A Private Issue Concern with the Works of the Second Scientific

Antimicrobial activity of total lipids extracted from Anchusa strigosa Lab.

Al-Salihi, F.G.* , Al-Ameri, A.K.** , Al-Juobory, T.S.***

* Department of Chemistry, College of Education for Women, Tikrit University
** Department of Biology, College of Education, Tikrit University
*** Department of Chemistry, College of Science, Tikrit University

Summary:

In this study, the total lipids of *Anshusa strigosa* L. dry flowers was extracted, and its constituents of phosphatidyl serine, phosphatidyl ethanol amine and tripalmitin were isolated by the two-dimensional thin layer chromatography.

The free fatty acids present in the extracted total lipids were estimated qualitatively and quantitatively by gas-chromatography technique.

The extracted total lipids were found to be active against different strains of bacteria, and the antibacterial effect was significant at different concentrations of the extracted total lipids. It was similar to the effect of different antibiotics on the bacterial growth.

Introduction

The first medicines were presumably discovered by accident, when prehistoric man realized that certain plants relieved pain or helped to heal wound. Gradually, what had at first been learned haphazardly by trial and error became an organized body of knowledge; herbals[(1)].

The ancient Iraqi people knew and used over 250 medicinal remedies and understood how much they could be supported by sound diet and good hygiene, one of these important plants was *Anchusa strigosa* Lab. (Boraginaceous) which called Lisan-el-thor in Arabic, and its dry flowers which are used locally for curing many kinds of diseases [(2)] .

In previous papers, we reported on the antibacterial activity of the water-extract and a pure proteins isolated from the water extracted dry flowers of this plant against different strains of bacteria. [(3,4)] . Ahmed et al. (1998) has reported that the aquons root extract of *Anchusa strigosa* Lab. was effective against gastric- ethanol- induced ulcer in laboratory animals, and that activity was related ti the presence of anchusin (a red- brown resinoid coloring material) [(5)] .

Later six new pyrrolizidine alkaloids and glycoside compounds have been isolated from *Anchusa strigosa* roots, and its antifeedant activity was investigated. [(6)]

Now, in this work and as apart of our effort to identify the substances responsible for the pharmacological activities attributed to *Anchusa strigosa*, which utilized in Iraq in popular medicine. We have studied the antibacterial activity of the lipid extracted from the dry flower. *Anchnsa strigosa* Lab.

Experimental

Plant Materials

Anchusa strigosa Lab. Flowers were collected from Tikrit university campus. The plant were identified in the herbarium of College of Science, Mosul University.

Extraction of total lipids

10gm of the dry flower was extracted with n-n-hexane (100ml), using soxhlet apparatus for 24 hours . The organic solvent was evaporated by rotatory evaporator, and the residne was considered as percentage of total lipids.

The lipid constituents have been separated from the total lipid extracted above by the two-dimensional Thin layer chromatography technique, with silica gel-G plate . The solvent in the first direction was chloroform : methanol : acetic acid : water (170: 25: 25: 4 ml), and that in the second , chloroform :methanol: 7M

NH_4OH (65: 30: 4) . The plate was dried and sprayed with 50% H_2SO_4 and then following by heating the plate at 100 °C to detect the spots .

Fatty acids analysis

0.1 gm. of the total lipids extracted above was dissolved using a 1:1 mixture of benzene and methanol, then the extracted suponified for two hours with a solution of 4N KOH in 1:1 methanol: benzene mixture.

The free fatty acids were released by acidifying the saponifical materials with 6N HCl to PH=2.0 and extracted with petroleum ether . The acid extract was then methylated by a solution of 14% BF_3 in methanol .

Analysis of fatty acid were carried on a perk in-Elmer sigma 300 capillary gas chromatography, with flame ionization detector (FID) using sp2100wcoJ column 25m . with He as a carrier gas (1.5ml/min), split less injection . Temperature program from 50 °C for 30 min. at rate 4°C/min .

Assay for antibacterial testing

The antibacterial activity of the lipid constituents against different bacterial strains was investigate of by a disc diffusion method . Petri plates containing 20ml . of nutrient agar medium were sealed with old cultures of the bacterial strains .

The lipid extract fraction was tested in concentration (0.01-10mg/ml) applying 10ml of each sample of to sterile filter paper discs (5mm in diameter) and placed on the surface of the medium.

The inoculums size was adjusted so as to deliver a final inoculums appnximately $5x10^7$ colony-forming units (CFU/ml) . Incubation was made at 37°C for 24hours. The assessment of antibacterial activity was based on the measurement of diameter of the inhibition zone formed around the disc.

Results and Discussion

The total lipid of dry flowers of *Anchusa strigosa* Lab. Were extracted by continuous sox let extraction and its percent was 4.42%.

The two-dimensional thin layer chromatography of the extracted total lipids showed the presence of two phospholipids types; phosphatidy ethanol amine and one triglyceride compound (Tripalmetin) as show in Fig. (1).

The free fatty acids present in total lipid extracted for the dry flowers of *Anchusa strigosa* Lab. Were estimated qualitatively and quantitatively by using Gas chromatography technique. The results were shown in Fig. (2). and Table (1).

Table (1) shows the presence of concentration of linoleic acid (4.6 ngm/100 gm dry weight) and palmitic acid (3.6ngm/100gmdry weight). These result was in good agreement with Iodine No. value we have already estimated for the

extracted total lipids [7.8]. This value of Iodine No. is related to coccunnt oil, which is rich in the fatty a cids: linoleic and palmitic acid [9] .

The antibacterial activity of the extracted lipid constituents against different bacteria strains, has been investigated as shown in table (2). This effect was significant at different concentrations of the extracted lipids (0.01-10mg/ml) and higher at Gram positive strains of bacteria in the sequence

Pseudomonas a eruginosa > streptococcus faecalis > staphylococcus anrense >Bacillus subtitles while the effect of Gram negative was in the flowing sequent proteins sp. > E. coli > Enterobacter sp. > Klebsiella sp. However, the effect of different antibiotics on the bacterial growth shows similar results to that of extracted total lipids, as shown in Table (3).

The biological and pharmacological activity of the medicinal plants is related to presence of different materials such as: alkaloids, glycosides, proteins of volatile oils, e.g. the antibacterial activity of *Tacoma stuns* L. is relented to the volatile oil (Laphacol)[10], *Mentha spicata* L. is dne to volatile oil (Carvone)[11]. Thymus Capital us L.is related to carvone as well[12], *foeniculum vnlgare* L. Mill is due to volatile oil (Anethol)[13] and the antimicrobial activity of *pucina granatum* fruits against methicillin-resistant staphylococcnc aurens strains was due to the ellagitanin punicalagin [14] . Hence, the investigating of the antibacterial effect of total lipids extracted, which is in consitent with our ealier observation , in which the antibacterial activity of water extract and a pure proteins isolated from dry flowers of *Anchusa strigosa* L. may provides an example of prospecting for new compounds, whicl may be effective agaist infections currently difficult to treat.

Table (1) : Fatty acids composition of total lipids extracted from dry flower of Anchnsa strigosa L.

C.atom	Fatty acids	µgm /100gm dry.wt
C_{14}	n-Tetradecanoic	0.6424
C_{15}	Pentadecanoic	0.7495
C_{16}	n-Hexadecanoic	3.6404
C_{17}	Heptadecanoic	1.2849
C_{18}	Octadecanoic	4.6040
C_{20}	Eicosanoic	0.7495
C_{21}	Heneicosapentanoic	0.6424
C_{22}	Docosanoic	2.1414

Table (2) : The antibacterial activity of total lipids extract for dry flower of *Anchnsa strigosa* L., as inhibition zone (mm.)

10 µg/ ml	**100 µg/ ml**	**500 µg/ ml**	**1000 µg/ ml**	**5000 µg/ ml**	**10000 µg/ ml**	**Bacteria strain**
0	1	3	6	8	10	Escherichia coil (Gr.-ve bacilli)
0	0	2	5	7	8	Enterobacter sp (Gr.-ve balilli)
0	1	2	3	6	8	Klebsiclla sp (Gr.-ve capsule forming)
1	4	7	9	11	15	Proteus sp (Gr.-ve bacilli)
4	9	11	14	18	22	Pseudomous aergin (Gr.-ve cocli)
1	4	5	5	6	8	Bacillus subtilis (Gr.-ve bacilli, spore formy)
3	4	5	8	11	16	Streptococcus faecalis (Gr.-ve cocli)
0	0	1	3	5	6	Streptococcus viri (Gr.-ve cocli)
0	0	3	4	6	7	Streptococcus epiderm (Gr.-ve cocli)
1	2	3	5	8	10	Streptococcus aurense (Gr.-ve cocli)

Table (3): The effect of standard antibiotics against bacterial growth, as inhibition zone (mm.)

Antibiotics / Bactcrial Strains	**E**	**K**	**TE**	**CP**	**NA**	**F**	**C**	**CR**	**AMP**	**P**
Escherichia coli	11	10	5	9	13	11	12	10	6	8
Enterobacter sp.	7	11	7	10	10	10	13	2	5	7
Klcbsiclla sp.	8	9	16	14	12	13	11	10	7	5
Proteus sp.	11	12	11	15	18	12	10	12	11	10
Ps. Acruginosa	10	8	6	12	13	17	16	14	12	6
Bacillus sp.	6	9	13	12	11	9	10	8	6	3
Staph. Aureus	4	11	8	17	7	10	9	10	9	7
Staph.Epidermidis	7	10	4	16	9	6	8	6	4	5
Strcpt. Sp.	12	9	10	16	8	12	9	6	10	7

P: Penicillin (10units); Amp: Ampicillin (10mg); CR: Carbenicillin(100mg);
C: Chloramphenicol (30mg); F:Nitrofurantion (300mg); NA:Nalidixic acid(30mg);
CP: Ccphalcxin (30mg.); TE: Tetracycline (30mg); K: Kanamycin (30mg);
E: Erythromycin (15mg)

References

1- Kalafallah, A.A.M. (1988) " Medicinal dramatic and poisonous plants in the Arab world " (Arab organization for agriculture development, Khartoum) pp. 160-161 "in Arabic" .
2- Chakravarty, H.L. (1976)" plant wealth of IRAQ" Vol. I (Ministry) of Agriculture and Agrarian reform, Baghdad) p.29.
3- Al-Hassan, I.A., Al-Salihi, F.G. and Al-Salihi, N.J. (1999)" *Anchusa strigosa* Lab., antibacterial activity and Biochemical studies" , Ibn Al-Haitham J. Pure and Applied sci., 10(1) , 62-70 .
4- Al-Salihi, F.G. (2000)" Isolation and purification of some proteins from *Anchusa strigosa* Lab. and study its biological activity",Tikrit J. of pure science, 6(1),105-117 .
5- Ahmad, M., Saleh, O. and Tamimi , G.M. (1998)" Effect of *Anchusa strigosa* root aqueous extract on gastric-ethanol-induced ulcer in laboratory animals", J. Enthnopharmacology, 60(3), 189-198 .
6- Braca, A., Bader, A., Sicilian, T. and Morelli, I. (2003)" New pyrolizidine alkaloids and glycoside *Anchusa strigosa*", plants Med, 69(9), 833-41 .
7- Harborn, J.B.(1984)" Phytochemical methods" 2nd ed., Chapman and Hall, Landon, New York.
8- Rois, J.L. , Recio, M.C. and Villar, A.J. (1988)" Antimicrobial activity of selected plants employed in the spanish mediterranean area". J. Enthnopharmacology, 23, 127.
9- Plummer, D.T. (1971)" An introduction to practical Biochemistry", McGraw-Hill Co, (NK), 1st ed., p, 362.
10- Lagrota, M.H., Wigg, M.D., Pereira, L.O. and fouseca, M.E. (1983)" The In vitro antimicrobial and antioxidant activities of the essential oil and methanol extracts of endemic *Thymus spathulifolius* " Rev. Microbiol. 14 (1), 21-26.
11- Rathee, P.S., Mirsha, S.H. and Kaushal, R. (1984)" In vitro antimicrobial activity and chemical composition of Sardinian Thymus essential oils " Indian J. pharm. Sci 44 (1), 8-10.
12- Vandenbrouke, C.O. and Leml., J.A.(1982)" Antimicrobial activity of aqueous and methanol extracts of Juniperus oxycedrus L. " J.A. planta medica 45(3), 188.
13- Risan, A.Z. (1994)" A study of some isolated contents of foenicnlum vulgar L. Mill (Umblliferae) and measuring their biological activities " M.Sc. thesis, college of science Basrah university.

14- Machada, T., Leal, I., Amoral, A.C., Santos, K.R., Silva, M.G. and Kuster, R.M.(2002)" Antimicrobial activity of *Viscum album* L. sub *Sp.Abietis* (wiesb) " J. Braz. chem. soc. 13 (5), 1-6.

Fig.(1) : chromatogram of total lipids found in n-hexane extract of dry flowers of *Anchusa strigosa* L., by two-dimensional chromatography on thin layer silica gel G-plate . solvent (1) : chloroform : methanol: acetic acid: water (170:25:25:4 ml), solvent (2): chloroform: methanol: 7M NH 404 (65: 30: 4 ml).

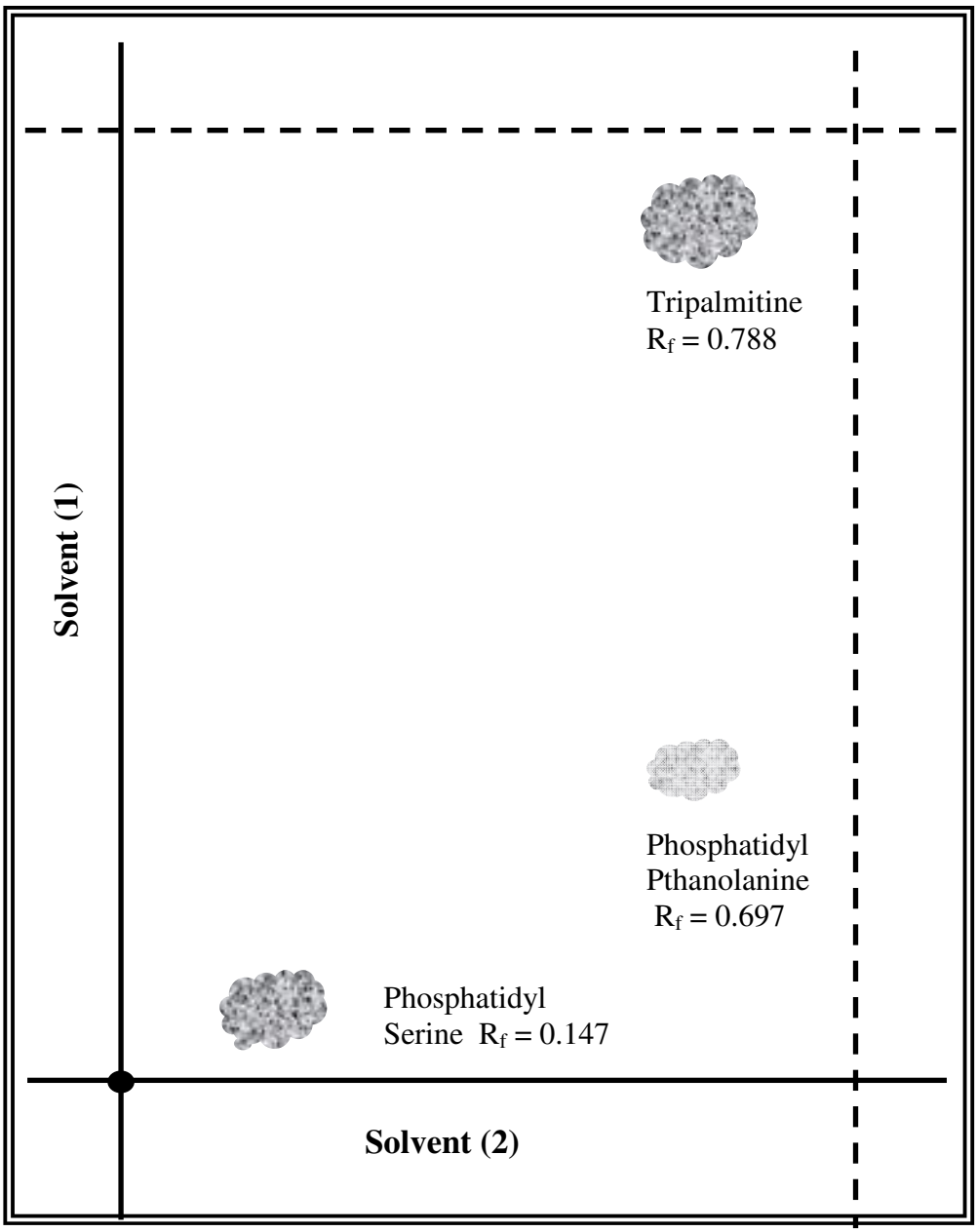
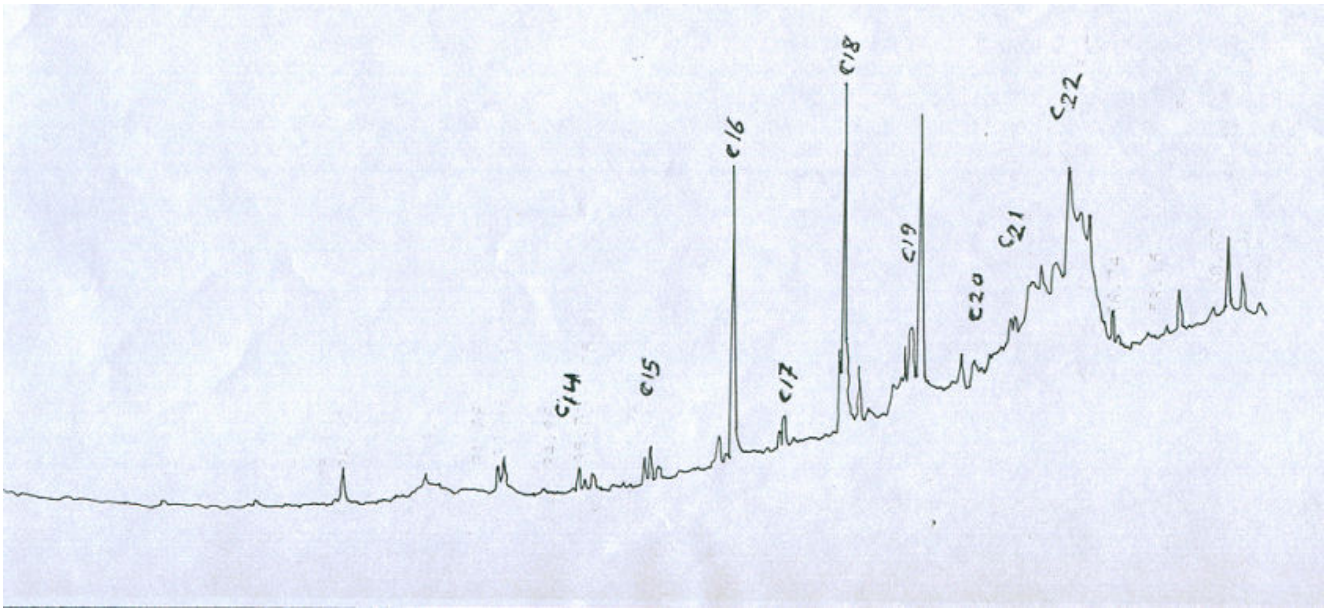


Fig. (2): Gas tiqnid chromatography separation of fatty acid methyl esters, using Sp 2100 wcoJ column 25 m.



الفعالية البايولوجية لليبيدات الكلية المستخلصة من ورد لسان الثور
Anshusa strigosa Lab.

فرام غالي الصالحي* ، احمد كمال الآمري ، طارق سليمان الجبوري*****

*** قسم الكيمياء/ كلية التربية للبنات – جامعة تكريت**
*** قسم علوم الحياة/ كلية التربية – جامعة تكريت**
*** قسم الكيمياء/ كلية العلوم – جامعة تكريت**

الخلاصة :

تضمن هذا البحث استخلاص الليبيدات الكلية من ورد لسان الثور، وعزل مكوناتها من فوسفوتيديل السيرين ، فوسفوتيديل الايثانول امين وثلاثي البالمتين بواسطة كروماتوغرافيا الطبقة الرقيقة ذات الاتجاهين. وتم ايضا تحديد مكونات هذه الليبيدات من الاحماض الدهنية الحرة نوعياً وكمياً من خلال تقنية الكروماتوغرافيا الغازية.

وشمل البحث قياس الفعالية البايولوجية لهذه الليبيدات المستخلصة ضد الاصناف المختلفة من البكتريا والتي اثبتت تاثيرها التثبيطي العالي ضد النمو البكتيري للعديد من الاصناف الموجبة والسالبة والذي يشبه تاثير العديد من المضادات الحيوية ضد النمو البكتيري.