

## Isolation of Rhamnocitrin Insecticide from Retamaraetam via Shephadex LH-20

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**Abstract:** *Retamaraetam* is a medicinal herb growing wild in the mountain alkurvillage was collected in the flowering stage and after treatment in ways different methods of chromatography for isolated Rhamnocitrin has been purified by Sephadex LH-20 and identified by spectroscopic methods (UV, NMR). showed that despite the differences in potency of *Retamaraetam* and Rhamnocitrin substances they were found to possess parallel regression lines. This may suggest that these extracts have the same mode of action against the tested insect larvae.

**Keywords:** *Retamaraetam*, Fabaceae, Alwajh, Rhamnocitrin, insecticide.

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### I. Introduction

Plants have formed the basis of sophisticated traditional medicine systems that have been used for thousands of years in countries, such as China [1] and India [2]. The use of plants in the traditional medicine of many other cultures has been extensively documented. These plant based systems continue to play an important role in health care and it has been estimated by the World Health Organization that approximately 80% of the world's inhabitants rely mainly on traditional medicines for their primary health care, while plant products also play an important role in the health care systems of the remaining 20% of the population mainly residing in developed countries [3].

*Retamaraetam* is a spontaneous shrub legume belonging to the Fabaceae; it is one of the most important plants in the east Mediterranean deserts. In Tunisia this shrub legume plays an important ecological role, it is widely used in dune stabilisation and soil fixation. In addition, *R. raetam* is able to produce important quantities of biomass that are used as forage and for treating various human and animal diseases. Due to their capacity to enter into symbiosis with legume nodulating bacteria (LNB) collectively called rhizobia, *R. raetam* could play an important role in the nitrogen cycle. They may be used to restore or increase fertility of degraded and eroded soils [4].

As the plant is being used extensively as an herbal medicine, it is necessary to have knowledge of the constituents of the plant.

In 2014, investigated *R. raetam* shoots antioxidant and antimicrobial activities and its natural antioxidant contents obtained from four fractions (petroleum ether, acetone 60%, ethyl acetate and water). Results showed that the ethyl acetate fraction exhibits the highest antioxidant activity as compared to the other ones. Moreover, RP-HPLC analysis showed that syringic acid and coumarin were the major phenolic compounds. Furthermore, this moderately polar fraction showed considerable antibacterial properties against human pathogen strains especially against *Escherichia coli* and *Bacillus cereus*. Finally, fractionation allows the identification of *R. raetam* most active molecules and therefore the optimization of their utilization. Our findings pointed out the appropriate solvent for extracting *R. raetam* potent phenolics which might provide a rich and novel source of natural antioxidants as food additives replacing synthetic ones in food industry [5].

Two new flavones were isolated from the aerial parts of *Retamaraetam* subsp. *raetam*. Their structures were established as luteolin 4'-*O*-neohesperidoside (1) and 5,4'-dihydroxy-(3'',4''-dihydro-3'',4''-dihydroxy)-2'',2''-dimethylpyrano-(5'',6'':7,8)-flavone (2) by means of spectroscopic methods. Also present was ephedroidin (4',5,7-trihydroxy-8-(2-hydroxy-3-butenyl)-flavone) [6].

### II. Materials And Methods

For the study, Aerial parts of the herb was collected from Al-Khur Village in Al-Wajh Province at Tabuk area, North- Western, Saudi Arabia. It is grown widely on mountains, during the rainy season (JAN, 2016). The original sample was classified and identified according to their family, genes and specie by Professor Amal Fakhry, Department of Botany, University of Tabuk.

#### **Chemical reagents and instrumentation**

**NMR spectroscopy:** <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy was performed on a Bruker 300 NMR spectrometer using deuterated solvents as internal standards.

**Steady state UV-Vis absorption spectroscopy:** Steady state absorption spectra were obtained using a spectrophotometer model UV – 240 (200-600 nm). All spectra were recorded using a quartz glass cuvette of 10x10 mm.

**General remarks:** Column chromatography was performed using Flash column Shephadex (LH-20) blocked Silica Gel (35-60 mesh). All chemicals were purchased from Sigma Aldrich and were of reagent grade.

**Extraction and isolation**

Dry herbs materials(4 Kg) were powdered and extracted by warm ethanol (80%). The solvent was evaporated by rotary evaporator and the residue (398g) was kept for further isolation chromatography methods and biological activities. The crude extract was chromatographed on a silica gel column ((6x70 cm) blocked Silica Gel (35-60 mesh) with (Benzene : Ethyl acetate, 9:1) to yield 100 fractions. The fraction number 93 was purified using Flash column Shephadex (LH-20) with absolute ethanol to afford compound **MR** (17mg).

**Tested insects**

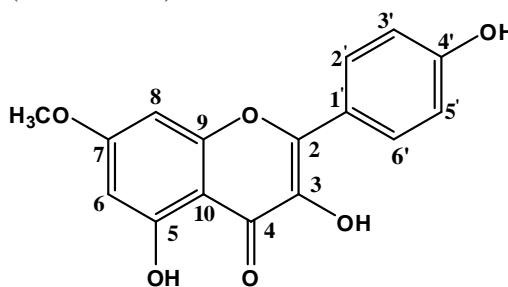
Preliminary toxicological bioassay tests were carried out to the selected plant extracts on tested insects according to a cited method after modification by Kamel, *et al.* 2005, Eldiasty, *et al.* 2014 and El-Ghaban, *et al.* 2015. Mosquitoes were maintained in a walk-insectaries under controlled conditions of temperature ( $27 \pm 2$  °C), relative humidity, R.H. (70%-80%) and light - dark period (16: 8 hrs.) under a fluorescent light. Larvae of the tested mosquito species were reared in white enamel pans (35-40 cm diameter and 10 cm depth) containing about 1.5 L of de-chlorinated tap water. Larvae were provided with tetra-amine (tropical fish food) sprinkled twice daily over the water surface of the breeding pans. The water containing larvae was gently transferred every 2 days into clean enamel pans to avoid formation of scum on the water surface or on the walls and bottoms of pans. The breeding water was gently aerated for about 5 minutes every day by means of a small air pump.

Developed pupa were collected and transferred daily to plastic cups containing saline water then introduced into the breeding screened wooden cages (30x30x30 cm<sup>3</sup>). Emerged adults were fed on 10% sugar solution. After three days adults were fed on blood to lay egg batches were transferred to the white enamel pans containing de-chlorinated tap water for hatching. When mosquito larvae developed to the 2<sup>nd</sup> instars, they were poured into clean pans and observed daily. Late third larval instars were used for toxicological studies as described previously for *Culex pipiens* [7].

**III. Results And Discussion**

**Identification of compound MR:**

**Name:** 7-O-Methyl Kaempferol (Rhamnocitrin)



**Weight:** 17 mg

**Molecular:** C<sub>16</sub>H<sub>12</sub>O<sub>6</sub>

**Molecular weight:** 300

**Shape:** powder

**Color:** yellow

**Melting point:** 224 °C

**Spectral Characteristics**

The ultraviolet/visible light absorption spectrum of compound **MR** is presented in Figure 1.

**Table 1:** UV Spectrum data ( $\lambda_{max}$  nm ) of Compound MR

MeOH	+ NaOMe	+ AlCl <sub>3</sub>	+ AlCl <sub>3</sub> /HCl	+ NaOAc	+ NaOAc /H <sub>3</sub> BO <sub>3</sub>
270, 363	278, 399	274, 305 sh, 348, 424	274, 305 sh, 348, 424	276, 364	276, 364

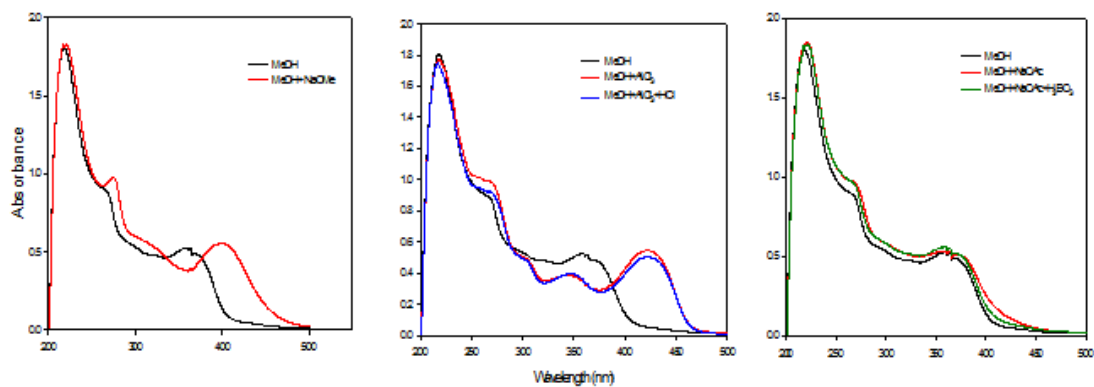


Fig.1.

Ultraviolet/visible light absorption spectrum of Compound MR

Compound MR was identified by using <sup>1</sup>H-NMR, <sup>13</sup>C-NMR. All spectroscopic data were resumed in table (3).

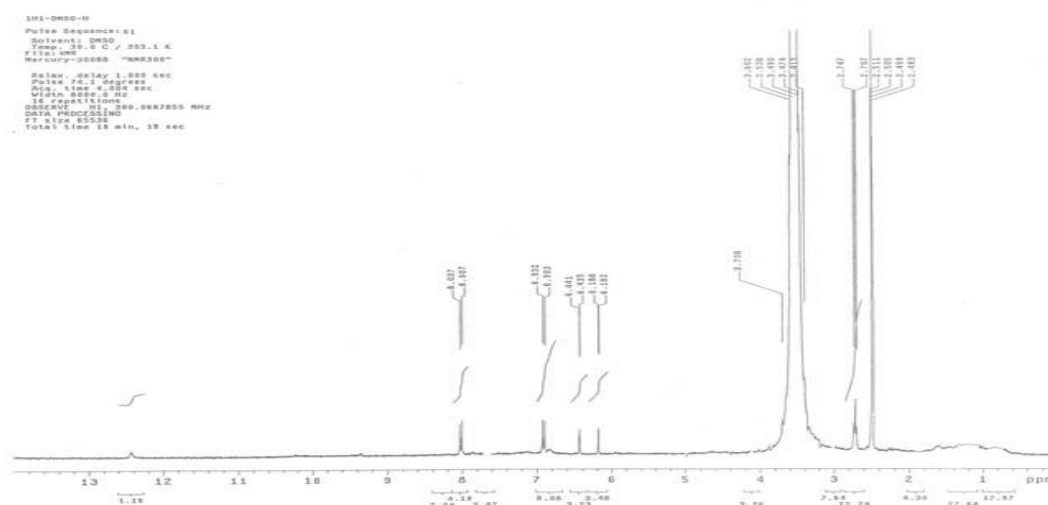


Fig. 2: <sup>1</sup>H NMR Spectrum of Compound MR

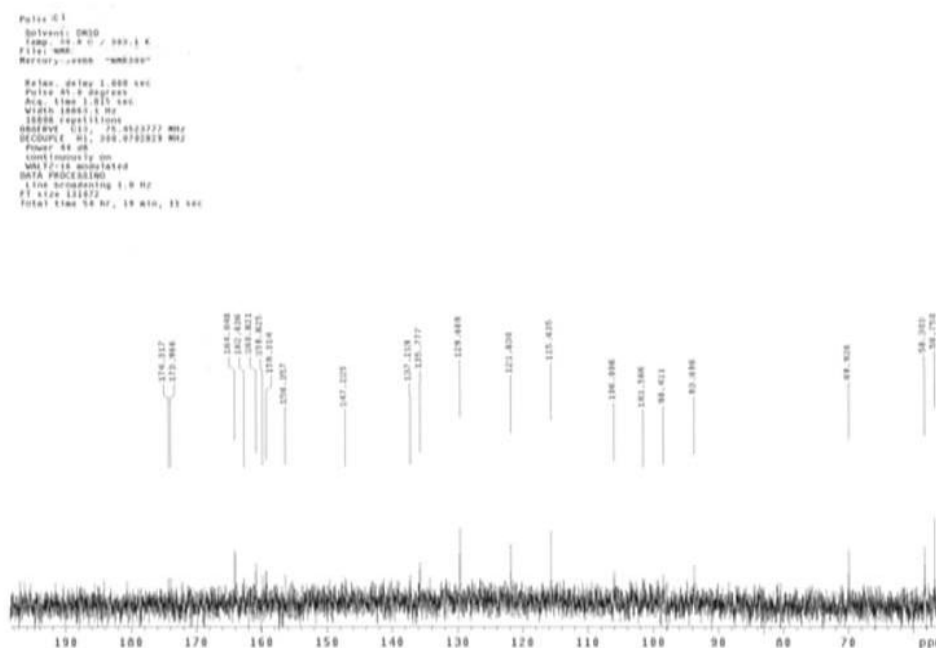


Fig.3: <sup>13</sup>C NMR Spectrum of Compound MR

**Table 2:** <sup>1</sup>H, <sup>13</sup>C NMR Chemical Shifts of MR compound

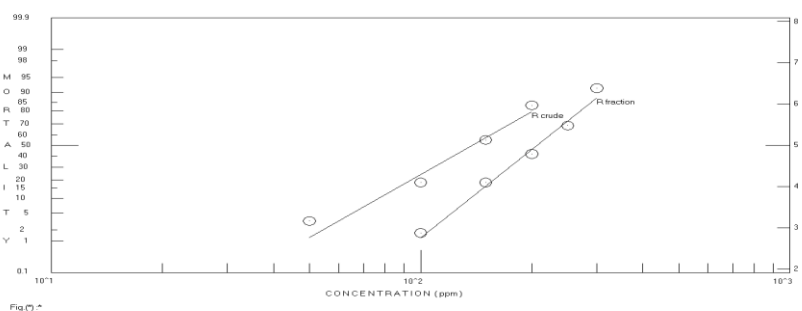
No. of H	Chemical shift	No. of C	Chemical shift
H-6	d 6.19 (J=2.1 Hz)	C-2	147.2
H-8	d 6.44 (J=2.4 Hz)	C-3	135.8
H-2'	d 8.03 (J=8.7 Hz)	C-4	176.0
H-3'	d 6.92 (J=8.7 Hz)	C-5	160.8
H-5'	d 6.92 (J=8.7 Hz)	C-6	98.4
H-6'	d 8.03 (J=8.7 Hz)	C-7	164.0
OCH <sub>3</sub> -7	s 3.46	C-8	93.7
OH-5	s 12.45	C-9	156.4
		C-10	106.0
		C-1'	121.8
		C-2'	129.7
		C-3'	115.6
		C-4'	159.3
		C-5'	115.6
		C-6'	129.7
		OCH <sub>3</sub> -7	56.7

**3.3.Evaluation of the larvicidal activity of Ethanol Extracts(Retamaraetam & Rhamnocitrin) on mosquito larvae:**

These experiments were carried out to evaluate the potency of *Retamaraetam* and Rhamnocitrin which extracted both by ethyl alcohol. The results from table (3) and fig. (4) showed that the *Retamaraetam* extract more potent than the Rhamnocitrin extract according to LC50 and LC95 values. The confidential limits of each of the tested extract were statistically calculated for LC50 and LC95 at P= 0.05. The LC50 values were 138.28 and 206.77 ppm for crude extract and the fraction respectively. The difference in potency between *Retamaraetam* extract and the Rhamnocitrin may be attributed to the synergistic effect between the components of crude extract. Several authors have used the plant extracts to synergize each other and the insecticidal activities of the traditional insecticides [8-11]. The present data showed that despite the differences in potency of crude and fraction substances they were found to possess parallel regression lines. This may suggest that these extracts have the same mode of action against the tested insect larvae. The slope function reflects the homogeneity reaction of tested insects toward the tested substance. The lowering of slope function that meaning the more homogeneity of tested population [12-13].

**Table 3:** larvicidal activity of Ethanol Extracts(Retamaraetam&Rhamnocitrin)on *Culex pipiens* larvae.

Ethanol Extracts	Conc. ppm	Mortality %	LC 50 (Co. Limits)	LC 95 (Co. Limits)	Slope Function
<i>Retamaraetam</i> (crude)	50	1.28	138.28 (126.72 – 150.91)	292.49 (237.59 – 360.24)	5.1
	100	23.82			
	150	57.1			
	200	97.12			
Rhamnocitrin (fraction)	100	1.7	206.77 (195.40 – 218.81)	352.80 (313.01 – 397.70)	7.1
	150	18.3			
	200	40.98			
	250	67.21			
	300	91.67			



**Fig. 4:** Susceptibility of *Culex pipiens* larvae to Ethanol Extracts(Retamaraetam&Rhamnocitrin)

#### IV. Conclusion

In conclusion, a combination of spectroscopic methods, chromatography, and biological testing was particularly useful for the rapid characterization of unknown active compounds of an extract. It is a great help in isolation and structure determination. Experiments suggest that substances have the same mode of action against the tested insect larvae.

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