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ISOLATION OF TWO BIOACTIVE COMPOUNDS FROM TWO EGYPTIAN WILD PLANTS

(With One Figure)

By

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فصل مركبين حيوي النشاط من نباتين بريين مصريين

رأفت فخر الدين عرفة

تـم تجمـيع النباتين موضوع الدراسة من أماكن نموهما الطبيعية في مصر وتم استخلاص المكونـات الكيميائية الموجودة في ٢٠٠ جم من المجموع الخضري المجفف والمطحون لكل مـنهم مرّه باستخدام الميثانول والميثلين كلوريد (١:١) (المستخلص العضوي) ومره أخري بالماء المقطر (المستخلص المائي) وتم التخلص من المذيبات باستخدام جهاز التبخير الدّوار. وقـد أجـري الاستقصـاء الحيوي لهذه المستخلصات الخام ضد بعض السلالات البكتيرية والفطـرية، ولـم تظهر هذه المستخلصات أية فاعلية ضد الفطريات و لكنها أظهرت نشاطاً فعـالاً ضد بعض السلالات البكتيرية المختبرة. وتم تجزئة المستخلصات ذات الفاعلية الضد بكتـيرية باسـتخدام مذيبات الهكسان، الميثلين كلوريد، خلات الايثيل والكحول البيوتانولي. وأجري الاستقصاء الحيوي لهذه المستخلصات المجزئة فأظهرت النتائج أن مستخلص خلات الايثـيل انه الأشد فاعلية في ايقاف نمو بعض السلالات البكتيرية المختبرة. وتم تجزئة هذا المسـتخلص باستخدام عمود الفصل اللوني وكروماتوجرافيا الطبقة الرقيقة وتنقيته باستخدام كروماتوجرافـيا العمود المعبأ بالسيفادكس ال اتش ٢٠ فتم فصل مركبين. وأجري للمركبين المفصولين الاستقصاء الحيوي ضد عديد من السلالات البكتيرية. وقد اظهر المركبين فاعلية في ايقاف نمو بكتيريا باسيلس سابتيلس فقط. وتم التعرف علي هذين المركبين باستخدام تقنية كروماتوجرافيا الغاز/مطياف الكتلة علي انهم مريستين (مركب فلافونويدي) وريزورسينول. وهـذه النـتائج مشجعه لأنها تعطى دلاله واضحة علي إمكانية استخدام مستخلصات بعض النـباتات المصرية كمضادات لبكتيريا الباسيلس سابتيلس شديدة الضراوة والتي تسبب فساد وتسـمم الأغذية مسببة خسائر مالية وبشربة جسيمة في أنحاء العالم باستخدام مركبات نباتية طبيعية متاحة ومأمونه بيئياً وغير مكلفة اقتصادياً.

SUMMARY

Two bioactive natural products were isolated through bioactivity guided fractionation assay from *Heliotropium diygnum* and *Zilla biparmata.* Antibacterial substances are identified through GC/MS spectral analysis

as myricetin (3,5,7,3`,4`,5`pentahydroxyflavone) and resorcinol (HO-C_6H_4-OH).

Key words: *Heliotropium diygnum and Zilla biparmata, Extraction, purification, Antibacterial activity and GC/MS analysis.*

INTRODUCTION

Heliotropium species is one of the family Boraginaceae and usually has a small flowers. In Egyptian species, the flowers are white or yellow, sessile in leafless (bracteate only in nods numbers 4 and 11) scorpioid, usually one side cymes. Style terminal on top of the fruit. Ovary of the plant species at maturity breaking up into four distinct nutlets (really drupelets), rarely these connate in pairs. *Heliotropium diygnum* is a small shrublets, leaves are yellow-green, flowers yellow and the petals mostly 1-2 mm long (Tackholm, 1974).

Zilla is one of the family Cruciferae (Brassicaceae) and characterized as spiny desert shrubs with pink or purple flowers. Pods indehiscent, globose or square with a long subulate spinescent beak. *Zilla biparmata* has a pod cuber purple. The peculiar shape of the pod has given rise to its name "biparmata", meaning 2 shields (Tackholm, 1974).

The previous phytochemical studies revealed that *Heliotropium* species is one of the richest plant genera by pyrrolizidine alkaloids. The principle alkaloids include europine, heliotropine, lasiocarpine and `s-acetyleuropine (Asibal, *et al.* 1989) as well as 7-angelheliotropine, 9-angelolyroncine and its N-oxide. The alkaloid compounds possess antibacterial (Jain, and Sharma, 1986 and Marquina,. *et al.* 1989) and antitumor properties (Jain, and Purchit, 1986). The pyrolizidine alkaloids from *Heliotropium* plants are very toxic and have been associated with the venoocclusive diseases of the herbal tea. The compounds are excreted through the milk in lactating animals and their presence in milk has been identified possessing potential toxicity hazards to neonates (Panter, and James, 1990). *Heliotropium indicum* used in treatment of fevers, eye lotion, threatened abortion, wound dressing and vermifuge (Daziel, 1937).

In Arabic herbal medicine, the leaves of *Heliotropium digynum* and the whole plant or the juice of *Heliotropium strigosum* used for treatment of skin diseases, crazy dog bite, laxative, pain relief for insects and snake bite and as a remedy for the human terminal parts (El-

Shanawany, 1996). Plants containing pyrolyzidine alkaloids have medicinal interest since at least the fourth century and they have been reported for using in different purposes (Rina, *et al.* 1995, Villaroel and Urzua 1991 and Marouina *et al.* 1989).

Family Brassicaceae is a very common plant family distributed worldwide specially Africa. In African herbal medicine, some of the family plants as *Armoracia lapathifolia*, *Brassica campestris*, *Lepidium sativum*, *Nasturtium officinale* and *Raphanus raphanistrum* are used in the treatment of many diseases. As Rubifacient, Bronchitis, Inflammation (Daziel, 1937 and Watt, *et al.* 1962), skin infection, snake bite and pot-herb (Watt, *et al.* 1962), Galactogogue, insect repellant, tonic (Iwu, 1986), skin diseases, embrocatin (Daziel, 1937, Dahr, *et al.*, 1968 and Watt, *et al.* 1962), bacterial and fungal infections, diarrhea, dysentery, cough, poison antidote. Anthrax remedy, hemorrhoids, malaria and skin diseases (Iwu, 1986).

In Arabian botanical medicine *Eruca sativa*, as one example of this family used as a broad spectrum antibacterial herbs and treatment of many diseases (El-Shanawani, 1996). Some of Brassicaceae member's plants as *Brassica napiform* is have been experimentally verified and showed indeed potent in the treatment of diabetes. It is significant, there fore, that a peptide designated as p-insulin has been reported from some plants of this family (Iwu, M. 1986).

Phytochemical screening of *Zilla* and *Heliotropium* species revealed the presence of many chemical substances as flavonoids, alkaloids (especially pyrolyzidine alkaloids), fatty acids, saponins and tannins (Rizk *et al.* 1991, Ismail, and Grass, F. 1989 and Karawya *et al.* 1974).

Research on bioactive substances from plant sources has a great scope and could lead to the provision of value added economics returns. Also established of a natural plant products industry, an export earning and betterment of the rural populations in terms of health care (Steven, and Russel, 1993).

Bacillus subtilis group of bacilli is widely distributed in the natural environment, being especially abundant in soil and on vegetation. This group of bacteria is of particular concern to the food processing and canning industries as the potential cause of contamination and spoilage problems because most strains secrete a range of highly active protolytic and saccharolytic enzymes (Mascart-lemone *et al.* 1985). Also, *Bacillus subtilis* was isolated from incidents of the food poisoning materials and

its able to synthesize peptide antibiotics during growth (Hanninen, 1981), that is inhibitory towards competing organisms, hence increasing the opportunities for proliferation (Doylee, 1989)

This study is one of my current major research interests including the development of chemotherapeutic agents based on Egyptian wild plants isolates for the treatment of local diseases and the industrial utilization of the Egyptian plants as a tool for the conservation of Egyptian native plant biodiversity.

Aim of the study:

This study aims to extract, isolate, purify and identify the bioactive plant natural products of Egyptian wild plants, *Heliotropium diygnum* and *Zilla biparmata*.

MATERIALS and METHODS

Plant material and extraction-Fresh plant materials were collected in suitable quantities from its natural habitats in Egypt. Professor Fayed, A., Assiut University confirmed identification. Two hundred grams of each plant extracted by Dichloromethane: Methanol in equal volume (organic extract). Another two hundred Grams of each plant was extracted by distilled water (aqueous extract) (Arafa, 2002).

Partition and fractionation-Both organic and aqueous extracts were partitioned through liquid-liquid extraction using hexane, dichloromethane, ethyl acetate and n- butanol for organic extract and by ethyl acetate and n-butanol for aqueous extract.

Fractionation was carried out using column chromatography packed by silica gel or sephadex LH-20 and the elution was achieved in a slow rate, using methanol followed by increasing concentration of water (Arafa, 1998).

Purification-The bioactive fractions were purified through column chromatography packed by Sephadex LH-20 (Arafa, 2003).

Bioassay-Antibacterial assay were carried out according to standard method of Bauer *et al.*, 1966 against four bacterial strains, two of them are Gram positive bacteria, *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 6538. The other three are Gram negative; *Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 27853.

Antifungal assay were carried out according to Daw *et al.*, 1994 and Rasooli and Razzaghi 2004 against two fungal strains, *Penicillium*

citrinum ATCC 10499 and *Mucor rouxii* ATCC 24905. In this methods. the fungal growth inhibition was calculated by considering the controls' and samples' mycelial dry weights.

GC/MS investigation-GC/MS analysis was carried out by GC/MS [Finnigan Mat SSQ 7000 mass spectrometer coupled with a Varian 3400 gas chromatogram according to Sharaf, *et al.* (2000).

RESULTS and DISCUSSION

The investigation of bioactive natural products has, in recent years, assumed a greater sence in response to the expanding human population and it is subsequent demands for food, good health and increasing areas of land and on which to live (Steven, and Russel, 1993).

Conducts research remains one of the main means of discovering bioactive compounds from wild plants (Block 1989). Natural product chemistry has always been concerned with nature and natural phenomena and, as a consequence bioligically active plant secondary products (Arafa, 2004).

The results obtained from this study revealed that only, aqueous extracts of each plant showed antibacterial activity against all tested Gram negative and Gram positive bacteria. Both organic and aqueous extracts failed to show antifungal activity. This may be due to the chemical composition of the extractable, which in turn had a selective inhibition effect which might linked with type of the microorganisms.

Partitioned aqueous and organic extracts of each plant were re-examined against the bacterial tested organisms. The results indicated that the ethyl acetate fraction of both aqueous and organic extracts of *Heliotropium diygnum* and *Zilla biparmata* showed the most potent antibacterial effects against *B. subtilis*.

Each bioactive fraction was fractionated through column chromatography packed by silica gel. Ethyl acetate fraction of *Heliotropium diygnum* and *Zilla biparmata* gave 5 subfractions; each one was examined against *B. subtilis*. Only one sub-fraction showed the strongest inhibition of *B. subtilis* growth and then subjected to a successive purification process using column chromatography (CC) packed by Sephadex LH-20.

Two sub-fractions of *Heliotropium diygnum* showed significant antibacterial activity and both of them refractioned to give 5 and 6 extra-subfractions successively and re-examined against *B. subtilis*. The two

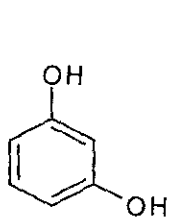
bioactive extra-subftractions of *Heliotropium diygnum* were gathered in only one fraction because they have the same R_f value.

Through different successive fractionation, purification and bioscreening through bioactivity guided separation techniques; the bioactive natural products of two investigated plants were isolated. The isolated bioactive compounds were purified using column chromatography packed by Sephadex LH-20.

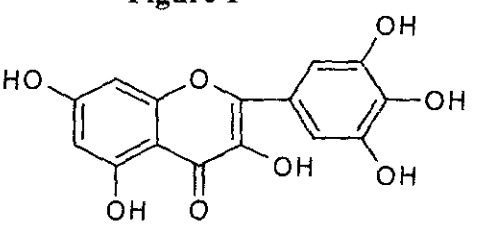
GC/MS analysis was carried out by injection the pure bioactive fraction onto GC/MS [Finnigan MAT SSQ 7000 mass spectrometer coupled with a Varian 3400gas chromatography]. The injection temperature was 250 °C using DB-5 column (5% phenyl) methyl polysiloxane, 30 m, I.D. 25 mm, helium was the carrier gas, with programmable temperature 50 °C, 3 min; 50-300 °C, 5 min; 300 °C , 15 min. The mass spectra were recorded in Elmode at 70 ev. The replication rate was 0.5 scan over a mass rang of 40-500 amu.

Peaks were identified by computer search of use-regenerated reference libraries and incorporation mass spectra. Peaks were examined by single-ion chromatographic reconstruction to confirm their homogeneity; mixed peaks were resolved by computer program aimed at resolving the mass spectral data of one compound from over lapping mass spectra of another. The two bioactive phytochemicals compounds responsible about growth inhibition of *B. subtilis* were identified through GC/MS spectroscopy as myricetin (Flavonoids compounds) and resorcinol (metahydroxy phenol) (HO-C_6H_4-OH), (Figure 1).

Figure 1



m-Hydroxyphenol



Myrcetin(3,5,7,3\',5\',7\'-pentahydroxyflavone)

B. subtilis inhabits soil, water, skins and respiratory and intestinal tracts and they have a great environmental importance (Tortora *et al.* 1995). So, the result of this screening of two plant extracts for antibacterial activity in combination with chemical identification of bioactive substances may provide new chemical analogues which may be used for controlling the growth of some undesirable bacterial strains (*B. subtilis*) at environments.

Briefly, the result of this study proves that antibacterial activity of investigated plant extracts and pure substances have an ecological value. Because its playing a role in inhibition of *B. subtilis* grwoth as environmental pollutants microorganisms.

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