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Isolation and Identification of Active Components in Some Plant Extracts and Their Effect on *Agrotis ipslon*(Hufn.)

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ABSTRACT

The impact of the two plant extracts of *Cymbopogon citratus* and *Rheum officinale* on the 2nd larval instar of the black cut worm, *Agrotis ipsilon* (Hufn). was evaluated. Three solvents: ethanol, ethyl acetate and hexane were used. The ethanolic extract of *C. citratus* and *R. officinale* were more effective than ethyl acetate extract. Hexane extract of *C. citratus* and *R. officinale* had no effect at all the tested concentrations. Also, isolation and identification of the active components of ethanolic extract of *C. citratus* and *R. officinale* were studied.

Key Words: Isolation, Identification, Active Components, Plant Extracts, Agrotis ipslon

INTRODUCTION

The black cut worm, *Agrotis ipsilon* (Hufn.) is a serious worldwide insect pest. In Egypt, it causes damage to seedlings of many winter and summer crops specially cotton, maize in addition to vegetable crops.

In Egypt, as in many countries, intensive studies have been carried out in search of ecological safe pesticides in particular among substances of plant origin as alternatives of the traditional pesticides.

Therefore, the aim of the present work is to study the effect of ethanolic extract of both *Cymbopogon citratus* and *Rheum officinale* on *A. ipsilon* larvae in addition to isolate and identificate of active components of each extract using GC/ MS, NMR and IR techniques.

MATERIALS AND METHODS

1. Plant materials:

a.Cymbopogon *citratus* (lemon grass) fresh leaves were obtained from El-Kanater, El Khairia farm. *b. Rheum officinale* was obtained as dried roots from local market Rubarb.

2. Extraction :

Hundred grams from each plant material was ground in a food grinder to a coarse powder, then extracted in soxhlet apparatus. Ethyl alcohol (96%), ethyl acetate (98%) and n-hexane (99%) were used. Each solvent was used at a rate of 2 ml/1gm plant material and for 8 hrs extraction period. Each solvent extract was evaporated to dryness under reduced pressure by a rotary evaporator of a water bath adjusted at 40°C-60°C. The residues were weighed and used to obtain different concentrations using diluent water. The concentrations tested were 5, 10, 20, 40 and 60%.

3. Isolation and identification of the active components of ethanol extract:

Thick layer chromatography (T.L.C) technique was used for separation of the ethanol extract of leaves of *C. citratus* and roots of *R. officinale*. Ethanol crude extracts of both plants were separated on T-L.C plates using solving system toluene/methanol (100/15 $^{v}/_{v}$).

The following techniques were used:

1- Thick layer chromatography T.L.C.: Is similar to column adsorption chromatography with the expection that it is perfarmed using a thick layer of absorbant spread eventy over a glass plate.
2- Infrarad spectroscopy (IR): Infrarad (IR) radiation refers broadly to the part of the electromagnetic spectrum between the visible and microwave regions.
3- Nuclear magnetic retention (NMR): Nuclear magnetic retention (NMR) is basically another form of absorption spectrometry a kin to IR or UV spectrometry.
4- Gas chromatography/mass spectrometry (GC/MS): Gas chromatography is similar to column chromatography except that a gas is used as the mobile phase is place of a liquid GC separation or based upon selective adsorption on a solid or upon partition between the gas and an immobile liquid phase.

Mass spectromoter is an instrument in which molecules of a substance are broken down into ions to the mass or mass-charge ratio.

4. Laboratory rearing of *A. ipsilon*

A colony of *A. ipsilon* was maintained since 1985 in the laboratories of Plant Protection Research Institute, Ministry of Agricultural, Dokki, Giza, without any insecticidal pressure. Couples of female and male moths were kept in a glass jars (9.5 cm diameter and 15 cm height) covered with muslin. In each jar 5-7 stripes of porous filter paper, each 3x10cm were hanged as ovipositing sites. Food was provided daily by using a cotton pad soaked with 10% honey solution. Laid eggs were daily collected and kept in a clean glass jar till hatching. Newly hatched larvae were individually reared in a plastic tray (10.8 × 22.7 cm.) containing 14 separated cells, each measured (5.2 × 3.2 × 3.7 cm). The cell had 5 small pages on its outer lateral walls for ventilation and its bottom was covered with a thin layer of saw dust. The trays were covered with transparent plastic sheets to avoid larval escape. Fresh castor oil leaves were cleaned and offered daily to the larvae till pupation. The pupae were placed in glass jars until adult emergence.

5. Larval treatment

Larvae were treated with plant extracts by (Leaf dipping tech.). Under laboratory conditions, castor oil leaves were dipped in the tested concentration for 1 minute and left to dry. For each concentration, fourty eight second instar larvae in four replicates were allowed to feed on the treated leaves for 2 days, thence the mortality was recored. Control experiment was carried out alongside treatments using water alone.

Mortality percentages were corrected according to Abbott's formula (Abbott, 1925). The data were then subjected to statistical analysis using computer to determine LC_{50} for each extract.

RESULTS AND DISCUSSION

Data presented in table (1) show that LC_{50} was 64-248 and 158.68 for *C. citratus* in ethanol and ethyl acetate extract. In case of *R. officinale*, LC_{50} was 91.029 and 851.88 in ethanol and ethyl acetate extract. Hexane extracts had no effect in all concentrations in both *C. citratus* and *R. oficinale* plant. So, the ethanolic extract of *C. citratus* leaves and *R. officinale* roots was the most toxic copmared to the other two solvent extracts. Similar results were obtained by Devaraj Urs and Srilatha (1990) who found that leaf extract of *C. citratus* had a repellent effect against the rice moth, *Carcyra cephalonica*. Also, Gupta *et al.* (1990) reported that *C. citratus* extract protected paddy against rice stink bug. Thus, ethanolic extract of both plants were selected for isolation and identification of the active components.

Table (1): Toxiciy data of the different extracts of *C. citratus* and *R. officinale* against 2nd larval instar of *A. ipsilon*

Solvent	Plant	LC_{50}	Slop
Ethanol	*C. citratus*	64.248	0.301 ± 0.153
	R. officinale	91.029	0.831 ± 0.251
Ethyl acetate	*C. citratus*	158.68	0.301 ± 0.153
	R. officinale	851.888	0.554 ± 0.274
Hexane	*C. citratus*	0.0	0.0
	R. officinale	0.0	0.0

R_f values of isolated active components of ethanolic extract of *C. citratus* and *R. officinale* were 0.3 and 0.8 which gave 100% mortality of *A. ipsilon* larvae after 1 day of treatment.

Preliminary phystochemical examination of the crude ethanol of *R. officinale* roots revealed the presence of alkaloids, tannins and glycosides. Similar results were obtained by Soad (1996) who mentioned that glycosides, alkaloids and tannins were existed in *piper nigrum* extract and they were effective against larvae of *A. ipsilon*.

Fractions isolated from ethanolic extract of *R. officinale* roots were mentiored for their activity against 2nd instar larvae of *A. ipsilon*. These fractions were physcion, chrysophenol, chrysophenol-antroo; their and aloe-emodin fig. (1).

Fractions isolated from ethanolic extract of *C. citratus* leaves by T.L.C were mentiored for their activity against 2nd instar larvae of *A. ipsilon*. These fractions were vobasan-17-oic acid, 3-oxo-, methyl ester; L, (IH)-pteridinone 2-amino 6-7 dimethy; 1,2 benzen dicarboxlic acid, 3-nitro and lumazine 8-ethyl 6,7-dimethyl are shown in Table (2) and illustrated in fig (2). The presence of amide group may be act as active

compounds. These results are in agreement with Su and Robert (1981) who isolated three amide which were effective against cowpea weevil.

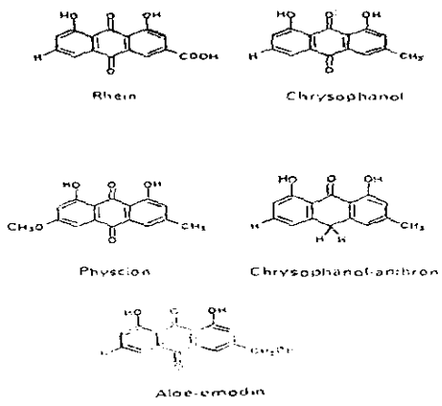

Fig. (1): The proposed chemical structure of the major components in *Rheum officinale* ethanol extract.

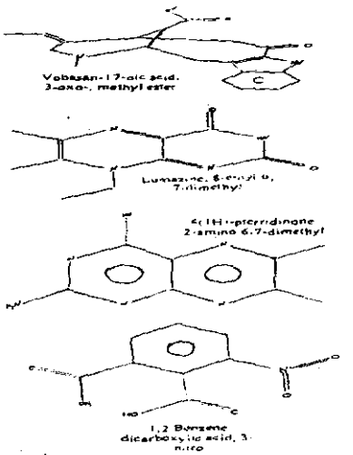

Fig. (2): The proposed chemical structure of the major components in *Cymbopogon citratus* ethanol extract

Table (2). Chemical structure of the major components in *C. citratus* ethanol extract :

Compounds	Molecular formula	M.W	Boiling point °C
Vobasan-17-oicacid, 3-oxo-, methyl ester	$C_{21}H_{24}N_2O_2$	352	180
4 (lH)-pterridinone, 2-amino 6-7-dimethyl	$C_{10}H_{12}N_4O_3$	191	192
1,2 Benzene dicarboxylic acid, 3-nitro	$C_8H_5NO_6$	211	149
Lumazine, 8-ethyl 6,7-dimethyl	$C_{10}H_{12}N_4O_2$	220	53

***R. officinale* extract:**

IR :

It is appeared from the I.R. (KBr) v max/cm^{-1} spectrum of *R. officinale* (ethanol extract) the presence of 3650 (free OH), 3425.3 (OH), 3150 (aromatic-CH), 2923.9, 2854.5, 2360.7 (aliphatic CH), 1725 (C = O), 1651 (C = C), 1465 (CH_2-bon), 1458.1, 1373.2 (CH_3-bond), 1095.5, 802.3 (= C-HOOP) .

NMR :

Spectrum of the compound is shown in fig. (3).

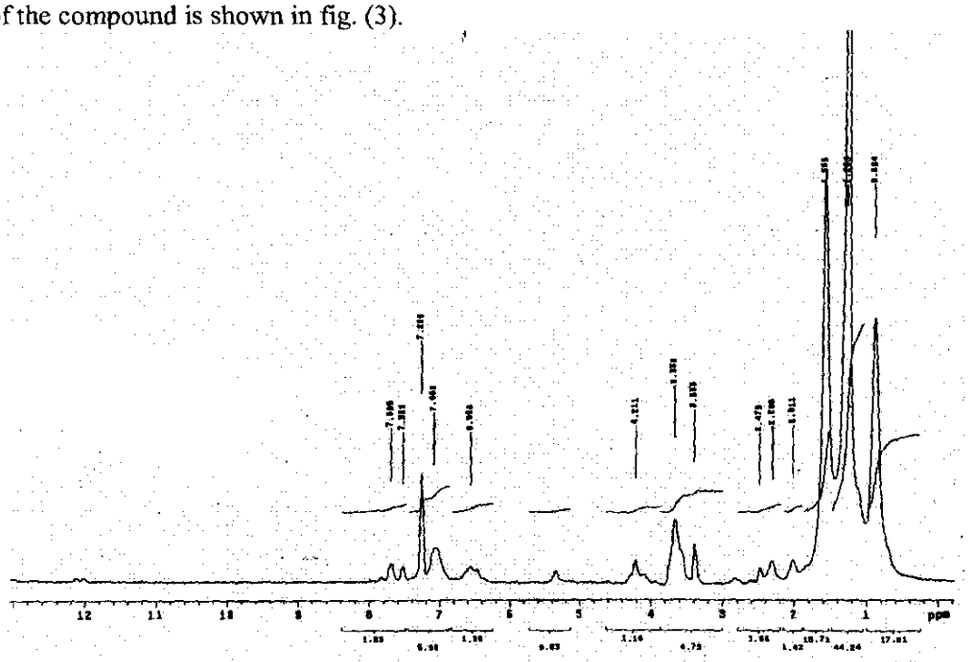

Fig. (3): Proton NMR spectrum of the active compound in *R. officinale* ethanol extract

MS:

Mass spectrum of the same extract is shown in fig. (4). Expected product m/z = 280 (M+). The exact molecular weight was determined by high-resolution mass spectrometry as 280 corresponding to molecular formula of $C_{15}H_8NO_6$. All these structure were confirmed by the National Research Center and Organic Chemistry Dept., Faculty of Science Cairo University. Fig. (1).

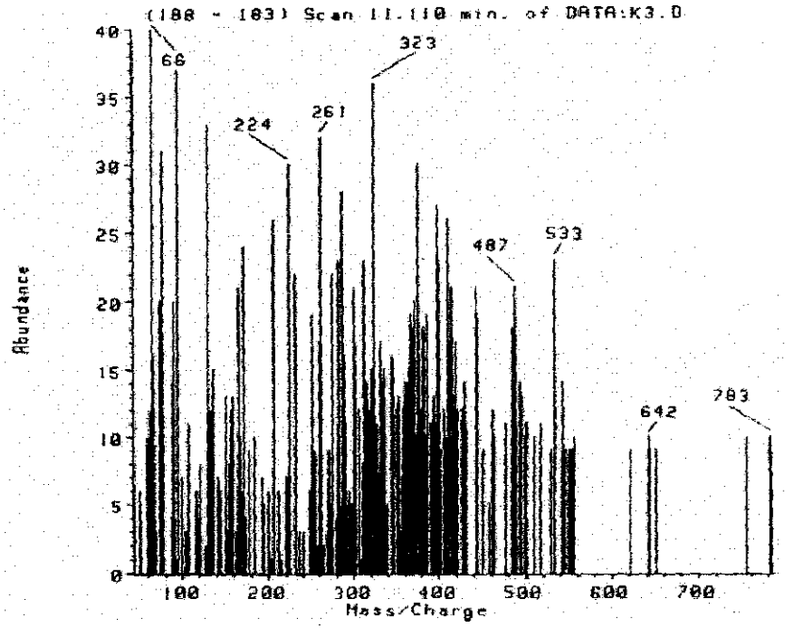

Fig. (4): Mass spectrum of the active compunds in *Rheum officienale* ethanolic extract

From all of these structures, the active components may be phenol or ketone or carboxylic. Similar results were obtained by Numata *et al.* (1983) who reported ketone derivatives found in *Osmunda Japonica* extract as antifeedant effect against larvae of the yellow better fly *Eurema hecabe mandarina.* Also, Butlery *et al.* (1984) reported that phenol derivatives found in *Trifolium protense* extract had attractant effect to bugs *Lygus spp.*

***C. citratus* extract:**

I.R :

The I.R spectrum of *C. citratus* ethanol extract. It is appeared from the I.R. (KBr) max/cm^{-1}: 3456.2 (NH, NH_2 or OH), 2923.9, 2864.5 (aliphatic CH), 165.0 (C = O), 1550 (NO_2, 1450 (CH_2), 1350 (NO_2), 1380.9 (CH_3), 1095.5,794.6 (= C-HOOP).

GC/MS:

The ethanol extract of *C. citratus* obtained by soxhelt apparatus, extraction of *C. citratus* was subjected to GC/MS analysis using GC conditions similar to that described. All MS were recorded in the electron impact (E.I) made at 70 e.v. The reconstructed iron chromatogram (RIC). Mass spectra of the major peaks are shown. From all of these structure the active component may be nitro or amino or terpene. Similar results were obtained by Chiu (1983) who reported that terpene found in *Celastrus angulata* extract had antifeedant effect to *Ostrinia furnacalis*.

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المكونات الفعالة لبعض المستخلصات النباتية وتأثيراتها على الدودة القارضة ***Agrotis ipsilon* (Hufn.)**

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تم دراسة بعض المستخلصات النباتية لأوراق نبات حشيشة الليمون وريزومات نبات الروند على العمر اليرقى الثانى للدودة القارضة · وقد أستخدمت ثلاثة مذيبات لعملية الاستخلاص وهى الاثيل اسيتات والهكسان وكان مستخلص الايثانول لكل من النباتين هو الأكثر تأثيراً· ولذلك تم فصل وتعريف المكونات الفعالة فى هذا المستخلص ودرس تأثير هذه المكونات على العمر اليرقى الثانى للدودة القارضة· وبالتحليل ظهرت مركبات Caroxlyic, Ketone Phenol لمستخلص ريزومات الروند و Amino, Nitro Terpene لمستخلص حشيشة أوراق الليمون كمركبات فعالة ضد الطور اليرقى الثانى للآفة·