

ANTIBACTERIAL ACTIVITY OF EXTRACTS,  
FLAVONOIDS AND ESSENTIAL OIL  
OF PULICARIA UNDULATA L.

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Different successive extracts prepared from the herb of Pulicaria undulata L. as well as flavonoid aglycones and essential oil were investigated for their antibacterial activity. Ether and ethyl alcohol extracts exhibited significant activity against both Staph. aureus and B. cereus. The four flavonoid aglycones tested showed variable significant effects against both Staph aureus and B. cereus. The lowest minimum inhibitory concentrations (MICs) on both organisms (22.4 and 1.8 µg/ml respectively) were met with aglycone A<sub>3</sub> (Rhamnetin).

The essential oil revealed more antibacterial activity against the tested organisms than benzyl penicillin and streptomycin sulphate.

The genus Pulicaria includes members which contain essential oils among other active constituents of important medicinal uses,<sup>1,2</sup>.

Karryew et al<sup>3</sup> mentioned the bactericidal and bacteriostatic effects of the extracts and essential oil of Pulicaria gnaphloides.

The essential oils obtained from Pulicaria gnaphloides, P. prostrata, P. uliginosa and P. salviaefolia possessed antibacterial properties and induced rapid healing when tested on infected rabbit's eye<sup>4</sup>.

Pulicaria undulata L. herb is used by the natives of some Upper Egypt's areas in the form of decoction as a substitute for tea and as a tonic drink.

In previous communications the authors reported the isolation and identification of some constituents of the material

under investigation<sup>5, 6</sup>.

In view of establishing the value of the extracts, flavonoids and essential oil of the plant under investigation, possibly as antibacterial agents, the following study was performed.

### EXPERIMENTAL

#### Materials:

- 1- Extracts: Crude successive petroleum ether, ether, chloroform and ethyl alcohol extracts were prepared from the total herb of *Pulicaria undulata* L.
- 2- Flavonoid aglycones: Four flavonoid aglycones designated A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub> and A<sub>4</sub> namely 7-methoxykaempferol (rhamnocitrin), 3,7-dimethoxyquercetin, 7-methoxyquercetin (rhamnetin) and dihydrokaempferol respectively, were obtained using the method of Bishay et al<sup>6</sup>.
- 3- Essential oil was prepared by steam distillation of the total herb using Egyptian Pharmacopoeia method<sup>7</sup>.

#### Determination of Antibacterial Activity:

The different successive extracts alongside with the pure corresponding solvents were tested for their antibacterial activity against Staph. aureus, B. cereus and E. coli, using the agar-cup plate diffusion method<sup>8</sup>.

The flavonoid aglycones were dissolved in dimethylformamide (DMF). Five mgs of each compound were dissolved in one ml of DMF, then five concentrations were prepared by two-fold serial dilution in the solvent. The obtained concentrations of each compound alongside with the pure DMF were tested on every organism and the minimum inhibitory concentrations (MICs) were determined.

The essential oil was tested for its antibacterial activity adopting the filter paper disc diffusion method<sup>9</sup>. The inhibitory effect of the oil was noted and compared with benzylpenicillin and streptomycin sulphate.

## RESULTS AND DISCUSSION

Investigation of antibacterial activity of different successive extracts (Table 1), showed that all extracts had no effect on E. coli. The ether and ethyl alcohol extracts exhibited the highest activity against Staph. aureus (19.17mm respectively) and B. cereus (14,18mm respectively).

However, the successive petroleum ether and chloroform extracts showed no significant effect on both organisms.

The antibacterial activity of flavonoid aglycones are shown in Tables 2, 3 and illustrated in Figs 1, 2 and 3, From Table (2) it is noticed that compounds A<sub>1</sub> and A<sub>2</sub> did not reveal any effect on E. coli. However, compounds A<sub>3</sub> and A<sub>4</sub> exhibited somewhat remarkable effects. Compound A<sub>4</sub> was more active than A<sub>3</sub>, where the MICs were 158 and 316 µg/ml respectively.

Concerning the antibacterial activity of the flavonoids on Staph. aureus and B. cereus, it was observed that compound A<sub>3</sub> produced the greatest effects on both organisms where the MICs were 22.4 and 1.8 µg/ml respectively followed by compound A<sub>2</sub> on Staph. aureus (50 µg/ml) and A<sub>1</sub> on B. cereus (158.5 µg/ml). Compounds A<sub>2</sub> and A<sub>4</sub> showed similar activity on B. cereus (316.2 µg/ml).

In general, the pure flavonoid aglycones showed more antibacterial activity than the extracts. The reduced activity of the extracts, specially petroleum ether and chloroform extracts, may be attributed to the organic constituents contained and which may inhibit the antibacterial activity of the flavonoid aglycones.

The antibacterial activity of the essential oil (Table 4) revealed similar effect to benzylpenicillin and streptomycin sulphate on both B. cereus and E. coli.

From the previous results it can be taken in consideration that P. undulata L. herb can be used as a good source for isolation of flavonoid aglycones and essential oil which

may play an important role in preparation of certain pharmaceutical products used for the treatment of staphylococcal infections and some types of food poisoning caused by B. cereus and enterotoxigenic staphylococci.

Table 1- Antibacterial activity of various successive extracts of P. undulata on different organisms

Extract in	Inhibition Zone in (mm)*					
	<u>Staph. aureus</u>		<u>B. cereus</u>		<u>E. coli.</u>	
	Exp.**	Control <sup>Δ</sup>	Exp.	Control	Exp.	Control
Petroleum ether	11	-	12	-	-	-
Ether extract	19	-	14	-	-	-
Chloroform extract	-	-	13	-	-	-
Ethyl alcohol	17	11	18	10	10	-

\* : Average of three observations. Diameter of the original cup = 9 mm.

\*\* : Inhibition zone produced by the extract

Δ : Inhibition zone of pure solvent.

Table 2--: Antibacterial activity of compounds A<sub>1</sub> , A<sub>2</sub> , A<sub>3</sub> and A<sub>4</sub> on different organisms

Conc. µg/ml	log C	Diameter of inhibition zone (mm) *															
		<u>Staph. aureus</u>				<u>B. cereus</u>				<u>E. coli</u>							
		A <sub>1</sub>	A <sub>2</sub>	A <sub>3</sub>	A <sub>4</sub>	A <sub>1</sub>	A <sub>2</sub>	A <sub>3</sub>	A <sub>4</sub>	A <sub>1</sub>	A <sub>2</sub>	A <sub>3</sub>	A <sub>4</sub>	A <sub>1</sub>	A <sub>2</sub>	A <sub>3</sub>	A <sub>4</sub>
5000	3.69	12	19	17	16	14	13	22	13	-	-	-	-	18	19	18	19
2500	3.39	11	18	16	14	12	12	21	12	-	-	-	-	17	18	17	18
1250	3.09	10	16	15	12	12	11	20	11	-	-	-	-	16	17	16	17
625	2.79	9.5	15	14	10.5	11	10	19	10	-	-	-	-	15	16	15	16
312.5	2.49	-	13	13	-	-	-	17.5	-	-	-	-	-	14	15	14	15

\* : Average of three observations.

N.B. Diameter of the original cup = 9 mm.

DME Showed no effect on Staph. aureus and B. cereus but it produced an inhibition of 14 mm on E. coli.

Table.3- : MICs of the isolated compounds(A<sub>1</sub>- A<sub>4</sub>) of *P. undulata* L. on different organisms.

Organism	MICs in µg/ml			
	A <sub>1</sub>	A <sub>2</sub>	A <sub>3</sub>	A <sub>4</sub>
<u>Staph. aureus</u>	631.00	50.12	22.40	446.68
<u>B. cereus</u>	158.50	316.23	1.80	316.23
<u>E. coli</u>	-	-	316.23	158.50

Table 4-: Antibacterial Activity of the Essential oil of *P. undulata* L. with reference to penicillin and streptomycin.

Organism	Zone of inhibition (mm)* caused by		
	Oil	S <sub>1</sub>	S <sub>2</sub>
<u>Staph. aureus</u>	23	24	28
<u>B. cereus</u>	24	16	17
<u>E. coli</u>	18	14	16

\* Average of three observations

S<sub>1</sub> Benzylpenicillin

S<sub>2</sub> Streptomycin sulphate. Diameter of the disc = 6 mm

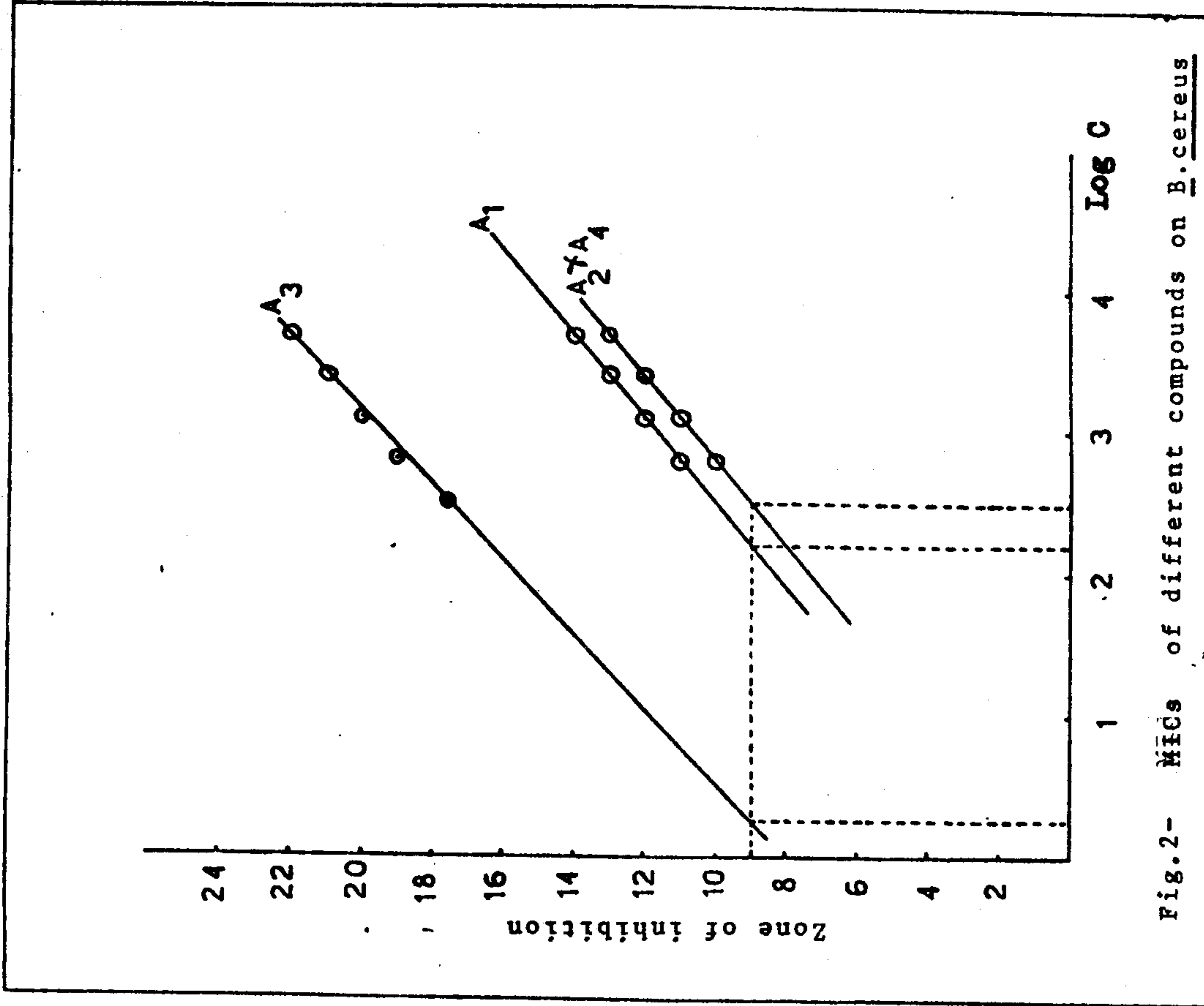


Fig.2- MICs of different compounds on *B. cereus*

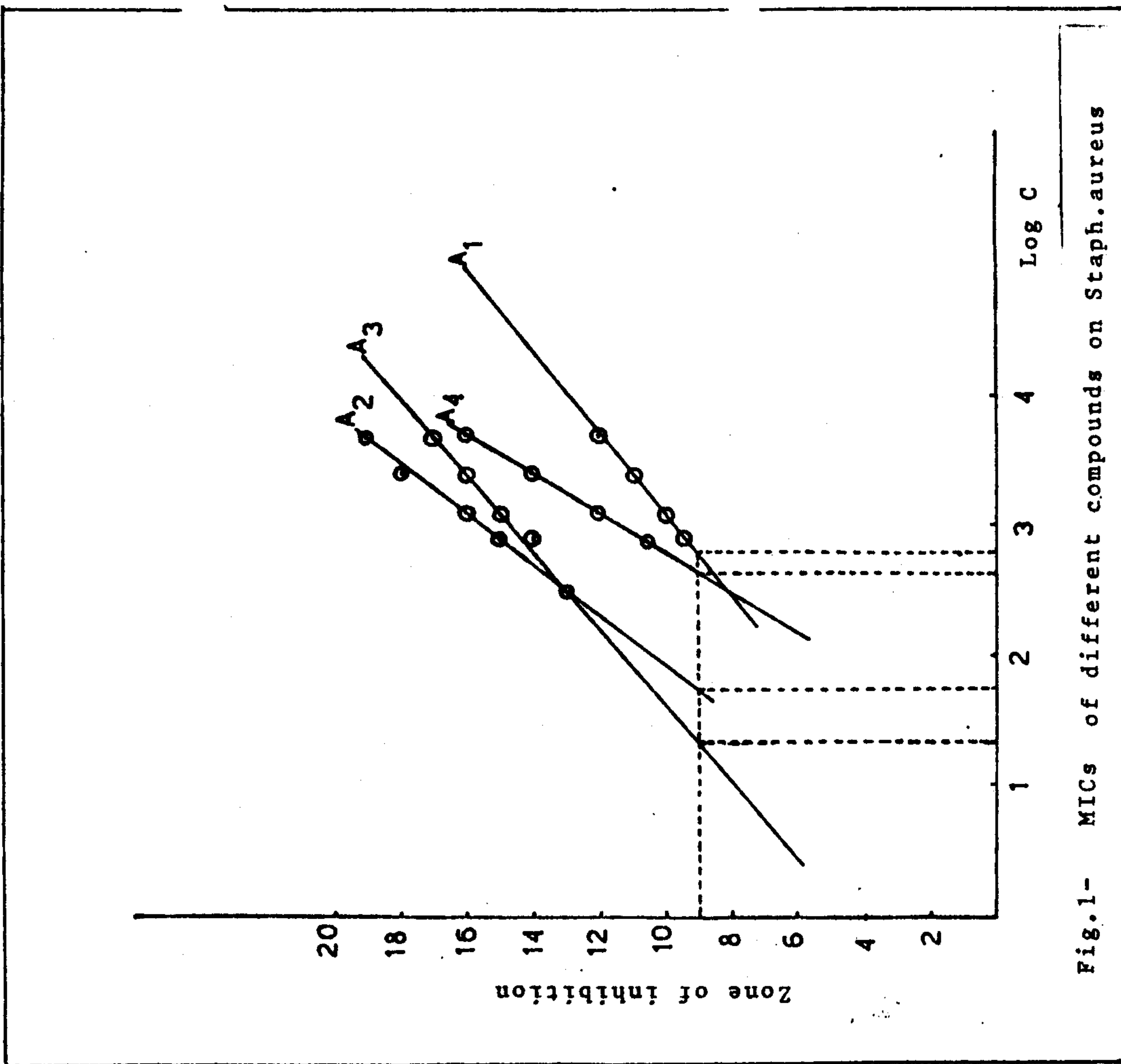
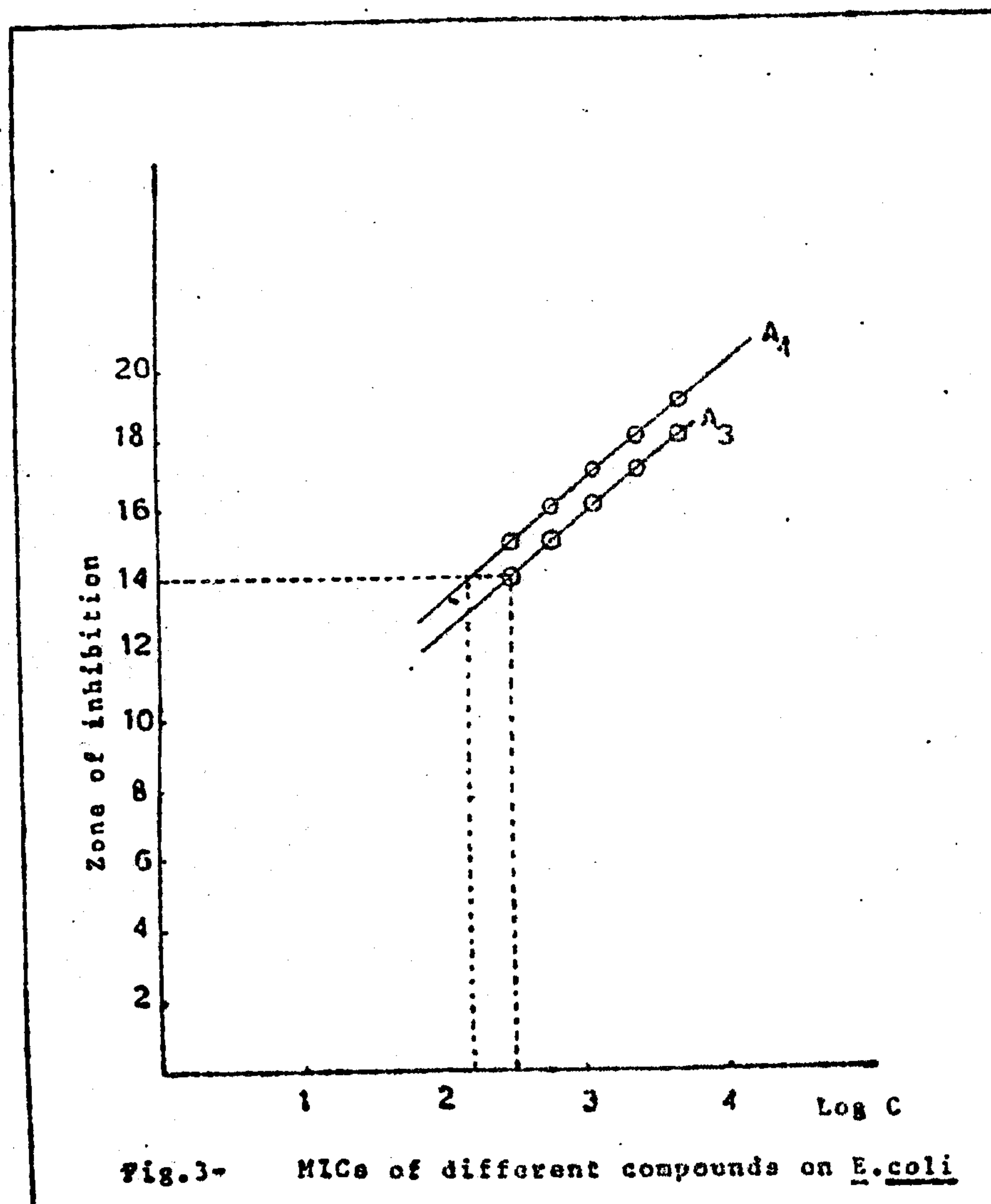


Fig.1- MICs of different compounds on *Staph. aureus*

*Antibacterial Activity Of Extracts Flavonoids  
And Essential Oil Of Pulicaria Undulata L.*





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دراسة التأثير البكتريولوجى لخلاصات وبعض المركبات  
 الفلافونيدية والزيت الطيارى لنبات الشاى الجبلى ( بوليكاريا انديولاتا )  
 داود ونيس بشاى - شعبان هاشم احمد - كاميليا سعيد جمعه - محمود حافظ  
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تسم فى هذا البحث دراسة تأثير بعض الخلاصات المحضرة بواسطة عملية  
 الاستخلاص المتعاقب للعشب بالمذيبات العضوية مثل الاثير البترولى - الاثير  
 الكلوروفورم والكحول الايثيلى .

وقد وجد أن خلاصتى الاثير والكحول الايثيلى لهما بعض التأثير  
 المضاد على نوعين من البكتريا .

وقد اظهرت الدراسة ايضا تأثير لبعض المركبات الفلافونيدية وان احداها  
 وهو مركب ( الرامنتين ) له تأثير واضح ومضاد لنوعين من البكتريا .

وقد اثبتت الدراسة ان الزيت الطيار لنبات الشاى الجبلى له  
 تأثير مضاد للبكتريا يشابه كثيرا تأثير البنسلين والستربتومايسين وقد  
 يفوق عنهما فى التأثير ضد نوعين من البكتريا المسببة للامراض