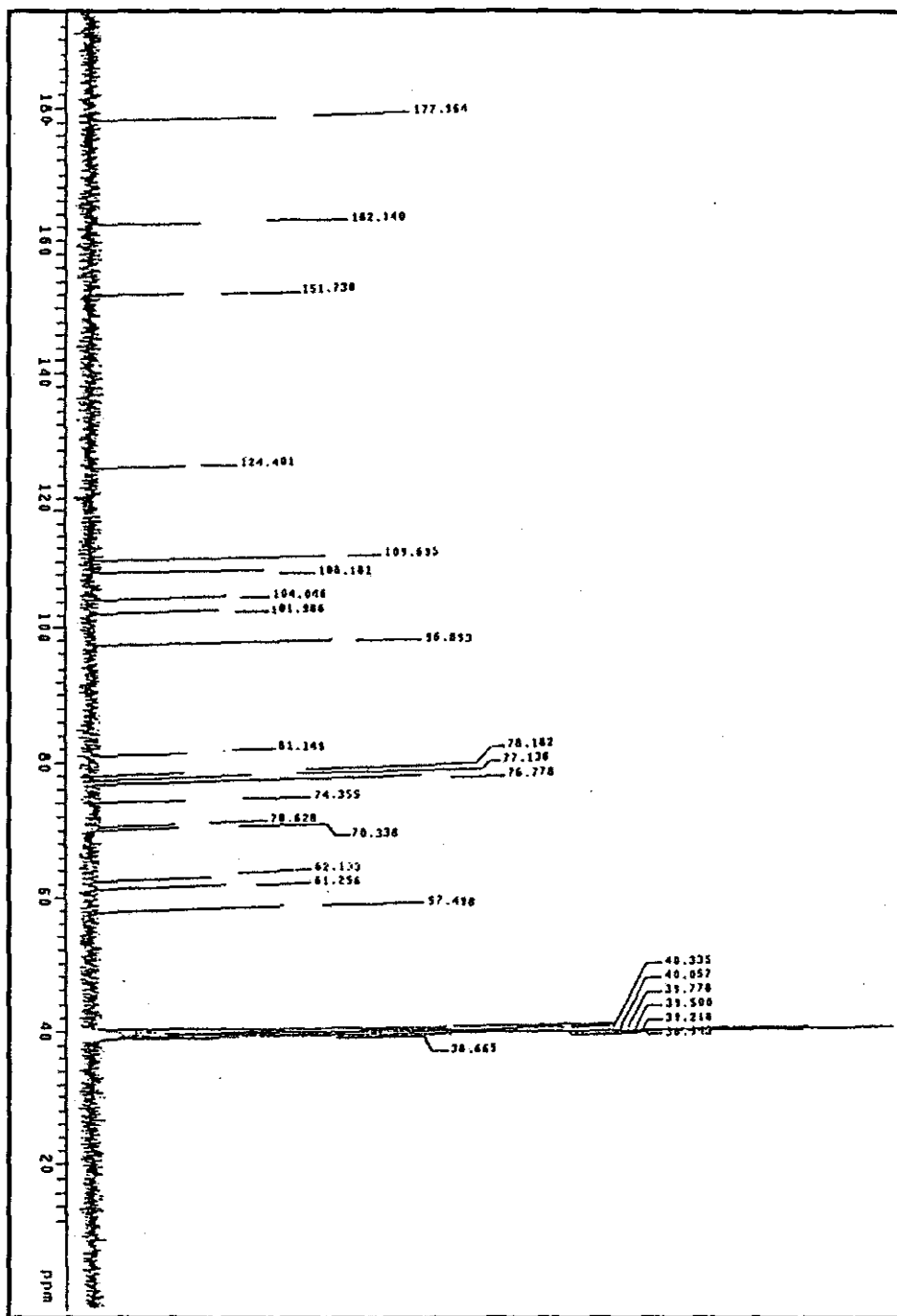
Fig (5): ¹H-NMR spectrum of compound (II) in CD₃OD
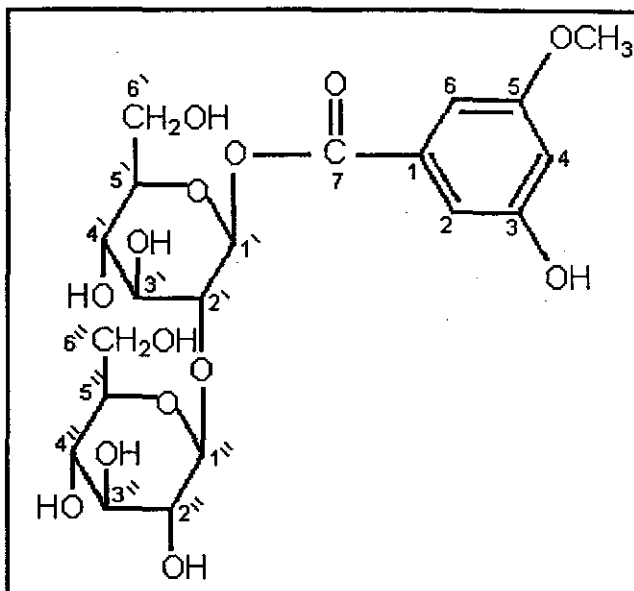
Fig (6): ^{13}C-NMR spectrum of compound (II) in CD$_3$OD

Fig (7): **Structural Formula of Compound (II).**

The isolated compound II (3-hydroxy-5- methoxy benzoyl- O- β-D- glucopyranosyl (1-2) β- D-glucopyranoside) exerted a strong fungicidal effect against the three phytopathogenic fungi but a more moderate effect against the three nematodes than compound I [Table (1)]

Benzoic acid and its derivatives have been reported to exhibit a wide range of biological activities, such as a strong antifeedant action for *Hylobius abietis* (L.) (Unelius *et al.*, 2006), strong inhibition of lettuce seed germination (Stobiecki *et al.*, 1993), and antimicrobial activity (Piscopo *et al.*, 1984).

Finally, this is the first study on the isolation and structural elucidation of both myricetin 3-O (2''-O galloyl))- α-rhamnopyranoside – 7 – methyl ether (compound I) and 3-hydroxy-5-methoxy benzoyl- O- β- D- glucopyranosyl (1-2) β- D-glucopyranoside [compound (II)] from *Acacia saligna* leaves with both fungicidal action against the three phytopathogenic fungi causing root rot and wilt diseases together with nematicidal effect against three nemaode speacies causing root knot and die back in many economically important crops.

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التعرف على التركيب الكيميائي لمركبات مفصولة من أوراق نبات الأكاسيا ساليجنا وتأثيرها على النيماتودا والفطريات

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تم فصل مركبات نقية من أوراق الأكاسيا ساليجنا وأختبـرت لنـشاط النيمـاتودا والفطريات ضد ثلاث أنـواع مـن النيمـاتودا: ميلـودجين جافانيكـا، روتيلنكيـولاس سميبنترانس، وتيلنكيولاس سيمبينترانس، وثـلاث أنـواع مـن الفطريـات: فيـوزاريم اوكسيبورام، سكلورانشيم روفلاى، وفرتسليم دالهي.

وقد تم التعرف على التركيب الكيميائي للمركبات المفصولة باستخدام طرق التعريف الموصوفة بواسطة فرانزوارث (١٩٦٦)، التحلل المائي في وسط حامـضي والطـرق الطيفية (أطياف الأشعة فوق البنفسجية والرنين النووي المغناطيـسى). لقـد تـم فـصل ميريسيتين-٣-O-(٢-O- جالويل)-α – رامانوبيرانوزيد – ٧ – مثيل إيثر (مركبI) باستخدام طرق الفصل الكروماتوجرافي والتعرف عليـه باستخدام الـرنين النـووي المغناطيسي للهيدروجين والكربون (جدول ٢). وتم فصل هذا المركب من قبل من أوراق نبات الأكاسيا كونفيوزا. وقد ثبتت فاعليته ضد أنواع النيماتودا الثلاثة المستخدمة وكـذلك الفطريات (جدول ١).

الدراسات السابقة أظهرت أن كلا من الميريسيتين ومـشتقات الجالويـل للمركـب المفصول (مركبI) مركبات على درجة عالية من السمية ولهـا العديـد مـن الأنـشطة البيولوجية، لكن لا توجد أي دراسـات سـابقة لنـشاط النيمـاتودا والفطريـات لهـذا المركب(مركبI).

تم فـصل مركـب ٣-هيدروكـسي- ٥- ميثوكـسي بنزوايـل – O – β – D – جلوكوبيرانوزايد (١،٢) β – D – جلوكوبيرانوزايد (مركب II) باستخدام طرق الفـصل الكروماتوجرافي والتعرف عليه باستخدام الـرنين النـووي المغناطيـسي للهيـدروجين والكربون (جدول ٣). هذه هى المرة الأولى التي يفصل فيها هذا المركب (مركب II) وقد أظهر هذا المركب تأثير قوي للفطريات ضد الثلاثة أنواع المستخدمة ولكن أظهر نـشاط متوسط للنيماتودا ضد الثلاثة أنواع من النيماتودا (جدول ١).

أثبتت الدراسات السابقة أن حمض البنزويك ومشتقاته له مدي واسع في النشاط البيولوجي ، لكن هذه هي المرة الأولى التي يفصل فيها (مركب II) من نبات الأكاسيا ساليجنا، وكذلك تأثيره على النيماتودا والفطريات (جدول ١).

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