

## Phytochemical screening and insecticidal activity of different extracts of *Acacia modesta* Wall. on adult *Culex pipiens* mosquito.

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### Abstract

**Background:** Insect-transmitted diseases remain a major source of illness and death worldwide. Mosquitoes alone transmit diseases in more than 700 million people annually. **The aim of the work:** is to perform phytochemical screening tests and to investigate the insecticidal effects of different extracts of the aerial parts of *Acacia modesta* (*A. modesta*) Wall. on adult *Culex pipiens* (*Cx. pipiens*) mosquito. **Materials and methods:** Different *A. modesta* Wall. extracts were bioassayed against adult *Cx. pipiens* mosquito to estimate their insecticidal effects. Three different extracts of *A. modesta* Wall. aerial parts were prepared (distilled water, ethanol and acetone extracts). Different concentration for each prepared extract were tested for their insecticidal activity as well as different duration of exposure e.g. one hour, 24 hours, 48 hours and 72 hours were also performed. **Results:** The efficacy of these extracts varies according to type of solvent used, concentration of extract and duration of exposure. With continuous exposure for 24 hrs; efficacy of *A. modesta* Wall. extract was elevated from 62–91% in the same manner. Expanding the exposure for 48 hrs; show continuous elevation up to 90–100 %. Meanwhile, the highest concentration of the tested plant extracted in acetone is one third (100 mg/ml) compared to other solvents (300 mg/ml), results showed that using acetone as a solvent is better than either distilled water or ethanol for its insecticidal activity. **Conclusion:** It was evident from the results that the plant is a potential source of botanical insecticides against adult *Cx. pipiens* mosquitoes and their toxic effects are time and concentration dependent. Moreover, phytochemical screening for the aerial parts of *A. modesta* Wall. resulted in the identification of carbohydrates and/or glycosides, tannins, flavonoids, unsaturated sterols and/or triterpens as well as saponins.

**Keywords:** *Acacia modesta* Wall., *Culex pipiens*, insecticides and phytochemical screening.

### Introduction:

Insect-transmitted diseases remain a major source of illness and death worldwide. Mosquitoes alone transmit diseases in more than 700 million people annually<sup>1</sup>.

Control of such diseases is becoming increasingly difficult because the over production of detoxifying mechanisms of resistance of chemical insecticides has reported for most mosquito species. On the other hand, majority of mosquito species have also developed high levels of resistance to microbial control agents. One alternative control approach is the use of natural insecticides from plant origin.

The botanical insecticides are generally pest specific and are relatively harmless to the non-target organisms including man<sup>2</sup>. They are also biodegradable and harmless to environment. One plant species may possess chemical substances with a wide range of activities, like extracts from *Azadirachta indica* which showed

antifeedent, antioviposition, repellent and growth regulating activity<sup>2</sup>.

*A. modesta*, commonly known as Phulai, is a member of the family Fabaceae (also called Leguminosae) and sub-family Mimosaceae. It is a deciduous, slow-growing small tree with thorny youngshoots and dark brown and black wood, reaching 7 meters height and 20 cm diameter<sup>3</sup>.

Reports on *A. modesta* aerial parts exposed the presence of flavonoids, alkaloids, terpenoids and tannins and long chain alcohols<sup>4</sup>. The aim of the current study is to perform phytochemical screening tests and to investigate the insecticidal effects of different extracts of the aerial parts of *A. modesta* Wall. on adult *Cx. pipiens* mosquito

### Materials and methods

**Insecticidal activity:**

**Plant materials: Collection and preservation of the plant:**

The aerial parts of *A. modesta* Wall. were collected in August 2015 from Giza zoo garden, the plant was kindly identified and authenticated by taxonomist Dr. Threse Labib consultant in the central gardening administration, Orman garden, Giza, Egypt. Voucher specimens had been deposited in the Pharmacognosy Department, Faculty of Pharmacy, Future University In Egypt, Cairo, Egypt.

**Preparation of the extract:**

90 grams of the air dried powder aerial parts of *A. modesta* Wall. were used in this experiment, each extract was achieved by soaking the ground plant material with the solvent for 48 hours then filtered with standard filter paper. 30 g of the aerial parts was extracted with distilled water, another 30g of the aerial parts was extracted with ethanol alcohol, the remaining 30 g of the aerial parts was extracted with acetone. Extracts were dried using rotary evaporator R-3 (Buchi, A.G., Switzerland).

**Bioassays requirements:** The insecticidal bioassays activities were carried out at the Institute of Medical Entomology, Ministry of health, Giza, Egypt.

The insecticidal activity of the extracts was conducted using modified CDC bottle assay. 250-ml Wheaton bottles with screw lids. Each bioassay concentration typically requires five bottles: four for replicates and one for control, graduated disposable plastic pipettes that can measure 1 ml, or micropipettes and tips, aspirator apparatus for collecting mosquitoes, containers for transferring/holding mosquitoes, bottles for concentrate solutions, timer capable of counting seconds, permanent markers for labeling bottles, caps, pipettes, masking tape for labeling bottles, caps, and pipettes, disposable gloves; Sheets, pens, and pencils for data recording.

**Procedure:**

Every sample bottled in the corresponding bottle and covered, rotation, and gentle shaking of the bottles takes place to ensure adequate layering of the sample to the inside wall of each bottle. Dryness of the bottle is helped by use of electric incubator at 35°C while the cap was removed with frequent rotation to ensure

homogenous film layering of the sample. After complete dryness of the sample, the bottles stored with loss cap tighten in the dark dry places for 24 hours to ensure complete dryness. Test was done in at least two replicate for tested concentrates, solvent control, and counter tested chemical insecticide.

Adult female non-blood feed *Cx. pipiens* mosquito of the same progeny group (20 – 40 in number) is delivered to every tested and control bottle for one hour exposure, 24, 48 and 72 hours for distilled water and ethanol extract, and for one hour, 24, and 48 hours for the acetone extract. Knockdown of the insects were followed up during exposure time every 10 min. (insect fall to the bottom or on its back or that one cannot move), and mortality calculated against time. The mortality rates were counted for: Test concentrates (different one), test solvent (test control distilled water, ethanol and acetone), pesticide control (ethanol) and standardized synthetic Pesticide (deltamethrine) as comparative mean. The mean for replicates is then plotted against time. Temperature and relative humidity were recorded at the beginning and end of the test. Results would be accepted for control bottle when mortalities were less than 5% percent (distilled water, ethanol and acetone), and rejected when the mortality rate was more than 20% percent. Five different concentrations were used for distilled water and ethanol extracts (300, 200, 100, 50 and 25 mg/ml) of *A. modesta* Wall. . The concentration of deltamethrine used in the experiment was (0.125 mg/ml) dissolved in ethanol. The solvent used (distilled water, ethanol and acetone; El Nasr Com., Cairo, Egypt). For the diethyl ether residue four different concentrations were used (100, 50, 25, 12.5 mg/ml of *A. modesta* Wall)

**Phytochemical screening:**

**Preparation of the extract:**

Twenty grams of *A. modesta* Wall. aerial parts were macerated in 70% methyl alcohol, filtered and kept in amber colored bottles for screening.

**Procedure:**

The total extract was screened via different phytochemical screening tests<sup>5-14</sup>.

**Statistical analysis**

The collected data were analyzed using the statistical analysis system SAS software package (version 9.0). Data were presented using frequency number and percent and mean

± SD as appropriate. Chi square test was used as appropriate, p value <0.05 were used as indicators of statistical significant difference.

### Results: The insecticidal activity:

Table (1) Effect of different concentrations of distilled water extract of *Acacia modesta* Wall. aerial parts on adult *Culex pipiens* mosquitoes after exposure for one hour.

Concentrations	Number of introduced mosquitos	Effect of one hour exposure				Statistical analysis	
		Living No.	Living %	Dead No.	Mortality %	$\chi^2$	P
300mg/ml	32	32	100	0	0	225.96	P<0.0005
200 mg/ml	33	33	100	0	0		
100 mg/ml	32	32	100	0	0		
50 mg/ml	28	28	100	0	0		
25 mg/ml	30	30	100	0	0		
Distilled Water	33	33	100.0	0	0		
Deltamethrine	40	5	12.5	35	87.5		
Ethanol	36	36	100	0	0		

No.; Number, % percentage, chi-square  $\chi^2 = 225.96$  and probability value  $P < 0.0005$ .

Table (1) shows that after one hour there were no effect of different concentrations of distilled water extract of the studied plant as well as the control on adult *Cx. pipiens* mosquitoes compared with the standard Deltamethrine (mortality rate was 87.5 %).

Table (2) Effect of different concentrations of ethanol extract of *Acacia modesta* Wall. aerial parts on adult *Culex pipiens* mosquitoes after exposure for one hour.

Concentrations	Number of introduced mosquitos	Effect of one hour exposure				Statistical analysis	
		Living No.	Living %	Dead No.	Mortality %	$\chi^2$	P
300mg/ml	32	32	100	0	0	189.06	P<0.0005
200 mg/ml	27	27	100	0	0		
100 mg/ml	29	29	100	0	0		
50 mg/ml	28	28	100	0	0		
25 mg/ml	30	30	100	0	0		
Deltamethrine	40	5	12.5	35	87.5		
Ethanol	36	36	100	0	0		

No.; Number, % percentage, chi-square  $\chi^2 = 189.06$  and probability value  $P < 0.0005$ .

Table (2) shows that after one hour, there were no effect of different concentrations of ethanol extract of the studied plant as well as the control on adult *Cx. pipiens* mosquitoes compared to the standard of Deltamethrine (mortality rate was 87.5 %).

Table (3) Effect of different concentrations of distilled water extract of *Acacia modesta* Wall. aerial parts on adult *Culex pipiens* mosquitoes and control after continuous exposure for 24 hours.

Concentrations	Number of introduced mosquitos	Effect of 24 hours exposure				Statistical analysis	
		Living No.	Living %	Dead No.	Mortality %	$\chi^2$	P
300mg/ml	33	20	60.6	13	39.4	14.79	P<0.05
200 mg/ml	31	22	71	9	29		
100 mg/ml	31	24	77.4	7	22.6		
50 mg/ml	40	32	80	8	20		
25 mg/ml	36	29	80.6	7	19.4		
Distilled water	28	28	100	0	0		

No.; Number, % percentage, chi-square $\chi^2$  =14.79 and probability value P<0.05.

Table (3) shows that after 24 hours exposure of mosquitoes to different concentrations of distilled water extract the mortality rates ranged from 19.4-39.4%. The highest percentage of mortality was found to be at concentration 300 mg/ml (39.4%), the insecticidal activity (% mortality) ranging from 19.4-39.4%, it is a function of increasing concentration.

Table (4) Effect of different concentrations of ethanol extract of *Acacia modesta* Wall .aerial parts on adult *Culex pipiens* mosquitoes and control group after continuous exposure for 24 hours.

Concentrations	Number of introduced mosquitos	Effect of 24 hours exposure				Statistical analysis	
		Living No.	Living %	Dead No.	Mortality %	$\chi^2$	P
300mg/ml	35	6	17.1	29	82.9	78.98	P<0.0005
200 mg/ml	33	8	24.2	25	75.7		
100 mg/ml	31	12	38.7	19	61.3		
50 mg/ml	33	23	69.7	10	30.3		
25 mg/ml	22	19	86.4	3	13.6		
Ethanol	38	38	100	0	0		

No.; Number, % percentage, chi-square $\chi^2$  =78.98 and probability value P<0.0005.

Table (4) shows that after 24 hours exposure to different concentrations of the ethanol extract of the studied plant, percentage of mortality of mosquitoes ranged from (13.6–83%). The highest effect (83%), was due to exposure to the highest concentration (300 mg/ml).

Table (5) Effect of different concentrations of distilled water extract of *Acacia modesta* Wall. aerial parts on adult *Culex pipiens* mosquitoes and control after continuous exposure for 48 hours.

Concentrations	Number of introduced mosquitos	Effect of 48 hours exposure				Statistical analysis	
		Living No.	Living %	Dead No.	Mortality %	$\chi^2$	P
300mg/ml	33	0	6	33	100	102.09	P<0.0005
200 mg/ml	31	2	6.5	29	93.5		
100 mg/ml	31	3	9.7	28	90.3		
50 mg/ml	40	4	10	36	90		
25 mg/ml	36	3	8.3	33	91.7		
Distilled water	28	24	85.7	4	14.3		

No.; Number, % percentage, chi-square $\chi^2$  =102.09 and probability value P<0.0005

Table (5) shows that after 48 hours exposure of mosquitoes to different concentrations of the plant in distilled water the mortality rates ranged from 90-100%. The highest rate was in concentration 300 mg/ml (100%). The difference of mortality was highly statistically significant.

Table (6) Effect of different concentrations of ethanol extract of *Acacia modesta* Wall. aerial parts on adult *Culex pipiens* mosquitoes and control group after continuous exposure for 48 hours.

Concentrations	Number of introduced mosquitos	Effect of 48 hours exposure				Statistical analysis	
		Living No.	Living %	Dead No.	Mortality %	$\chi^2$	P
300mg/ml	35	2	5.7	33	94.3	95.21	P<0.0005
200 mg/ml	33	3	9.1	30	90.9		
100 mg/ml	31	10	32.3	21	67.7		
50 mg/ml	33	23	69.7	10	30.3		
25 mg/ml	22	15	53.3	7	46.7		
Ethanol	38	37	97.3	1	2.7		

No.; Number, % percentage, chi-square $\chi^2=95.21$  and probability value  $P<0.0005$

Table (6) shows that after 48 hours exposure to different concentrations of the ethanol extract of the studied plant, mortality rates of mosquitoes ranged from (47–94.3 %). The highest effect (94.3%) was due to exposure to concentration (300 mg/ml).

Table (7) Effect of different concentrations of *Acacia modesta* Wall. aerial parts ethanol extract on adult *Culex pipiens* mosquitoes and control group after continuous exposure 72 hours.

Concentrations	Number of introduced mosquitos	Effect of 72 hours exposure				Statistical analysis	
		Living No.	Living %	Dead No.	Mortality %	$\chi^2$	P
300mg/ml	35	0	0	35	100.0	80.25	P<0.0005
200 mg/ml	33	2	6.1	31	93.9		
100 mg/ml	31	11	35.5	20	64.5		
50 mg/ml	33	13	39.4	20	60.6		
25 mg/ml	22	10	45.5	12	54.5		
Ethanol	38	34	89.5	4	10.5		

No.; Number, % percentage, chi-square $\chi^2= 80.25$  and probability value  $P<0.0005$ .

Table (7) indicates that after continuous exposure for 72 hours to the ethanol extract of *A. modesta* aerial parts. The mortality rates of mosquitoes ranged from (54.5–100%). The highest effect was due to concentration (300mg/ml) (100%).

Table (8) Effect of extract of different concentrations of *Acacia modesta* Wall. aerial parts acetone extract on adult *Culex pipiens* mosquitoes and acetone after one-hour exposure.

Concentrations	Number of introduced mosquitos	Effect of one hour exposure				Statistical analysis	
		Living No.	Living %	Dead No.	Mortality %	$\chi^2$	P
100mg/ml	33	13	39.4	20	60.6	16.14	P<0.01
50mg/ml	30	13	43.3	17	56.7		
25mg/ml	39	25	64.1	14	35.9		
12.5mg/ml	21	15	71.4	6	28.6		
Acetone	21	18	85.7	3	14.3		

No.; Number, % percentage, chi-square $\chi^2=16.14$  and probability value  $P<0.01$

After one-hour exposure to the extract of *A. modesta* Wall. aerial parts in acetone, table (8) shows good effectiveness of the plant extract on adult *Cx. pipiens* mosquitoes the (mortality rates of mosquitoes ranged from 28.6–60.6%) compared with control. The difference is statistically significant. The highest effect was due to concentration (100mg/ml) (60.6 %).

Table (9) Effect of different concentrations of *Acacia modesta* Wall. aerial parts acetone extract on adult *Culex pipiens* mosquitoes and acetone after 24 hours exposure.

Concentrations	Number of introduced mosquitos	Effect of 24 hours exposure				Statistical analysis	
		Living No.	Living %	Dead No.	Mortality %	$\chi^2$	P
100mg/ml	33	3	9.1	30	90.9	42.8	P<0.0005
50mg/ml	30	8	26.7	22	73.3		
25mg/ml	39	6	15.4	33	84.6		
12.5mg/ml	21	8	38.1	13	61.9		
Acetone	21	18	85.7	3	14.3		

No.; Number, % percentage, chi-square  $\chi^2=42.8$  and probability value  $P<0.0005$

Table (9) shows that after 24 hours exposure to the extract of *A. modesta* plants in acetone, high effect of the plant extract on adult *Cx. pipiens* mosquitoes (the mortality rates of mosquitoes ranged from 62–91%) compared with control.

The mortality percentages difference is statistically significant. The highest effect was due to concentration (300 mg/ml) (91 %).

Table (10) Effect of different concentrations of *Acacia modesta* Wall. aerial parts acetone extract on adult *Culex pipiens* mosquitoes and acetone after exposure for 48 hours.

Concentrations	Number of introduced mosquitos	Effect of one 48 hours exposure				Statistical analysis	
		Living No.	Living %	Dead No.	Mortality %	$\chi^2$	P
100mg/ml	30	0	0	30	100	120.49	P<0.0005
50mg/ml	33	0	0	33	100		
25 mg/ml	39	0	0	39	100		
12.5mg/ml	21	0	0	21	100		
Acetone	21	18	85.7	3	14.3		

No.; Number, % percentage, chi-square  $\chi^2=120.49$  and probability value  $P<0.0005$ .

Table (10) declared that after 48 hours exposure to the extract of *A. modesta* Wall. plant in acetone, the best effect occurs in all concentrations (the mortality rates of mosquitoes were 100 %).

#### Phytochemical screening:

Table (11): Results of the phytochemical screening tests for *Acacia modesta* Wall. aerial parts.

Active constituents	Result	Test	Reference
Carbohydrates and/or glycosides	+ve	Molish test	5
Tannins	+ve	Ferric chloride test	6
Flavonoids	+ve	Schinoda's test	7
Unsaturated sterols and/or triterpens	+ ve	Lieberman-Burchard's test Salkowski's test	8-9
Saponins	+ve	Froth test	10
Anthraquinone glycosides	- ve	Borntagers test and modified Borntagers tests	11-12
Alkaloids	-ve	Mayer's test	13-14

**+ve; positive, -ve; negative**

Table (11) Illustrated results of phytochemical screening of *A. modesta* wall. aerial parts. It

revealed the presence of the carbohydrates and/or glycosides, tannins ,flavonoids , unsaturated sterols and/or triterpens as well as

saponins meanwhile the absence of Anthraquinone glycosides as well as alkaloids.

### Discussion

Synthetic chemical pesticides have some serious flaws to environmental and health related concerns. It is expected that only in America about 200 people are dying every year due to this pesticidal poisoning<sup>15</sup>. These problems resulted in renewed interest in formulating new botanical pesticides, which could be nonhazardous, effective, biodegradable, and of low cost and pose less threat to the environment<sup>16</sup>.

Chemical pesticides have shown very good results at the beginning, but with the passage of time, the need was realized to use the natural plant sources for the safe control of insects. The current study will provide minor contribution towards it. The aim of the present study was to evaluate the insecticidal activities of different concentrations of *A. modesta* Wall. extracts in many solvents such as distilled water, ethanol and acetone to detect the suitable solvent as well as concentrations

Results of this study showed that exposure of the adult *Cx. pipiens* mosquitoes to the cold extract of different concentrations *A. modesta* Wall. aerial parts extracted with distilled water as well as those dissolved in ethanol has no significant effect on *Cx. pipiens* mosquitoes after exposure for one hour meanwhile the exposure to the plant extracted with acetone for the same period have induced mortalities among exposed insects, (mortality rates of mosquitoes ranged from 28.6–60.6%). The highest mortality rate was due to concentration (300 mg/ml) 61 %. Increasing duration of exposure also was in favor of the plant dissolved in acetone. The mortality rates of mosquitoes after 24 hours of exposure ranged from 62–91% compared with control. The difference is statistically significant. The highest effect was due to concentration (300 mg/ml) (91 %). meanwhile the exposure to the ethanol extract for the same period (24 hours) have induced lower mortalities among exposed insects, (mortality rates of mosquitoes ranged from 13.6–83%) and also the exposure to the distilled water extract for the same period have induced the lowest mortalities among exposed

insects, (mortality rates of mosquitoes ranged from 19.4–39.4%),

In addition, after continuous exposure for 48 hours of all concentrations of the acetone extract, the percentage of mortality of mosquitoes were 100 % in all concentrations. Meanwhile, the exposure to the ethanol extract for the same period of time had induced lower mortalities among exposed insects, (mortality rates of mosquitoes ranged from 47–94.3 %), and also the exposure to the distilled water extract for the same period have led to lower mortalities among exposed insects, (mortality rates of mosquitoes ranged from 90-100%).

Although, the highest concentration of the tested plant extracted in acetone was one third (100mg/ml) compared to other solvents (300 mg/ml), results showed that using acetone as a solvent was most effective than either distilled water or ethanol for its insecticidal activity. Similar results were obtained by **Nazeefullah et al.**<sup>17</sup> using different cold water extracts dilutions of *A. modesta* when tested against other insects *Tribolium castaneum* and *Lemna minor*. The extract dilutions of the plant caused mortality of the *T. castaneum*, which showed direct proportionality with time and concentrations of plant extracts. The maximum mortality percentage was after 72 hrs. While, minimum was after 24 hrs. as compared to control. Also, the mortality percentage of the insect increased with the increase in concentration of plant extracts. Phytotoxic activity showed that dilutions of *A. modesta* Wall. extracts significantly inhibited the growth of *L. minor*.

The obtained result is in agreement with the results of **Kundu et al.**<sup>18</sup> and **Khani and Asghari**<sup>19</sup>. **Ahmad et al.**<sup>20</sup> who reported that the crude methanol extract and various fractions were inactive against *T. minor*. Also, methanol extract of *A. modesta* Wall. was assessed for insecticidal effects against *Callosbruchus analis*, *Rhizopertha dominica* and *Tribolium castaneum*<sup>21</sup>. All test samples exhibited no activity towards *T. castaneum*. While against *R. dominica*, low activity was observed in case of chloroform and n-hexane fractions. Also, other samples were inactive. Significant activity of 60% was seen with n-hexane fraction against *C. analis*<sup>21</sup>.

Results of current study showed that the mortality percentage was also directly proportional to concentration. This agrees with the findings of **Kunduet *al.***<sup>18</sup> who worked on the medicinal plants for insecticidal activity using *T. castaneum*.

It was evident from their results that *A. modesta* Wall. is a potential source of botanical insecticides against adult *Cx. pipiens* mosquitoes and *T. castaneum*. Their toxic effects were time and concentration dependent. Phytochemical screening of the aerial parts of the studied plant revealed the presence of carbohydrates and/or glycosides, tannins, flavonoids, unsaturated sterols and/or triterpens as well as saponins.

### Conclusion

Synthetic insecticides are of great threat to the environment due to their toxic effects. Therefore, environment friendly insecticides from natural resources is for great importance. The present study reveals that *A. modesta* Wall. had good insecticidal properties and can be used as a natural insecticide. The plant extracts could be a new source for pesticides with minimum noxious effects on the environment. Further studies may also lead to isolate and characterize the active compounds of the plant extracts and to elucidate their biological mechanisms of action.

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