

## First study of larvicidal activity of Algerian *Oudneya africana* extracts against *Culex pipiens* larvae

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**Received:** 15-Jan-2022, **Manuscript No.** UJE-22-51696; **Accepted:** 07-Feb-2022, **Pre QC No.** P-51696;

**Editor assigned:** 17-Jan-2022, **Pre QC No.** P-51696; **Reviewed:** 27-Jan-2022, **QC No.** Q-51696;

**Revised:** 01-Feb-2022, **Manuscript No.** R-51696; **Published:** 17-Feb-2022.

Mosquitoes are factors in several parasitic diseases that are dangerous for humans. *Culex pipiens* is one of the mosquito species, which causes many of the health problems in Algeria. The chemical fight against these mosquitoes causes several environmental problems. Biological control is an alternative considered by the use of secondary plant substances. Saharan plants can provide toxic secondary metabolites against these mosquitoes. With the objective of valuing plants from southern Algeria, the *Oudneya africana* plant collected from the Ghardaïa region was used as a natural insecticide.

Acetone, ethanol and methanol were used to prepare the plant extract using cold maceration method. The larvicidal power of the three extracts against *Culex pipiens* larvae was studied. Qualitative phytochemical screening of organic extracts is studied.

The mortality rates varied considerably and significantly with the concentrations of extract, solvent used and the time of exposure. The acetone extract exhibits the highest larvicidal effect and present the lower LC<sub>50</sub> (0.10 g/L) and LT<sub>50</sub> (10 h) values while the methanolic extract have a less toxic (LC<sub>50</sub>=0.36 g/L, LT<sub>50</sub>=77 h). The phytochemical screening confirms that Alkaloids, Tannin and Steroids were responsible for larvicidal activity.

This study shows that the extracts of *Oudneya africana* can be used as a natural larvicidal against mosquitoes instead of using chemical insecticidal that has negative effects on the environment and health because this natural insecticide is biodegradable. LC-MS analysis suggested that rutin, caffeoylquinic acid and chlorogenic acid are responsible for larvicidal activity of acetonic extract of *Oudneya africana*.

**Keywords:** *Oudneya africana*, Extracts, Solvents, Larvicidal, Toxicity, *Culex pipiens*.

### Introduction

The Culicidae are insects distributed in different biotopes in the world (Aïssaoui and Boudjelida, 2017). Mosquitoes are number more than 3200 species, which are divided into three genera Aedes, Anopheles and Culex. The Culex genus is the most common (Zerroug et al, 2017). *Culex pipiens* is the most dominant species in Algeria (Bouguerra et al, 2019).

This species can adapt to different urban and rural biotopes, in polluted and clean waters (Faraj et al, 2016).

Mosquitoes are involved in the transmission of several diseases such as malaria, leishmaniasis, yellow fever and dengue fever, hemorrhagic fevers, lymphatic filariasis, and zika virus (Matoug et al, 2018; Sedaghat et al, 2010). These diseases affect more than 700,000,000 people worldwide each year (Raveen et al, 2017). In addition, mosquitoes cause other problems: they irritate humans, attack farm animals, and carry avian malaria (Ali et al, 2020).

In the fight against Mosquitoes, high quantities of chemical larvicidal are released into the aquatic environment (Matoug et al, 2017). The chemicals insecticides cause several ecological problems, for example the resistance of mosquitoes, negatives effects on non-target organisms and human health (Anyaele and Amusan, 2003). Therefore, biological control is an effective alternative in natural environments to protect the environment from all kinds of pollution (Matoug et al, 2017). The use of plant extracts is a biological control technique against mosquito larvae in aquatic environments to stop their life cycle (Lokesh et al, 2010). Plants have important bioactive compounds, which are a source of insecticidal agents (Kumar et al, 2012). Botanical insecticides are

biodegradable and less expensive than chemical insecticides. These pesticides have no harmful effects on non-target organisms and humans (Alouani et al, 2017).

The Algerian Sahara is recently represented by a spread of the Culicidae fauna (Merabti et al, 2016). *Culex pipiens* represents a West Nile virus vector. In Algeria, the West Nile virus caused a major epidemic in the Timimoune region in 1994 (Zerroug et al, 2017).

*Oudneya Africana* is a Saharan plant of the Brassicaceae family. This plant is endemic to Algeria, Tunisia and Morocco and is used in different fields: the stabilization of mobile dunes, the rehabilitation of degraded ecosystems, the breeding of goats and camelids, and the treatment of various diseases (lesions of the stomach, colon, liver, fevers and skin diseases) and against scorpion bites (Quezel and Santa, 1963). To our knowledge, no research has been carried out on the larvicidal power of extracts of this plant.

As part of the valuation of medicinal plants and the protection of the environment against pollution, the main purpose of this study was to perform phytochemical analysis and the larvicidal activity of *Oudneya africana* extract by using various organic solvents against the larvae of the fourth instar (L4) of the larvae of *Culex pipiens*.

## Materials and Methods

### Study area

The study site is represented by Ghardaïa that is located 600 km south of Algiers in the central northern part of the Algerian Sahara at the gates of the desert at 32°30 North latitude and 3°45 longitudes. The geomorphological formations that characterize the Ghardaïa region are Chabka du M'Zab, dayas region and Ergs region. The Ghardaïa region is characterized by a Saharan climate, which is distinguished by large thermal amplitude between day and night, summer and winter.

### Biological materials

The larvae of mosquitoes of the *Culex pipiens* species (Diptera: Culicidae) were collected in a site of an untreated wastewater accumulation pit at a place called Kef El-Doukhan, downstream of the M'zab river (El-Atteuf, Ghardaïa 32°28'39"N, 3°44'52"E) during the month of April to the month of May 2021. The identification of the species *Culex pipiens* was made according to the identification keys those of (Himmi et al, 1995). *Oudneya africana* was identified according to the identification keys those of (Quezel and Santa, 1963).

The aerial part of the plant was collected from Zone of Oued Drine (Metlili, Ghardaïa 32°16'22"N, 3°37'39"E) during the months of January and February 2021. The voucher specimens were prepared and deposited at the Biological and Agronomical Sciences Laboratory (LSBA) of Laghouat University. The part used was dried in the shade and at room temperature for 30 days. It was ground into a very fine powder and then stored in hermetically sealed glass bottles protected from light and moisture until further use.

## Methods

**Breeding technique:** Identified larvae were kept in mass rearing in the laboratory at room temperature of 40°C and a daily photoperiod in plastic bowls containing water and fed with a yeast-biscuit mixture (1:3, p/p). The breeding is carried out in cages of 50 cm × 50 cm.

**Extraction methods:** The maceration was carried out using ethanol, methanol and acetone as solvent. 100 g of plant powder were added to 500 mL of each organic solvent, then left to stir in a shaker (Precise Shaking Incubator WIS-10, Korea) for 24 hours at room temperature. The resulting mixture was filtered three times through Whatman paper. The organic extracts obtained were evaporated to dryness using a rotary evaporator at 45°C. The dry extract was then collected, weighed, labeled and stored at 4°C until use.

**Phytochemical screening:** Phytochemical screening consists of performing qualitative phytochemical tests based on coloring or precipitation reactions to determine the presence of groups of chemical families (EL-Haoud et al, 2018; Lerato et al, 2017). The test results are represented as continuations +++ strongly positive; ++: Moderately positive; +: weakly positive; -: negative.

**Flavonoid:** A volume of 3 mL of each extract was treated with a volume of 1 mL of a 10% NaOH solution. The presence of flavonoids is determined by the formation of an intense yellow color (Lerato et al, 2017).

**Cyanidine:** A volume of 5 mL of hydrochloric ethanol was added to the volume of 5 ml of each extract. The presence of leucoanthocyanins was determined by a cherry red or purplish color; the presence of catechols was determined by a brown-red tint.

**Anthocyanin:** A volume of 2 mL of each extract was added to the volumes of 2 ml of 2N HCl and 2 ml of ammonia. The presence of anthocyanin was determined by the appearance of a pink-red color turning to blue-violet (Lerato et al, 2017).

**Quinones:** A few drops of 1% NaOH were added to one volume of each extract. The presence of quinones is determined by the appearance of a yellow, red or purple color (EL-Haoud et al, 2018).

**Coumarin:** A volume of 3 mL of 10% NaOH was added to the volume of 2 ml of each extract. The presence of coumarins was determined by the formation of a yellow color (Lerato et al, 2017).

**Alkaloids:** A volume of 3 mL of each extract was added to the volume of 3 mL of 1% HCl. This mixture was heated for 20 min. After the mixture cooled, a volume of 1 mL of Mayer's reagent was added dropwise. The presence of alkaloids is determined by the formation of a greenish or cream-colored precipitate (Lerato et al, 2017).

**Glycosides:** A volume of 1 mL of each extract was added to the volume of 10% KOH. The presence of glycosides was determined by the formation of a brick red color.

**Steroids:** Volumes of 5 mL of chloroform and 5 mL of H<sub>2</sub>SO<sub>4</sub> were added to the 0.5 mL volume of each extract. The presence of steroids was determined by a color change from purple to blue or green or a ring of blue/green or if the top layer turned red and the sulfuric layer was yellow with green fluorescence (Lerato et al, 2017).

**Tannins:** A few drops of 1% FeCl<sub>3</sub> solution were added to the volumes of 1 ml of each extract, 1 mL of distilled water.

The appearance of a dark green color indicates the presence of catechetal tannins and the appearance of a blue-green color indicate the presence of gallic tannins (EL-Haoud et al, 2018).

**Reducing compounds:** 1 mL of the extract was added to 2 mL of Fehling's liquor, then heat in a water bath (70°C, 2 min). The presence of reducing compounds was determined by the formation of a brick red precipitate (Trease and Evans, 1987).

**Saponosides:** A few drops of distilled water were added to the 2 mL volume of each extract, and then the mixture was stirred. After 20 min, the saponosides content is evaluated according to the following criteria: No foam = Test negative, foam of 1-2 cm = Test positive (Trease and Evans, 1987).

## Toxicity tests

The toxic effect of the different concentrations of each extract (0.0335-0.335 g/L) was tested on the larvae of the 4th instar larvae. The concentrations were prepared by solubilizing the dry extracts in appropriate volume of DMSO (99.9%). No mortality was recorded in larvae of *Culex pipiens* mosquitoes with the control batch containing DMSO at 0.5%.

Six lots are prepared for these tests (one lot is control and each lot of the others is characterized by a concentration). 20 larvae were immediately placed in plastic cups which contained 100 ml of distilled water and the target concentration of the extract.

The toxicities tests were maintained under the same conditions of the breeding followed for the lavas. The mortality kinetics is monitored for 3 days after 3, 6, 12, 24, 48 and 72 hours. Each lot has three repetitions.

The percentage of observed mortality of the control and treated larvae of the individuals tested was determined according to the following formula:

$$\text{Mortality (\%)} = \frac{\text{Number of dead larvae after treatment}}{\text{Number of larvae introduced}} \times 100$$

The lethal concentrations, LC<sub>50</sub> and LC<sub>90</sub> which kill 50% and 90% respectively of the number of individuals exposed to the extracts. The percentages of mortality corrected were transformed into Probits, and the test concentrations of toxicity were transformed into a decimal logarithm. Equations of the dose log regression lines as a function of the Probits have been established. The same method was followed to determine LT<sub>50</sub> and LT<sub>90</sub> values, but the times of exposure were transformed into a decimal logarithm.

## Results

### Extraction yield and phytochemical screening

The extraction yield varied considerably with the organic solvent used. Methanol gives the high yield (13.37%) compared to the ethanol (4.93%) and acetone (2.06%). The results of the phytochemical screening of different extracts are indicated in Table 1. According to the results the ethanolic and methanolic extracts contains more secondary metabolites than acetonic extract. All extracts are rich in alkaloids and steroids. In addition, acetonic extract contain a high amount of tannin compared to others extracts.

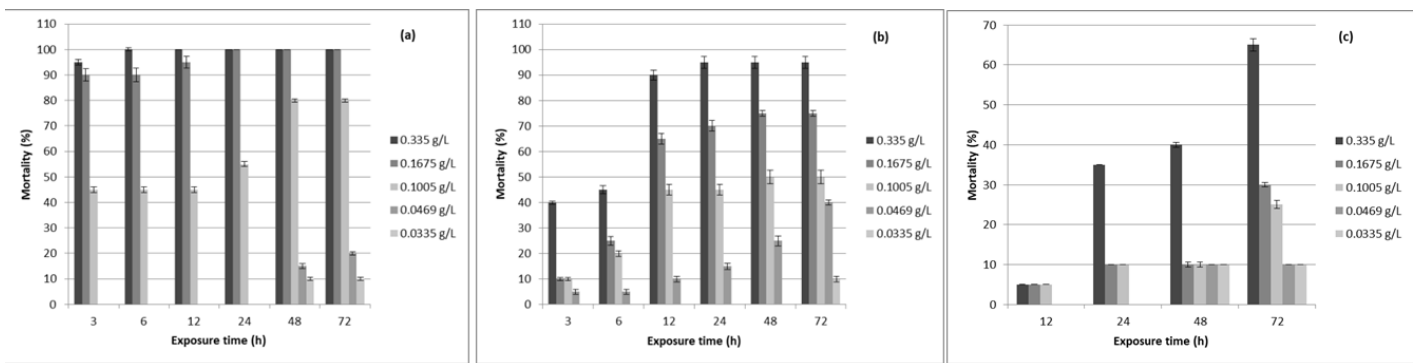
**Table 1.** Phytochemical screening of various extracts.

| Phytochemical Compounds | Solvent |         |          |
|-------------------------|---------|---------|----------|
|                         | Acetone | Ethanol | Methanol |
| Flavonoids              | -       | ++      | ++       |
| Cyanidine               | +       | ++      | ++       |
| Anthocyanin             | -       | -       | -        |
| Quinones                | -       | ++      | ++       |
| Coumarin                | -       | ++      | ++       |
| Alkaloids               | ++      | ++      | ++       |
| Glycosides              | -       | ++      | +        |
| Steroids                | ++      | +++     | +++      |
| Tannins                 | ++      | +       | -        |
| Reducing compounds      | -       | -       | -        |
| Saponosides             | -       | -       | -        |

### Toxicity of plant extracts

According to Fig. 1a-c, which show the variation of mortality rates observed of *Culex pipiens* at increasing concentrations and the exposure time for all extracts of *Oudneya africana*.

The results of the previous figure show that the larvicidal effect of the acetone extract results in high mortality rates after all times of exposure. Maximum mortality (100%) was recorded after 6 h by the concentration of 0.335 g/L of this extract. The ethanolic and methanolic extracts result in varying degrees of mortality depending on the time of exposure and even the concentration used. The concentration 0.335 g/L causes a mortality of around 95% after 24 h for the ethanolic extract and a mortality of around 65% after 72 h for the methanolic extract.



**Fig. 1.** Variation of mortality rates observed of *Culex pipiens* at increasing concentrations of the different extracts of *Oudneya africana*. (a) Acetone extract, (b) Ethanolic extract and (c) Methanolic extract.

**Larvicidal efficacy of plant extracts**

The efficacy of each extract of *Oudneya africana* is presented profoundly with the toxicological parameters, lethal concentrations (LC<sub>50</sub> and LC<sub>90</sub>) and lethal time (LT<sub>50</sub> and LT<sub>90</sub>) which are estimated values by the Probit method are shown in the Tables 2 and 3. The acetonic extract has the lowest values for LC<sub>50</sub> and LC<sub>90</sub> and LT<sub>50</sub> while the methanolic extract present the highest values. Whereas, the methanolic extract has the lowest LT<sub>90</sub> value.

**Table 2.** Larvicidal potential of different solvent of *Oudneya africana* against *Culex pipiens* larvae after 24 h of exposure period.

| Extract  | LC <sub>50</sub>         | LC <sub>90</sub>         | R <sup>2</sup> |
|----------|--------------------------|--------------------------|----------------|
| Acetone  | 0.10 ± 0.00 <sup>a</sup> | 0.13 ± 0.00 <sup>a</sup> | 0.99           |
| Ethanol  | 0.10 ± 0.00 <sup>a</sup> | 0.27 ± 0.00 <sup>b</sup> | 0.99           |
| Methanol | 0.36 ± 0.05 <sup>b</sup> | 0.64 ± 0.00 <sup>c</sup> | 0.97           |

**Notes:** LC<sub>50</sub>: lethal concentration (g/L) that kills 50% of the exposed larvae; LC<sub>90</sub> (g/L): lethal concentration that kills 90% of the exposed larvae; R<sup>2</sup>: Regression Coefficient; Data represent mean ± SE. a, b, c: indicate significant differences between extracts at P<0.05 based on one-way ANOVA followed by Tukey’s HSD test.

**Table 3.** LT<sub>50</sub> and LT<sub>90</sub> values of different extracts of *Oudneya africana* against *Culex pipiens* larvae at 0.1005 g/L.

| Extract  | LT <sub>50</sub>       | LT <sub>90</sub>         | R <sup>2</sup> |
|----------|------------------------|--------------------------|----------------|
| Acetone  | 10 ± 2.81 <sup>a</sup> | 303 ± 0.00 <sup>a</sup>  | 0.89           |
| Ethanol  | 51 ± 0.88 <sup>b</sup> | 1151 ± 0.00 <sup>b</sup> | 0.94           |
| Methanol | 77 ± 1.89 <sup>b</sup> | 198 ± 0.00 <sup>c</sup>  | 0.86           |

**Notes:** LT<sub>50</sub>: lethal time (h) that kills 50% of the exposed larvae; LT<sub>90</sub> (h): lethal time that kills 90% of the exposed larvae; R<sup>2</sup>: Regression Coefficient; Data represent mean ± SE. a, b, c: indicate significant differences between extracts at P<0.05 based on one-way ANOVA followed by Tukey’s HSD test.

**Discussion**

Control of vector mosquitoes, using chemical insecticides, is not encouraged due to the rapid increase in mosquito resistance (Alouani et al, 2017). Therefore, biological control is an effective alternative in natural environments to protect the environment from all kinds of pollution (Ali et al, 2020).

Different types of extracts from plants have been used against mosquitoes as an adulticide, larvicide, growth regulator all over the world (Sedaghat et al, 2010). The insecticidal effect of plants depends on the method of extraction and particularly the nature of the solvent used.

Therefore, in this present work, the larvicidal activity of *Oudneya africana* was investigated for the first time in fourth instar (L4) larvae of *Culex pipiens* using different solvent of extraction.

Several factors influence the extraction yield such as plant species, plant stage, organ used and solvent used (Kemassi et al, 2018). In the present study, the maceration had variable yields depending on the polarity of the solvents.

The results obtained showed that the all tested extracts had a variable larvicidal effect translated by low mortality rates to high mortality rates with the increase in the concentrations used. These larval mortality rates were also shown to be directly related to exposure time. The statistical analysis using Tukey’s test shows that the acetone extract shows a high toxic effect on the larvae of *Culex pipiens* than the other extracts (P <0.05).

To determine the groups of chemical components responsible for this larvicidal activity, qualitative phytochemical screening is studied for the different extracts. The results obtained indicating a difference in the presence of groups of chemical compounds between the different extracts studied. The richest extract is the ethanolic extract and the least rich extract is the acetone extract, while the acetone extract is the richest in tannins. Alkaloids, Steroids and Cyanidine are presented in all extracts.

The research carried out by (Hajlaoui et al, 2019) studies the identification and quantification of chemical compounds by HPLC-MS of acetone and ethanolic extracts of *Oudneya Africana*. This latest study concluded that a total of ten phenolic acids, eight flavonoids and only two tannins were characterized in the acetone extract, and nine phenolic acids, seven flavonoids and one tannin were identified in the extract at 1 ethanol, respectively. The major compounds that have been identified in both extracts are chlorogenic acid, 4-O-caeoylquinic acid, caffeic acid, and 4,5-di-O-caffeoylquinic acid for phenolic acids and rutin for flavonoids.

Phenolic compounds are responsible for insecticidal activity by reducing insect herbivory, reducing fertility and shortening insect life (Al-Massarani et al, 2019). This shows that the groups of chemical compounds of the extracts studied are responsible for the larvicidal activity shown in this study. According to (Arab et al, 2018), the secondary metabolites rich in functional groups most often cause disturbance in insects. Tannins have a direct toxic effect for several insect pests, by influencing their growth, development and fertility. Alkaloids, steroids and flavonoids are insecticides in low concentrations with varying modes of action. Usually, these compounds act on acetylcholine receptors in the nervous system and on membrane sodium channels in the nerves (Acheuk and Doumandji-Mitiche, 2013). Rutin significantly blocked the growth and proliferation of mosquito larvae. The activity could be due to a synergistic effect between the different phenolic compounds (Ben Nasr et al, 2021). Indeed, the larvicidal activity of acetonic extract of *Oudneya africana* is attributed to its high content of rutin, caffeoylquinic acid and chlorogenic acid compounds.

The lethal concentrations 50 and 90 estimated for the acetone extract in this study is very low than that obtained by (Al-Solami, 2021) who concluded that the acetone extract of *Lantana camara* had insecticidal activity against the fourth instar larvae (L4) of *Culex pipiens* larvae with an LC<sub>50</sub> and LC<sub>90</sub> of 0.26 g/L and 0.84 g/L, respectively. This work also shows that the values of LC<sub>50</sub> of the same extract of *Rhazya stricta*, *Acalypha fruticosa* and *Ruta chalepensis* are of the order of 0.29 g/L, 0.44 g/L and 0.61 g/L respectively, While the values of LC<sub>90</sub> of the same plants are: 1.29 g/L, 2.17 g/L and 2.28 g/L respectively. The research of (El-Bokl, 2016) determined that the lethal concentrations 50 of the larvicidal activity of the acetone extracts of *Artemisia herbalba*, *Lavandula multifida* and *Peganum harmala* after 24 h of exposure are low than that obtained in this study against *Culex pipiens* with values of the order of 0.07 g/L, 0.08 g/L and 0.02 g/L respectively. The values of LC<sub>90</sub> of the acetone extracts of *Artemisia herbalba* and *Peganum harmala* is of the order of 0.18 g/L, while that of the extract of *Lavandula multifida* is of the order of 0.13 g/L. This last value is the same with that obtained by the extract of this research.

The ethanolic extract of *Amorpha fruticosa* used in the research for (Liang et al, 2015) had a larvicidal effect against *Culex pipiens* after 24 h of exposure with LC<sub>50</sub> and LC<sub>90</sub> values of the order of 0.02 g/L and 0.11 g/L respectively. These latter values are low than those obtained by the ethanolic extract studied in this work. The study by (El-Monairy, 2015) determined that the ethanolic extract of *Colocasia esculenta* had an insecticidal effect against the fourth instar larvae (L4) of *Culex pipiens* with LC<sub>50</sub> and LC<sub>90</sub> values of around 0.11 g/L and 0.39 g/L respectively after 48 h.

The research of (Farag et al, 2021) showed that the methanolic extract of *Sesamum indicum* has a lethal concentration 90 lower than that obtained by the methanolic extract of this study with a value of 1.0178 g/L unlike the LC<sub>50</sub>, which is higher than that obtained in this research with a value of 0.1617 g/L.

## Conclusion

Qualitative phytochemical screening in the present study determines the presence of several active compounds in acetone, ethanolic and methanolic extracts of *Oudneya africana*. The study of the toxicity of the larvae of the fourth instar (L4) by the various extracts tested, shows that these extracts exhibited significant larvicidal power. Mortality rates are positively correlated with the concentrations used and the time of exposure to the extracts. The results obtained have shown that the plant extracts are of very great interest in the biological fight against mosquito larvae and in producing a new natural insecticide alternative to chemical control. Further studies are required in order for identification of the compounds responsible of larvicidal activity of acetone extract.

## Acknowledgement

This work was supported by the Algerian General Directorate of Scientific Research and Technological Development (DGRSDT).


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#### Citation:

Rouari, L., Gouzi, H., Ghermaoui, M., Benaceur, F., Kemassi, A., Merabti, B., Messahli, I., Rezzoug, A., Rouari, A., Chaibi, R. (2022). First study of larvicidal activity of Algerian *Oudneya africana* extracts against *Culex pipiens* larvae. *Ukrainian Journal of Ecology*. 12:65-70.

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