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SCRENNING OF *PLANTAGO MAJOR* ORGANIC EXTRACTS AGAINST SOME GRAM POSITIVE AND GRAM NEGATIVE BACTERIA

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ABSTRACT :

The bioactivity-guided separation technique of organic extracts is described. Ethyl acetate fraction crude extract showed most potent antibacterial activity. Through fractionation and purification of ethyl acetate fraction using column chromatography (CC), the semi-pure and pure active fractions were collected and biologically evaluated and identified as Luteolin-7-O-glucoside using Co-chromatography and Ultraviolet spectrophotometer.

INTRODUCTION :

The genus Plantago L. is the largest genus of the family Plantaginaceae with about 265 species [1]. Tackholm [2] described 21 Plantago species, which were distinguished on the basis of characters of stem, leaves, bracts, spike, corolla lobes and habit. *Plantago major* (Lissan-Elhamal) is a seasonal herbaceous plant grown in cultivated areas and road banks in Egypt. It's tall glabrous or minutely pubescent herb with narrow green spikes. Many flavonoid compounds were isolated from *Plantago* species as apigenin, luteolin, scultellarein-7-glucoside, hispidulin-7-glucoside. 5,7,4',5'-tetrahydroxyflava-none -3'-O-glucoside was also reported as being present in *P. major* [3].

According to the World Health Organisation (WHO), a medicinal plant is defined as any plant which contains substances that can be used for therapeutic purposes or which are precursors of chemopharmaceutical semisynthesis [4].

In African traditional medicine, *Plantago lanceolate* used as expectorant, laxative, antibacterial and for treatment of colds and asthma [5]. In Egyptian folk medicine, the leaves of *P. major* used for treatment of the injuries and the hot extract of shoot system treated stomach troubles and malaria [6].

The open air-dried powdered leaves and inflorescences of *P. major* was extracted with 70% ethanol. The concent-

rated extract was partitioned with n-hexane, methylene chloride, ethyl acetate and n-butanol. Antibacterial activity of both crude organic and partioned extracts was carried out against *Bacillus subtilis*, *Staphylococcus aureus* as Gram-positive bacteria and *Echerichia coli* & *Pseudomonas aurginosa* as Gram-negative bacteria. The primary bioassay indicated that ethyl acetate extract exhibited the strongest antibacterial activity than other crude and partitioned extracts.

The present study deals with antibacterial activity of crude and partitioned organic extracts. Through the bioactivity-guided separation techniques, a trial was made to isolate the bioactive natural products of *P. major*.

MATERIALS AND METHODS :

1-Plant material. Fresh leaves and inflorescences of *P. major* were collected from El-Fayoum at March, 22, 1992. It was open air dried at room temperature and then powdered. The powdered samples packed in plastic bags until using.

2-Preparation of organic extract and fractions. One hundred grams of dry powdered leaves and inflorescences of *P. major* were percolated with 70% ethanol (crude organic extract). The yield extract was partitioned using n-hexane, methylene chloride, ethyl acetate and n-butanol. Each fraction was evaporated under reduced pressure and kept under refrigeration until further antibacterial investigation [7].

3-Fractionation. Active crude extracts were fractionated using open column chromatography (CC) packed by Silica gel. Elution was carried out at slow rate using methanol followed by increasing concentration of water. Each fraction (150 ml) was biologically investigated after evaporation of solvents under reduced pressure. Several consecutive fractions were carried out before reaching pure compound [8].

4-Antibacterial assay. In vitro, all extracts and fractions were monitored for antibacterial activity by diffusion method against *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus subsp. aureus* ATCC 5638 as Gram-positive bacteria and *Escherichia coli* ATCC 8739 and *Pseudomonas areuginosa* ATCC 27835 as Gram-negative bacteria. Antibacterial activity was determined using diffusion disc assay (10 mm S&S filter paper 3740-E) according to Bauer [9]. The plates with bacteria were incubated overnight at 35°C. After the period of incubation was terminated the radii of either the inhibition zones or intermediate zone were measured in mm. Each bioassay was carried out together with the untreated blanks as acontrol (Solvent only).

5-Acid hydrolysis. Complete acid hydrolysis was carried out for 40-50 minutes at 100 ^{o}C using 2N hydrochloric acid. The hydrolysate was then extracted with ethyl acetate, and the extracts subjected to paper chromatographic investigation to detect the aglycones. The mother liquor was carefully neutralized, and then subjected to paper chromatographic studies to detect the sugars [10].

RESULTS :

Results of bioassy indicated that crude and partitioned fractions (except n-butanol extract) of *P. major* showed significant antibacterial activity against most tested bacterial species (Table 1). The most potent partition extract against *E. coli* and *B. subtilis* was ethyl acetate fraction.

Ethyl acetate fraction (100 mg) gave 5 sub-fractions through open column chromatography. Bioassays of sub-fractions were carried out using 4 mg/disc against *E.coli* and *S. aureus* (Table 2). Sub-fractions 1, 2, 3 & 4 failed to show any antibacterial activity against the previous two species respectively. Sub-fractions 4 and 5 exhibited moderate antibacterial activity and sub-fraction 2 was found to have the strongest antibacterial activity against *E.coli*. Also, sub-fractions 1, 3 and 5 showed moderate antibacterial activity to *S. aureus*.

The purification of sub-fraction 2 was carried out using open column chromatography (CC) packed by Sephadex LH-20. Identification was carried out through Co-chromatography with authentic samples of Apigenin, Luteolin, Kaempferol, Quercetin and Luteolin-7-O-Glucoside (Apin Chemical LTD, Oxon, UK) as Luteolin-7-Glucoside (Figure 1), (R_f= 0.36 using silica plates and n-butanol: acetic acid: water (4:1:5). Identification was confirmed using UV, and the spectral data obtained as the following (MeOH) λmax 260,355; +NaOME:263,300,394; +NaOAc: 260,365$_{sh}$, 400; +NaOAc/H_3BO_3: 260, 372; + $AlCl_3$: 274,328,432; +$AlCl_3$/HCl: 273, 358,387 nm. The obtained data were compared to those reported for Luteolin-7-O-Glucoside and were found identical [11]. Pure compound was biologically evaluated against *E. coli* and *S.* aureus and it showed a good antibacterial effect.

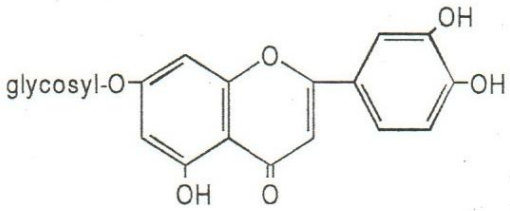
Table (1): Antibacterial activity of crude and partitioned organic extracts (2mg/disc) against four bacterial species. Measurements of inhibition zones were in mm.

Extracts	*B. subtilis*	*S. aureus*	*E. coli*	*P. areuginosa*
70 % ethanol	17.00	-Ve	11.00	16.00
n-hexane	15.00	14.00	-Ve	10.00
Methylene chloride	12.00	11.0	9.00	-Ve
Ethyl acetate	18.00	17.00	20.00	11.00
n-butanol	-Ve	-Ve	-Ve	-Ve

Table(2): Antibacterial activity of ethyl acetate sub-fractions (4 mg/disc) against two bacterial species. Measurements of inhibition zones were calculated in mm.

Sub-fractions	*E. coli*	*S. aureus*
1	-Ve	15.00
2	20.00	-Ve
3	-Ve	11.00
4	12.00	-Ve
5	13.00	10.00

Figure (1) : Structural formula of Luteolin-7-O-glucoside



DISCUSSION :

Higher plants are still regarded as potential sources of new medicinal compounds. Worldwide, plants are used traditionally to treatment many ailments, particularly infection diseases, such as diarrhea, fever and colds, as well as for the purposes of birth control and dental hygiene [12]. In addition , many psychoactive substances used in traditional medicine are of plant origin [13].

Numerous secondary compounds are fungicides or antibiotics protecting plants from fungal or bacterial invasion. Also, large numbers of these plant products are toxic to animals or insects. Those, such as alkaloids and carcinogenic glycosides that also have a bitter taste act as feeding deterrents [14].

In my laboratory program of screening of Egyptian plants for biologically active natural products. The screening assay of organic extracts was carried out against some Gram-negative and Gram-positive bacteria. The results showed that n-butanol fraction failed to show any antibacterial activity against the tested species (*B. subtilis, S. aureus subsp. areus, E. coli* and *P. areuginosa*). Other fractions (70 % ethanol & n-hexane) fractions exhibited slightly significant activity against the tested bacteria. Otherwise, both of them failed to exhibit activity against *E. coli*. Methylene chloride fraction showed almost weak antibacterial activity against all tested organisms except *P. areuginosa*. Only, ethyl acetate fraction exhibited a significant activity against all tested bacterial species.

Fractionation of the most potent fraction (ethyl acetate) using column chromatography (CC) packed by Silica gel afforded 5 sub-fractions. Each sub-fraction was biologically evaluated against *E. coli*. The most potent sub-fraction was number 2 which was then purified through CC packed by Sephadex LH-20.

Identification of pure sub-fraction (No. 2) was carried out using Co-chromatography with authentic samples as Luteolin-7-glucoside (5,3'-4'-trihydroxy-flavone-7-O-glucoside). The identification was confirmed by UV spectroscopy. The presence of 4-hydroxy group in the isolated flavonoids was established by the bathochromoc shift and hyperchromic effect of NaOMe. In Luteolin7-glucoside, the ortho-hydroxy system (3'-4') was indicated by the hypsochromic shift by $AlCl_3$ /HCl as compared to the band position in $AlCl_3$ and by bathochromic shift in $NaOAc/H_3Bo_3$. The position of sugar residue in luteolin glucoside was deduced from the lack of effect of NaOAc addition, indicating that the 7-OH group is not free.

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دراسة استقصائية لتأثير المستخلصات العضوية لنبات لسان الحمل على البكتيريا موجبة وسالبة الجرام

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فى الطب الشعبى المصرى تستخدم أوراق نبات لسان الحمل فى علاج كثير من الأمراض مثل : التهابات الجروح والتهاب الأذن البسيط منها والمتوسط ، وعلاج الأمراض الصدرية مثل : السل والسعال الديكى والربو ، وكذلك لمعالجة سوء الهضم الناتج من اضطرابات المعدة والكبد ، ولطرد الديدان المعوية ، ولمعالجة التهابات المثانة.

ولقد عزلت عديد من المركبات الكيميائية الفلافونية والجلكوسيدية من نبات لسان الحمل مثل : ابيجين ، ليتيولين ، ليتيولين–٧–جلوكوسيد ، تريسين ، كريزورول ، هيسبديولين–٧–جلوكوسيد والايكوبين جلوكوسيد ، والمركب الأخير مضاد للإسهال ويسبب تجلط الدم.

مما سبق يتضح مدى ثراء نبات لسان الحمل بالمركبات الكيميائية النباتية الأصل ذات النشاط الحيوى ، وكذلك مدى استخدامه فى الطب الشعبى المصرى ؛ ولذلك تم استخلاص المكونات الكيميائية الموجودة فيه باستخدام ٧٠٪ كحول ايثيلى (المستخلص العضوى) ، ثم جزى هذا المستخلص باستخدام بعض المذيبات العضوية الأخرى مثل الهكسان وكلوريد المثلين وخلات الاثيل وكحول البيوتانول ، ومن ثم دراسة النشاط الحيوى لهذه المستخلصات.

ومن خلال أسلوب النشاط الحيوى الموجة تم دراسة النشاط الضد بكتيرى لهذه المستخلصات باستخدام بعض السلالات البكتيرية مثل *Bacillus subtilis*, *Staphylococcus aureus subsp. aureus*, *Escherichia coli* and *Pseudomonas areuginosa.*

وقد أظهرت النتائج أن مستخلص خلات الاثيل ذو نشاط ضد بكتيري مميز جدا، بينما أظهرت المستخلصات الأخرى نشاطا متوسطا أو ضعيفا. ولذلك تم عزل المكونات الكيميائية الموجودة فيه باستخدام عمود الفصل اللونى فأعطى خمسة تحت أجزاء.

ومرة أخرى تم إجراء النشاط الضد ميكروبى لهذه الأجزاء الخمسة لمستخلص خلات الاثيل ، وتم تنقية الأجزاء الفعالة بيولوجيا باستخدام عمود الفصل اللونى ، ومره أخرى تم دراسة النشاط الضد بكتيرى لها ، وتم تعريف الجزء ذى أقوى نشاط ضد ميكروبى باستخدام كروماتوجرافيا المقارنة ومطياف الأشعة فوق البنفسجية على أنه ليتولين–٧–جلوكوسيد.