CHEMISTRY AND BIOLOGY OF PHENOLICS ISOLATED FROM *Reaumuria vermiculata* (TAMARICACEAE)

A Thesis Submitted By

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كيمياء و بيولوجيا الفينولات المفصولة من نبات روميريا فرميكيولاتا العائلة التماريكسية

رسالة مقدمة من

إيمان شيرين مصطفى

بكالوريوس العلوم الصيدلية

كلية الصيدلة جامعة مصر الدولية

و ذلك لاستكمال متطلبت الحصول على

درجة الماجستير فى العلوم الصيدلية (عقاقير)

قسم العقاقير

كلية الصيدلة جامعة عين شمس

العباسية القاهرة

جمهورية مصر العربية

٢٠١٢

تحت إشراف

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GENERAL SUMMARY

In accordance with the recent world-wide interest in plant phenolics which emerge from their broad range of biological activities, particular emphasis has been focused, in the present thesis, on the constitutive phenolics of the extract of a phenolic rich plant, namely, *Reaumuria vermiculata* (Tamaricaceae).

The present study was divided into two parts:

Part I: Isolation and identification of the phenolic constituents of the aqueous methanolic aerial parts extract of *Reaumuria vermiculata* which has not been subjected previously to a comprehensive phytochemical study.

Part II: Investigation of the antioxidant, cytotoxic and antimicrobial activities of the aqueous/methanolic aerial parts extract of *Reaumuria vermiculata.*

Part I:

Phytochemical investigation of *Reaumuria vermiculata* aerial parts extract :

The phytochemical study of *Reaumuria vermiculata* included successive column chromatographic investigation of the aqueous/methanolic aerial parts extract, separation of individual phenolics, repeated purification of these individuals and establishment of their homogeneity by paper chromatography.

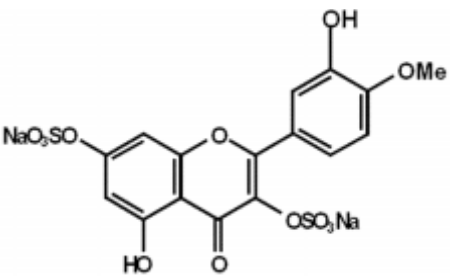
For the structure elucidation of the isolated phenolics, the required structural information was obtained through chromatographic analysis, application of chemical degradation methods and conventional spectroscopic techniques of analysis as well.

However, the recent advances in mass spectrometric and nuclear magnetic resonance spectroscopic analytical techniques were extensively applied in this

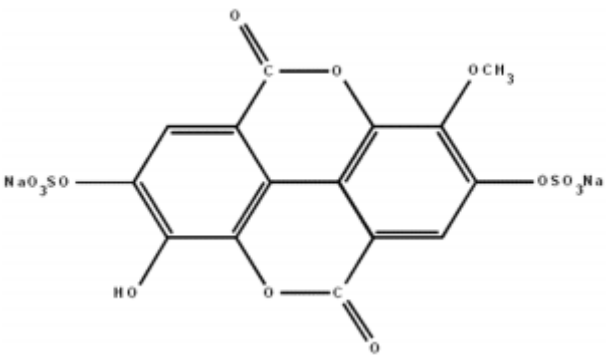
thesis, either to unravel the chemical structure of the isolated new natural phenolics, to clarify the full structure of some of the known phenolics or because there were no previously reported spectral data for some others.

As a result of this intensive study, **20** phenolic constituents were individually isolated and identified, **18** of which have not been previously identified in *Reaumuria vermiculata* ; among them **3** were found to be a new natural products which has not been reported before to occur in nature and **1** was found to be a novel one. The new compound was identified to be:

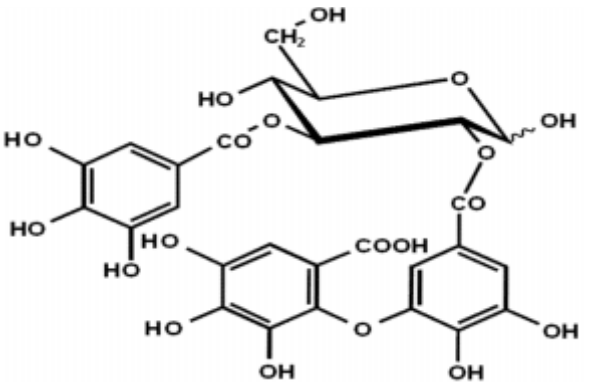
Compound **(2)**, a new sulphated flavonoid ; tamarixetin 3,7-disodium sulphate. This is a flavonoid structure which has not been reported in nature before.



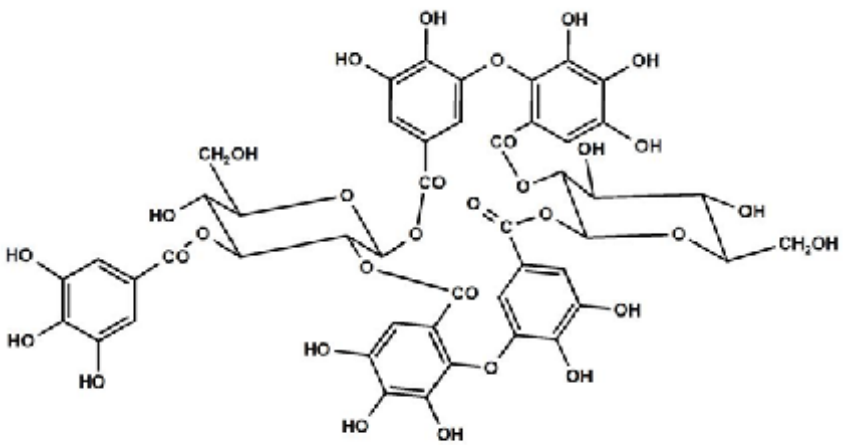
Compound **(4)**, a new sulphated ellagitannin ; ellagic acid 3-methyl ether 4.4'-di-sodium sulfate. This is a sulphated ellagitannin structure which has not been reported in nature before.



Compound **(9)**, a new ellagitannin; 2-*O*- dehydrodigallic acid monocarboxyloyl-3-*O*- galloyl-(α/β)glucopyranose. This is an ellagitannin structure which has not been reported in nature before.

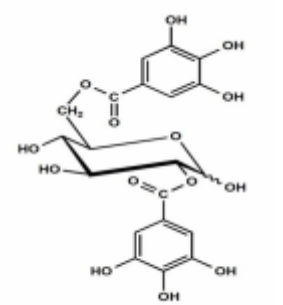
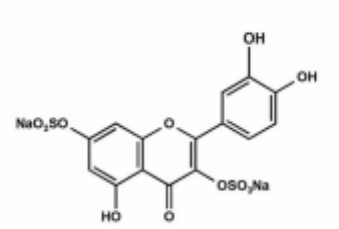


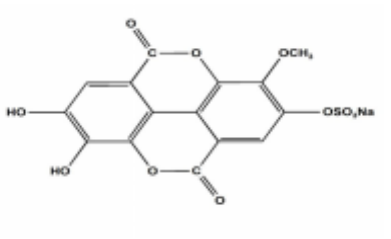
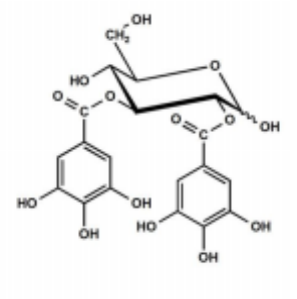
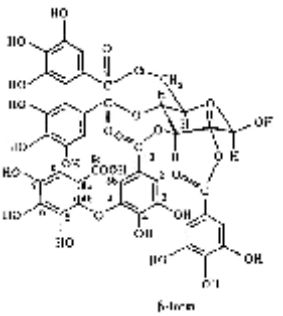
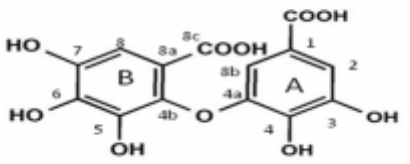
Compound **(13)**, a novel ellagitannin; vermiculatin. This new compound is of special interest as it represents a novel dimeric cyclic ellagitannin.

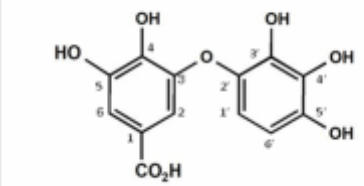
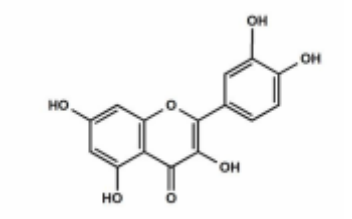
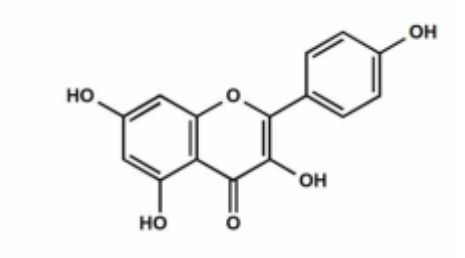
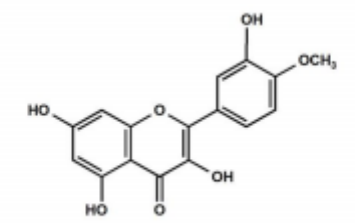


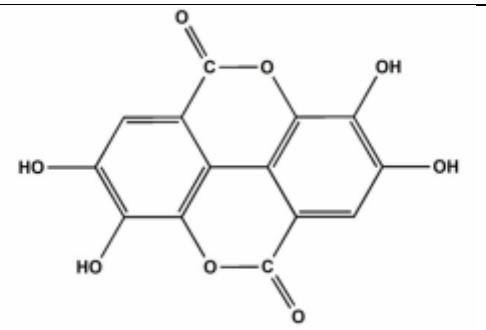
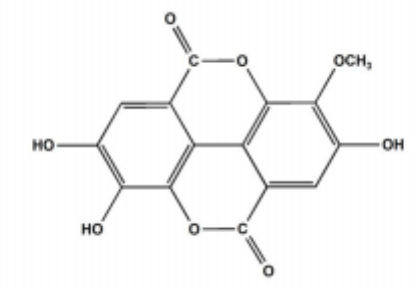
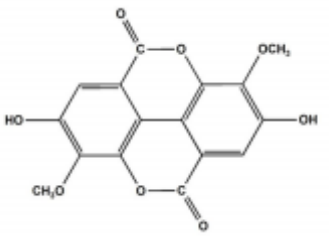
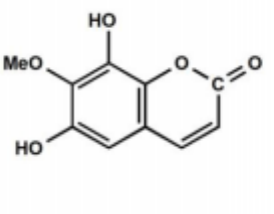
In addition, the (**16**) known compounds, were also isolated and identified by applying the conventional and spectral methods of analysis. It should be noted however that, compound (**3**) was isolated, previously from the same plant, but were identified on the basis of the conventional methods of analysis only.

Table (34): Compounds isolated for the first time from *Reaumuria vermiculata*

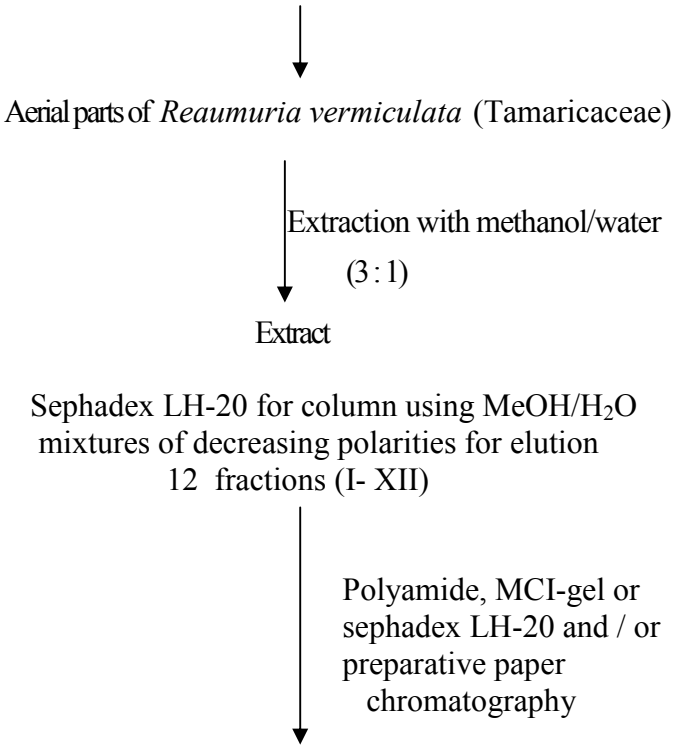
Compound (1)	 **2,6-di-*O*-galloyl-(α/β)-$^4C_1$- glucopyranose**
Compound (5)	 **Quercetin 3,7-disodium sulphate**

Compound (7)	 **Ellagic acid 3-methyl ether 4-mono-sodium sulfate side**
Compound (8)	 **2,3-di-*O*-galloyl-(α/β)-$^4C_1$-glucopyranose**
Compound (10)	 **Tamarixellagic acid**
Compound (11)	 **Dehydrodigallic acid**

Compound (12)	 **l '-decarboxydehydrodigal-lic acid**
Compound (14)	 **Quercetin**
Compound (15)	 **Kaempferol**
Compound (16)	 **Tamarixetin**

Compound (17)	 **Ellagic acid**
Compound (18)	 **3-methoxyellagic acid**
Compound (19)	 **3, 3'-dimethoxyellagic acid**
Compound (20)	 **6,8-dihydroxy-7-methoxycoumarin**

Scheme of work



Fraction I: the material of this fraction showed that it is a non phenolic material

Fraction II:

Compound (1): 2,6-digalloyl glucose

Compound (2): **New natural product, Tamarixetin 3,7-disodium sulphate**

Fraction III:

Compound (3): Kaempferol 3,7-disodium sulphate

Compound (4): **New natural product, Ellagic acid 3-monomethyl ether 4,4'-di-sodium sulfate**

Fraction IV:

Compound (5): Quercetin 3,7-disodium sulphate

Compound (6): Gallic acid

Compound (7): 3-Mono methoxy ellagic 4-sodium sulphate

Fraction V:

Compound (8): Nilocitin

Compound (9): **New natural product, 2-*O*- dehydrodigallic acid monocarboxyloyl-3-*O*-galloyl-(α/β)glucopyranose**
Compound (10): Tamarixellagic acid

Fraction VI:
Fraction VII:
Compound (11): Dehydro digallic acid
Fraction VIII
Compound (12): l'-decarboxydehydrodigallic acid
Fraction IX:
Compound (13): **Novel natural product, Vermiculatin**
Fraction X:
Compound (14**):** Quercetin
Compound (15): Kaempferol
Compound (16)**:** Tamarixein
Fraction XI:
Compound (17)**:** Ellagic acid
Compound (18): Ellagic acid 3-monmethyl ether
Fraction XII:
Compound (19):),Ellagic acid 3,3'-dimethyl ether
Compound (20): 6,8-dihydroxy-7-methoxycoumarin

Part II: Biological investigation of *Reaumuria vermiculata* aerial parts extract:

The aqueous methanolic aerial parts extract of *Reaumuria vermiculata* has been subjected in the present work to some (*in vitro*) biological studies including:

1-Antioxidant activity against the stable free radical DPPH. *Reaumuria vermiculata* proved high values for absorbance inhibition at the different concentrations used, when compared to the reference standard ascorbic acid. It had an IC_{50} of 5.8±0.22 μg/ml which is very close to the reference standards used [ascorbic acid(IC_{50} =1.83±0.32 μg / ml)].

2- Antioxidant activity of the more sensitive ORAC assay. the ORAC assay demonstrated a distinguishable anti-oxidant capacity of the crude extract and some fractions at the different concentrations used. The most active fraction is (**VI**) which showed the overall highest activity in this assay due to the presence of compound **9** as a main constituent in this fraction, among other minors. Also, the half life time of fluorescein after protection with 12.5 µg/ml test demonstrated a moderate anti-oxidant capacity of the crude extract and higher activities of the isolated compounds **9** and **13**.

3- Cytotoxic activity against four different solid tumor cell lines. *Reaumuria* extract showed comparable potencies against all solid tumor cell lines used with IC_{50} ranged from 1.3±0.15 to 2.4±0.22 µg/ml. The cytotoxicity pattern was gradual with relatively lower Hill-type co-efficient (2.38) in Huh-7 liver cancer cell line. The extract possesses the highest IC_{50} in Huh-7. However, the resistant fraction of Huh-7 was the lowest (0%) among all tested cell lines.

4- The Cell viability using HaCaT and *MTT assay* method was done for the extract, new compounds (**9**) and (**13**). The extract diminished the viability of the non tumorigenic cells only in concentrations higher than 3.1 µg/ml and only in a moderate degree. The extract showed stimulation and increase in cell proliferation at the lowest concentration used (0.8 µg/ml). The extract, new compounds (**9**) and (**13**) showed a dose dependent decrease in cell viability but never reached 50% ,so we can't calculated the IC_{50}.

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5- The antimicrobial activity was screened using agar diffusion method. It is noticed that extract showed an obvious antimicrobial effect on bacteria and yeast, though no effect was detected on fungi. The most sensitive strains were *Candida albicans* , *Staphylococcus aureus* and *Bacillus subtilis*. The minimum

inhibitory concentrations extract of were ranged from 5.0–15.0 mg/ml . The lowest MIC was detected with *Candida pseudotropicalis* ATCC 4135, *Candida albicans* ATCC 10231, *Staphylococcus aureus* and *Bacillus subtilis*.