



## ANTITERMITE PRINCIPLES ISOLATED FROM THE WILD HERB, *PSORALEA PLICATA* Del.

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### ABSTRACT :

The total extract and seven compounds isolated from the indigenous herb *Psoralea plicata* ,Leguminosae ,were evaluated against *Amitermes desertorum* which causes wide range of damage for trees and woody household furniture in upper Egypt. The study revealed the promising activity of plicatin-B as termiticidal within one week. On the other hand, plicatin-B was proved to be potent antibacterial against the bacteria isolated from the gut of the termite , where the insect is dependable on digestion of its food.

### INTRODUCTION :

The wild herb *Psoralea plicata* Del. which is known in Arabic as مرميد "Marmeed" or "Mermeed", is commonly grown in Allaqi area south east of Aswan . The plant is widely eaten by grazing animals in the area for its palatable green leaves [1].

It has been used in folk medicine as skin photosensitizer, anthelmintic, antipyretic, analgesic, anti-inflammatory, diuretic, diaphoretic and it is also useful in bilious infections, in liprosy and in menstrual disorders [2,3] .

Phytochemical studies on the plant revealed the presence of interesting compounds including furocoumarins, chromenes ,isoflavonoids, phenolic cinnamates, cinnamate dimers, flavonoid glycosides, monoterpenoids and triterpenoids in addition to tocopherol [4-10].

The subterranean termite *A. desertorum* (Desneux), builds its nests underground or in tree hearts ,and attacks dead wood and live trees [11]. It has been reported that *A. desertorum* is a very adaptable species and has a wide distribution in Egypt, especially in arid

and semi-arid localities [12-14]. The same species has been recorded to infest living trees and shrubs in Aswan governorate [15].

From the current literature, plant extractives, have been bioassayed for one or more antitermitic effects including toxicity to the insect and/or gut symbionts, feeding deterrence, non-preference or repellency against one or more termite species [16-18]. The above mentioned facts arouse our interest to study the effect of *Psoralea plicata* extractives and isolated components against *A. desertorum*.

## EXPERIMENTAL :

### Materials and Methods :

#### Termites :

A colony of the termites was collected from infected *Ficus* tree at Kima area in Aswan city. Termite workers (externally undifferentiated insects beyond the 3rd. stage) from that colony of subterranean termite *Amitermes desertorum* (desneux) was used as test insect.

#### Extraction and isolation of plant constituents :

Air-dried aerial parts of *Psoralea plicata* Del. herb (2 kg), collected from Wadi Allaqi (Aswan, Egypt), were powdered and exhaustively extracted with 75% MeOH by maceration. The alcohol extract was concentrated under reduced pressure to a syrupy consistency (179 g). The solvent free residue (50 g) was mixed with 200 ml water and 100 ml MeOH, transferred to a separatory funnel and partitioned between hexane, ethyl-acetate and

n-butanol respectively. Each fraction was dried over anhydrous sodium sulphate and condensed to syrupy residue (10 g hexane residue; 3.6 g ethylacetate residue and 5 g n-butanol residue).

Each fraction was subjected to flash silica gel column chromatography, with hexane-ethylacetate gradient. Plicatin-B, angelicin, psoralen and chromene compounds were isolated from the hexane soluble fraction. Plicatin-A, p-dimethylcoumaric acid and  $\alpha$ -duplicatin-B were isolated from the ethyl-acetate soluble fraction and roseoside, daidzin (isoflavonoid), psoralic acid and isovitexin (flavonoid) were isolated from the n-butanol soluble fraction.

#### Isolation of the bacteria from the termite gut :

A culture medium was prepared by dissolving 31g of nutrient agar powder in 1 L. distilled water, autoclaved at 120°C for 15 min. Slands were prepared from this medium and inoculated by the organisms isolated from the termite gut by homogenization in sterilized distilled water. The slands were incubated at 30°C for 24 hrs and were investigated [19].

#### Antimicrobial effect of the isolated compounds :

Small discs of filter paper were immersed in different concentrations of the selected compounds, then placed in petri dish containing nutrient agar medium and inoculated with the isolated organisms and incubated at 30°C for 24 hrs, then the clearing zones were inspected.

### Force-feeding test :

A round plastic container of 5 cm in diameter and 3.5 cm in height was packed with 50 g of sterile sand moistened with 7 ml of distilled water to keep relative humidity near saturation. Five levels of seed extracts were tested (15, 30, 75, 150 and 300 ppm). Three replicates of small pieces of filter paper (3 cm in diameter) were dipped in the methanolic extracts for each level for a period of 10 sec., and dried at room temperature for evaporation of the solvent. The treated papers were set up on sand surface and 100 worker termites were added to each container. Untreated filter papers were fed to termites as a control. The containers were incubated at 25°C for 5 weeks and the number of surviving termites was recorded in each replicate.

Filter papers treated with two concentration levels of the isolated compounds of *Psoralea plicata* (75 and 175 ppm), were evaluated against termites and the number of surviving termites was taken at 2, 6, 10, 14 and 18 days respectively.

The results were statistically analysed and the means were compared according to both the t-test and Duncan's multiple range test [20].

## RESULTS AND DISCUSSION :

The first part of the present work shows the bioassay experiments of crude extract from *Psoralea plicata*, on feeding and survival of *A. desertorum*, which depends in its digestion on

some symbiotic organisms (either bacteria or protozoa) living in their gut.

The mean survival of *A. desertorum* feeding on filter papers treated with different levels (ppm) of crude extract of *Psoralea plicata* during 5 weeks is given in Table (1). It was observed that the mean survival was level and exposure period dependent. The termite lived approximately for two weeks only after feeding on two levels (75 and 150 ppm), and the remaining insects died before the end of the third week; but other levels (15 and 30 ppm), showed weak anti-termite effect and the mean survival was not significantly different during the same period.

Increasing the concentration of the plant extract to 300 ppm, does not significantly increase the mean of mortality relative to other concentrations (75 or 150 ppm) till the end of the second week, but during the third and fourth week, the mean survival values was greatly decreased and 100% mortality was observed by the end of the fourth week. On the other hand, it was observed that the termites avoid eating the papers with concentration level of 300 ppm, and hide under the sand.

Some of the isolated compounds from *Psoralea plicata* viz., plicatin B, flavonoids, psoralen, angelicin, isovitexin, chromene E,  $\alpha$ -duplicatin B and total extract were tested for anti-termite effect.

Data presented in table (2) showed the mean survival percentages of *A. desertorum*

feeding on filter papers treated with two concentration levels (75 and 150 ppm) of the isolated compounds during 18 days. Figures 1&2 showed that, plicatin B, was the most effective compound since it showed significant decrease in the survival of the termites after two days at the two applied levels. After six days of treatment, most of the termites are

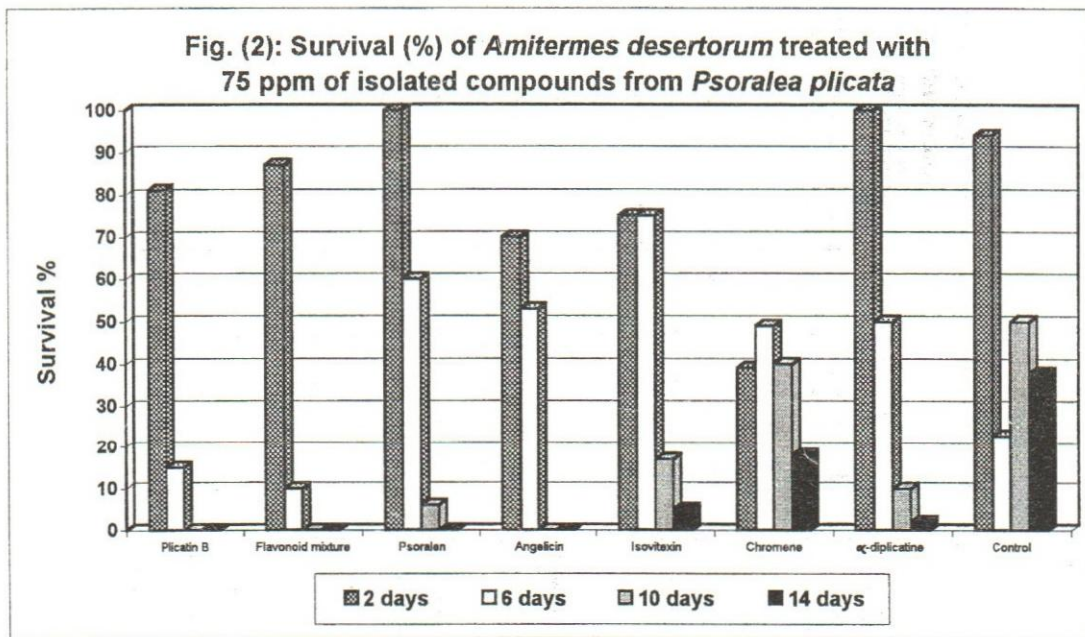
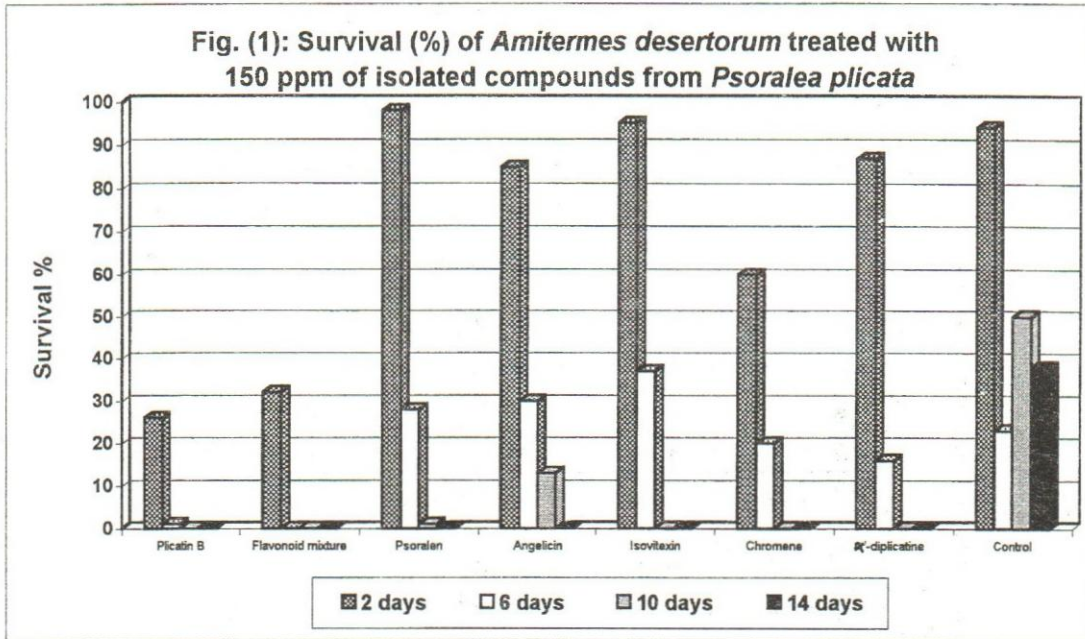
died at the level of 150 ppm, then no survival was recorded after 7 days at the same concentration. A clear reduction was noticed in the individuals size, probably due to the dehydration effect of the applied compound after two days of feeding ; at the same time the activity of the termites had greatly reduced at the same period.

Table (1) : Survival % of workers of *Amitermes desertorum* after exposure to *Psoralea plicata* extract for 5 weeks at five ppm conc. levels.

Level ppm	Exposure Week	Survival %					
		I	II	III	Total	Mean	±SD
0	1	100	100	100	300	100	0
	2	91	92	93	276	92	0.82
	3	68	87	81	236	78.7	7.93
	4	25	73	11	109	36.3	26.5
	5	6	7	1	14	4.7	0.66
15	1	98	91	86	275	91.7	4.92
	2	98	88	76	262	87.3	8.99
	3	75	71	62	208	69.3	5.44
	4	43	15	6	64	21.3	15.76
	5	1	2	1	4	1.3	0.47
30	1	98	81	97	276	92	7.79
	2	78	67	88	233	77.7	8.58
	3	12	63	65	140	46.7	24.53
	4	11	1	22	34	11.3	8.85
	5	5	0	7	12	4	-
75	1	12	11	13	36	12	0.82
	2	5	1	1	7	2.3	1.90
	3	0	0	0	0	0	-
	4	0	0	0	0	0	-
	5	0	0	0	0	0	-
150	1	35	18	17	70	23.3	8.26
	2	22	15	12	49	16.3	4.19
	3	0	0	0	0	0	-
	4	0	0	0	0	0	-
	5	0	0	0	0	0	-
300	1	100	88	87	275	91.7	5.91
	2	85	81	77	243	81	3.27
	3	6	23	11	40	13.3	7.13
	4	3	13	5	21	7	4.32
	5	0	0	0	0	0	-

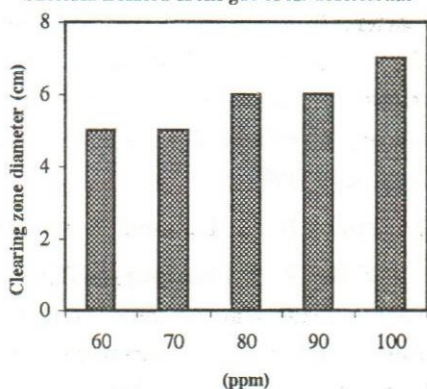
Table (2) : Survival % of *Amitermes desertorum* fed on filter papers with two concentrations (75 and 150) ppm of seven compounds and total extract.

Component's key	Component fraction name	Con.	Survival during 2 days			Survival after 6 days			Survival after 10 days			Survival after 14 days			Survival after 18 days																				
			R1	R2	R3	Total	Mean	R1	R2	R3	Total	Mean	R1	R2	R3	Total	Mean	R1	R2	R3	Total	Mean													
(1)	Plicatin B		26	25	27	78	26	1	3	1	5	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0							
(2)	Flavonoid mixture		32	97	18	147	49	0	0	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0						
(3)	Psoralen		98	99	97	294	98	28	20	37	85	28	1	0	0	1	0.3	0	0	0	0	0	0	0	0	0	0	0	0	0					
(4)	Angelicin	150	85	73	98	256	85	30	38	37	105	35	13	0	0	13	4	0	0	0	0	0	0	0	0	0	0	0	0	0					
(5)	Isovitexin	ppm	95	100	64	259	86	37	30	34	101	37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
(6)	Chromene		60	71	64	195	65	20	21	31	72	24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				
(7)	$\alpha$ -dipicatin		87	89	98	274	91	16	23	40	79	29	0	1	1	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0				
	Total ext.		81	88	85	254	85	33	10	18	61	20	0	4	0	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
Control			94	100	100	294	98	23	66	68	157	52	50	61	61	172	57	38	28	21	87	29	31	12	11	54	18								
(1)	Plicatin B		81	98	82	261	87	15	30	16	61	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
(2)	Flavonoid mixture		87	91	100	278	93	10	11	10	31	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
(3)	Psoralen		100	100	88	288	96	60	72	90	222	74	6	10	8	24	8	0	3	1	4	1	0	1	0	1	0	1	0.3						
(4)	Angelicin	75	70	71	66	207	69	53	27	42	122	41	0	15	0	15	5	0	10	0	10	3	0	0	0	0	0	0	0	0	0	0	0	0	0
(5)	Isovitexin	ppm	75	94	72	241	80	75	29	28	132	44	17	19	16	52	17	5	8	10	23	8	0	1	0	1	0	1	0.3						
(6)	Chromene		39	61	65	165	55	49	43	87	179	60	40	31	56	127	42	18	10	19	47	16	0	0	0	0	0	0	0	0	0	0	0	0	
(7)	$\alpha$ -dipicatin		100	88	85	273	91	50	37	48	135	45	10	6	6	22	7	2	4	0	6	2	1	0	0	1	0	0.3							
	Total ext.		100	100	100	300	100	60	61	66	187	62	20	9	18	47	16	14	6	9	29	10	1	0	0	1	0	0.3							



In case of flavonoid mixture, the mean survival of the termites was greatly decreased after two days of feeding at the two concentration levels 75 and 150 ppm, but the survival percent was much decreased at level 150 ppm comparatively to level 75 ppm. After nine days, all the individuals were dead at the two levels. It was observed that most of the termites started to avoid feeding on the filter papers and hide under damp sand after two days of exposure to the treated papers.

Fig. (3): Effect of Plicatin B in-vitro on the bacteria isolated from gut of *A. desertorum*



In case of chromene compound, the concentration level of 150 ppm, was more effective than the 75 ppm level, since all the termites were killed after nine days of exposure. The same effect was noticed with concentration levels 75 and 150 ppm in the first two days, but differ after six days.

Psoralen and angelicin, were similar in their effects on termites survival during feeding at levels 75 and 150 ppm.

Among the tested compounds of *Psoralea plicata*, only plicatin B, flavonoids and chromenes respectively showed the strongest termiticidal activity at concentration level of 150 ppm. Unlike these three *Psoralea* compounds, it was found that the other compounds are more or less similar in action on termites with apparent weak antitermite efficiency.

The statistical analysis of the obtained data indicated that the survival of termites is significantly different according to the concentration of the compound applied (in case of plicatin B and flavonoids), and the survival difference was clear with other compounds (psoralen, angelicin, isovitexin and  $\alpha$ -diplicatin B) especially at level 150 ppm after two days of feeding.

The action of plicatin B on the anaerobic bacteria isolated from the gut of *A. desertorum* was demonstrated by clear zone, when it is applied to nutrient agar medium inoculated with the respective bacteria after 24 hrs.

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## المواد المقاومة للنمل الأبيض والمفصولة من نبات " البسوراليا بليكاتا "

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تم فى هذه الدراسة إجراء التجارب الحيوية على مقاومة خلاصة النبات وسبعة من المركبات المفصولة من نبات " البسوراليا بليكاتا " المعروف باسم المرميد على النمل الأبيض ، الذى يسبب تدميراً شديداً للأشجار والأثاث المنزلى فى منطقة مصر العليا.

وقد أثبتت التجارب فاعلية مركبات البليكاتين - ب وكذلك المواد الفلافونية على نمو النمل الأبيض ، حيث قضت تماما على الحشرات فى فترة لا تتجاوز أسبوع واحد .

كما تأكد بالتجارب فاعلية مادة البليكاتين ب فى القضاء على البكتيريا المفصولة من أمعاء الحشرة خلال أربعة وعشرون ساعة ، والتي تعتمد عليها الحشرة فى هضم الغذاء .