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Ziziphus spina-christi (L.) as a Source of Environmentally Friendly Biocides: Phytochemical Characteristics and Insecticidal Potential of Leaves and Stem Bark Extracts on *Aphis fabae* Scopoli (Aphididae)

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Abstract
The present study aimed to assess the content of bioactive phytochemicals (total phenols, flavonoids, tannins, saponins and alkaloids), and to determine the best extract for potent insecticidal activity in the leaves and stem bark of the medicinal plant *Ziziphus spina-christi* (Rhamnaceae) from south-east Algeria against the aphid *Aphis fabae* (Aphididae). The extraction was done using different solvents (*N*-hexane, DCM, MeOH, and Water). The insecticidal activity of the extracts was determined using a contact toxicity test on *Aphis fabae* larvae and adults. Our results show that for leaves, the highest levels of polyphenols and saponins were observed in the MeOH extract (58.27 mg GAE/g DW and 320.93 mg CE/g DW respectively), with the highest mortality in larvae and adults (89.411 and 81.538% respectively) at the 60 mg/ml concentration, and LD_{50} values of (17.747 and 21.819 mg/ml respectively). In addition, the MeOH extract of stem bark was the richest in polyphenols and saponins (185.96 mg GAE/g DW and 662.47mg CE/g DW respectively), resulting in higher toxicity than the MeOH extract of leaves. On the basis of these results, we believe that *Z. spina-christi* is an excellent source for the isolation of compounds as alternative insecticidal agents that are safer than the use of chemical pesticides.
Keywords: *Ziziphus spina-christi*, *Aphis fabae*, Phytochemicals, Extracts, Insecticidal activity, Toxicity, Algeria.

1. Introduction

Pesticides are chemical or naturally synthesized products that manage and eliminate many pests (Pathak et al., 2022). Pesticides are of great importance in our contemporary world because they are widely used to protect crops and ensure growing global food demand (Tang et al., 2021). Pesticides make up the largest proportion of synthetic chemical input into the

environment, which has grown faster than any other single driver of global environmental change over the last five decades (Mansfield et al., 2024). In parallel with the importance of pesticides in agriculture, recent studies indicate that they harm the environment and human health. Moreover, their extensive use has contributed to the development of pest-resistant varieties (Perry et al., 2011). This situation has sparked widespread controversy and intense debates in the political and scientific communities (Jacquet et al., 2022; Struelens et al., 2022).

Many nations now share the goal of reducing pesticide use as a major concern in public policies (Jacquet et al., 2022). As a result, global interest in developing alternative strategies has increased (Khan et al., 2017). The plant's secondary metabolism substances are considered important sources of biopesticides; they are a better alternative than chemical insecticides for pest control because they are environmentally friendly and safe for human health (Isman, 2006; Tariq et al., 2010).

Ziziphus spina-christi (L.) is a deciduous tree belonging to the family Rhamnaceae, known globally as Christ's Thorn Jujube and Nebeq or Sidr in the Middle East (Shahat et al., 2001). Tropical and subtropical climate regions are home to *Ziziphus spina-christi* (L.). The distribution of this species is currently extensive, encompassing North Africa, the Mediterranean region, southern Europe, Australia, the subtropical regions of America, as well as the eastern, southern, and Middle Eastern parts of Asia (Asgarpanah and Haghighat, 2012).

Z. spina-christi has long been used in folk medicine to maintain health (El-Ghazali et al., 1994). Recent scientific research indicates that extracts of *Z. spina-christi* have shown various pharmacological activities such as antioxidant (Hassan et al., 2021), anti-inflammatory (Alsayari and Wahab, 2021), antiviral and antidiabetic (Hassan and Abdel-Gawad, 2010), anti-leishmanial (Albalawi, 2021), antimicrobial (Mervat et al., 2018), antifungal (El-Shahir et al., 2022), and anticancer (Soliman et al., 2019).

Researchers have already found that *Z. spina-christi* leaf extract has many plant-based chemicals in it, including flavonoids, alkaloids, tannins, triterpenoids, and phytosterols (Kadioglu et al., 2016). *Z. spina-christi* has been described as a plant rich in saponins and/or alkaloids (Yousef and EI-Kassas, 2013). The phytochemical composition of *Z. spina-christi* has revealed the presence of four saponin glycosides and alkaloids as molluscicides (Anthony, 2005; Shahat et al., 2008).

There is only a limited amount of previous literature on the insecticidal activity of this plant. The aqueous leaf extract has shown molluscicidal activity (Yousef and EI-Kassas, 2013). The

evaluation of the insecticidal potential of different root extracts of *Z. spina-christi* has been studied against *Lasioderma serricorne* (Elaloui et al., 2021). The insecticidal activities of the methanolic leaf extract have been studied on *Tribolium castaneum* (Elaloui et al., 2022). To our knowledge, this is the first report on the phytochemical analysis and insecticidal activity of *Z. spina-christi* from southeastern Algeria.

Therefore, this study was conducted to analyze the bioactive phytochemical components and evaluate the insecticidal activity of different extracts (*N*-hexane, DCM, MeOH, and aqueous) from the leaves and stem bark of *Z. spina-christi*. For this purpose, we chose to test this activity on a harmful insect, *Aphis fabae*, a pest insect that is becoming increasingly resistant to synthetic insecticides.

2. Materials and methods

2.1. Collection and preparation of samples

The leaves and stem bark of *Z. spina-christi* were collected in October 2022 at the University of El Oued, Southeast Algeria. The geographical coordinates of the collection site are as follows: (33°23’41.835" N) and (6°51’28.648" E). Taxonomic identification of the plant was carried out by Prof. Youcef Halis, a specialist from the Center for Scientific and Technical Research on Arid Regions (CRSTRA) in Touggourt, Algeria. The leaves and stem bark were washed with tap water, followed by distilled water, and then air-dried at 37°C for 10 days. Subsequently, they were ground into powder using an electric grinder. The resulting material was sieved through a 2 mm mesh and conserved in a hermetically sealed glass bottle for future use.

2.2. Preparation of extracts

The plant material was extracted using solvents of varying polarity following the protocol by Chaouche et al. (2015), with some modifications. Ten batches of plant powder were successively macerated in *N*-hexane (100 ml), then dichloromethane (DCM, 100 ml), then methanol (MeOH, 100 ml), and distilled water (100 ml) for 24 hours at room temperature with magnetic stirring. Each solvent (organic and aqueous phases) was filtered using Whatman filter paper, and subsequently, the obtained filtrates were concentrated under a vacuum at a temperature not exceeding 45°C. The resulting material was transferred to a sample vial and stored in the dark at 4°C until further use.

2.3. Secondary metabolite contents

2.3.1. Total phenolic quantification

The total phenolic content of the different extracts was determined using the Folin-Ciocalteu method as described by (Slinkard and Singleton, 1977; Singleton et al., 1999). 125 µl of each extract was mixed with 500 µl of distilled water, followed by the addition of 125 µl of the Folin-Ciocalteu reagent. After 3 minutes, 1250 µl of freshly prepared 2% sodium carbonate solution and 1 ml of distilled water were added to the melange and then incubated at room temperature for 90 minutes in the dark. The absorbance of the resulting blue color was measured at 765 nm. Gallic acid was used as a standard, and a range was also prepared with concentrations ranging from 0 to 0.5 mg/ml. The result is expressed in milligrams of gallic acid equivalent per gram of extract dry weight (mg GAE/g DW).

2.3.2. Total flavonoids quantification

The total flavonoid content of each extract was measured using the method described by Turkoglu et al. (2007)with some modifications. 250 µl of each extract to be analyzed was added to 2550 µl of methanol (95%), 0.1 ml of acetic acid solution (1M), and 0.1 ml of aluminum nitrate (10%). After agitation, the mixture was incubated at room temperature in the dark for 40 minutes. The absorbance was read at 415 nm using a quercetin calibration curve (range: 0-0.2 mg/ml). The result is expressed in milligrams of quercetin equivalent per gram of extract dry weight (mg QE/g DW).

2.3.3. Condensed tannins quantification

The content of condensed tannins was evaluated following Hagerman's method (Hagerman, 2002). 1 ml of the sample (prepared in methanol) was added to 5 ml of the analysis reagent, which consisted of a mixture of 2.5 ml vanillin solution (1%) and 2.5 ml HCl solution (8%) (prepared by diluting 8 ml of HCl to 100 ml with methanol). The mixture was vigorously agitated. After 1 minute, 5 ml of a 4% HCl solution was added. The mixture was then placed in a water bath at 30°C and left for 20 minutes, and absorbance was measured at 500 nm. A calibration curve was established using catechin served to quantify condensed tannins (range: 0-0.3 mg/mL). The result is expressed in milligrams of catechin equivalent per gram of extract dry weight (mg CE/g DW).

2.3.4. Total saponins quantification

Total saponins were quantified using the procedure indicated by Hiai et al. (1976), with some modifications. A volume of 0.1 ml of each extract was added to 0.5 ml of an 8% vanillin

solution (w/v) prepared by dissolving 800 mg of vanillin in 10 ml of ethanol (99.5%) freshly prepared (v/v). Next, 5 ml of a 72% sulfuric acid solution (v/v) was added to the inner wall of the tube. The mixture was heated in a water bath at 60°C for 10 minutes, followed by cooling the tubes in an ice-water bath for 3 to 4 minutes. Absorbance was measured at 544 nm relative to the blank reagent. A calibration curve was established using diosgenin to quantify saponins (range: 0-0.1 mg/ml). The result is expressed in milligrams of diosgenin equivalent per gram of extract dry weight (mg GAE/g DW).

2.3.5. Total alkaloid quantification

The gravimetric determination of alkaloids follows the procedure proposed by Harborne (1998) with some modifications. Approximately 5 g of dry leaf powder and stem bark of *Z. spina-christi* were dispersed in 50 ml of acetic acid in methanol (10%). The solution was shaken in an incubator with a shaker at 120 revolutions/minute at a temperature of 30°C, then left to rest for 4 hours. The suspension was filtered using filter paper, and the filtrate was evaporated to ¼ of its original volume using a heating plate. Concentrated ammonium hydroxide (30%) was added dropwise to precipitate the alkaloids from the filtrate. The precipitate was filtered using pre-weighed filter paper and washed with an ammonium hydroxide solution (1%). The filter paper containing the alkaloids was dried and reweighed, and its content was determined by the difference in weight of the filter papers and expressed in milligrams per gram of dry weight.

2.4. Insect rearing

We collected plants from a fava bean culture infested with Aphis fabae and placed them in plastic boxes with lids made of stretchable food film paper with meshed holes to allow light penetration and air exchange. The boxes were raised in a growth chamber at a temperature of 25 ± 1°C with a relative humidity of 60 ± 10% and 16 hours of exposure to artificial light at an intensity of about 4000 lux (Salari et al., 2012).

2.5. Insecticidal activity bioassay

The effects of hexane, dichloromethane, methanol, and aqueous extracts from the leaves and stem bark of *Z. spina-christi* were tested on the larvae and adults of *Aphis fabae* Scopoli. Four experimental treatments and one negative control were prepared. The range of experimental treatments was prepared with a 10% dimethyl sulfoxide (DMSO) solution using extracts at 7.5, 15, 30, and 60 mg/ml. The negative control was prepared with a 10% DMSO solution. Each of

the extracts was tested for insecticidal activity using a contact toxicity test according to the method of Pascual-Villalobos and Robledo Miras (1999), with some modifications. An aliquot of 250 µl from each concentration of each extract was applied to separate filter paper discs in Petri dishes (5.5 cm in diameter × 0.5 cm in height), and they were left for 3 to 5 minutes to absorb the aliquots. Ten healthy and active insects of the same size and age for both larvae and adults were placed in each dish using a clean brush. Five repetitions for each concentration and the control treatment were included.

All experiments were conducted under the same laboratory conditions, at a temperature of 25±1°C, relative humidity of 60±10%, and a photoperiod of 16:8 (L:D) (Salari et al., 2010). The mortality of larvae and adults of Aphis fabae was observed and recorded after 24 and 48 hours after the start of exposure to the treatments. The Abbott formula was used to correct the observed mortality results for control mortality (Abbott, 1925):

$$Ma\ (\%) = [(100 - Mc)(Mt - Mc)] \times 100$$

Where:

Ma: corrected mortality percentage.

Mt: percentage of mortality in the treatment group.

Mc: percentage of mortality in the control group.

2.6. Statistical Analysis

The data were collected and analyzed using IBM SPSS Statistics (Version 27.0.1). The MANOVA test was used to analyze and compare the results between the extracts, followed by the Tukey test. The *t*-test was used to analyze and compare the results of the alkaloid content between plant parts. The effects of the extracts on mortality were analyzed and compared by one-way analysis of variance (ANOVA) followed by the Tukey test. The LD_{50} values were calculated using Probit analysis by SPSS Statistics (Sakuma, 1998).

3. Results and discussion

3.1. Secondary metabolites contents

The obtained results showed that the total content of phenols, flavonoids, condensed tannins, and saponins varies from one part to another and from one extract to another (Tables 1 and 2). The MANOVA test revealed a highly significant difference ($P < 0.001$: ***) between the

different extracts and the total contents of phenols, flavonoids, condensed tannins, and saponins, both in the leaves and the stem bark of *Z. spina-christi*. In other words, solvent types significantly affect the quantity of secondary metabolites extracted, depending on their polarity levels.

The results of the present study show that the MeOH extract from *Z. spina-christi* leaves is very rich in phenols and saponins compared to other extracts, as it represents very significant contents of 58.27±0.618 mg GAE/g DW and 320.93±1.475 mg DE/g DW, respectively. In contrast, the DCM extract has the lowest values for phenols, tannins, and saponins compared to those found in other extracts, which are 8.49 ± 0.974 mg GAE/g DW, 0.002 ± 0.0005 mg CE/g DW, and 23.83 ± 1.552 mg DE/g DW, respectively (Table 1). As for the MeOH extract from the stem bark of *Z. spina-christi*, it is very rich in secondary metabolites, representing the highest values for phenols, flavonoids, tannins, and saponins, which are 185.96 ± 1.254 mg GAE/g DW, 8.92 ± 0.319 mg QE/g DW, 0.014 ± 0.0002 mg CE/g DW, and 662.47 ± 4.838 mg DE/g DW, respectively, almost 2 or 3 times higher than in the aqueous extract. However, the extracts obtained from *N*-hexane and DCM have extremely low values, representing less than 15% of the substances found in the MeOH extract (Table 2). According to El Maaiden et al. (2019) the phytochemical contents of the different extracts vary depending on the solvent used and its polarity, which plays a key role in increasing the solubility of these components.

Saponins represent the most abundant component compared to other phytochemical constituents in the leaves and stem bark of *Z. spina-christi* (Tables 1 and 2). In the same context, a recent study by Albalawi (2021) confirmed the presence of large quantities of saponins in the leaves. Also, phytochemical analysis of the ethanolic extract from the stem bark revealed abundant saponins (Taghipour et al., 2020).

The *t*-test revealed a highly significant difference (P = 0.009: **) in alkaloid concentration between the leaves and stem bark of *Z. spina-christi*"Figure 1". Alkaloid content varies across different parts of the plant. The leaves exhibit a value of 56.70 ± 3.245 mg/g DW, which is higher than that reported in previous studies by Khaleel (2018). Phytochemical substances are highly sensitive to environmental factors (Azwanida, 2015) and vary based on environmental conditions (Vaidya et al., 2014). Conversely, the stem bark contains a lower alkaloid quantity (43.50 ± 3.50 mg/g DW) than found in the leaves. The distribution of saponins among different plant parts depends on the plant's age (Kharkwal et al., 2012).

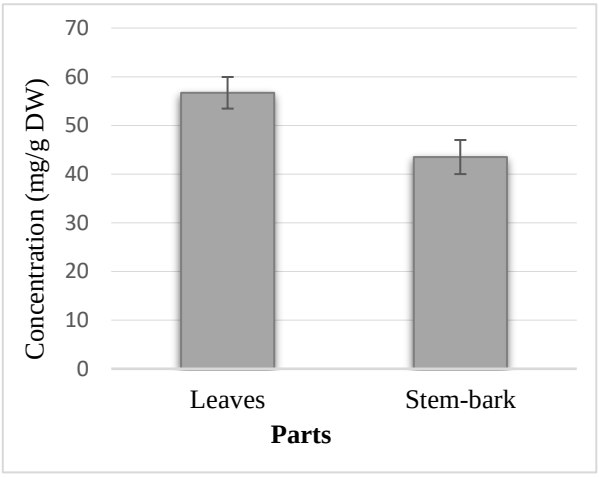

Figure 1. Total alkaloid concentration in the dry leaves and the stem bark of Z. spina-christi. Alkaloid content is expressed as the mean ± SE (n=3). Statistical significance was calculated by t-test at a threshold of 0.05.

Table 1. Contents of major bioactive compounds of leaves were extracted using solvents of various polarities.

Extracts	TPC [A]	TFC [B]	CTC [C]	TSC [D]
***N*-hexane**	11.86 ± 0.945 [bc]	28.62 ± 0.603 [a]	0.012 ± 0.0002 [a]	49.11 ± 1.215 [b]
DCM	8.49 ± 0.974 [c]	5.69 ± 0.654 [d]	0.002 ± 0.0005 [c]	23.83 ± 1.552 [d]
MeOH	58.27 ± 0.618 [a]	20.04 ± 0.998 [b]	0.002 ± 0.0001 [c]	320.93 ± 1.475 [a]
Water	12.82 ± 2.473 [b]	11.78 ± 0.524 [c]	0.005 ± 0.0001 [b]	41.22 ± 2.383 [c]

The contents are expressed as the mean ± SD (n=3). Statistical significance was calculated by one-way ANOVA test (P < 0,001: ***). Different letters in the same column indicate significant differences between the different extracts for each parameter determined by Tukey's test.

[A] Total phenolic content expressed as mg of gallic acid equivalent per g of dry weight (mg GAE/g DW).

[B] Total flavonoid content expressed as mg of catechin equivalent per g of extract (mg QE/g DW).

[C] Condensed tannins content expressed as mg of catechin equivalent per g of extract (mg CE/g DW).

[D] Total saponin content expressed as mg of diosgenin equivalent per g of extract (mg DE/g DW).

Table 2. Contents of major bioactive compounds of stem bark were extracted using solvents of various polarities.

Extracts	TPC [A]	TFC [B]	CTC [C]	TSC [D]
***N*-hexane**	10.07 ± 0.238 [c]	2.87 ± 0.479 [c]	0.005 ± 0.0003 [b]	3.05 ± 2.383 [d]
DCM	1.75 ± 0.357 [d]	0.61 ± 0.079 [d]	-	92.43 ± 2.178 [c]
MéOH	185.96 ± 1.254 [a]	8.92 ± 0.319 [a]	0.014 ± 0.0002 [a]	662.47 ± 4.838 [a]
Water	72.09 ± 0.545 [b]	5.50 ± 0.138 [b]	0.001 ± 0.0001 [c]	205.95 ± 2.011 [b]

The contents are expressed as the mean ± SD (n=3). Statistical significance was calculated by one-way ANOVA test (P < 0,001: ***). Different letters in the same column indicate significant differences between the different extracts for each parameter determined by Tukey's test.

[A] Total phenolic content expressed as mg of gallic acid equivalent per g of dry weight (mg GAE/g DW).

[B] Total flavonoid content expressed as mg of catechin equivalent per g of extract (mg QE/g DW).

[C] Condensed tannins content expressed as mg of catechin equivalent per g of extract (mg CE/g DW).

[D] Total saponin content expressed as mg of diosgenin equivalent per g of extract (mg DE/g DW).

3.2. Insecticidal activity bioassay

The insecticidal potential of leaf and bark extracts of *Z. spina-christi* was tested at different concentrations by contact against *A. fabae* larvae and adults. Mortality results and toxicity values after 48 hours for leaf extracts are presented successively in Tables 3, 4 and 5, and those for stem bark are presented successively in Tables 6, 7 and 8.

According to the Tukey test, significant mortality values were recorded for larvae and adults treated with high concentrations (60 and 30 mg/ml) compared to the effects of treatments at lower concentrations (15 and 7.5 mg/ml), most of which resulted in low mortality rates (less

than 30%). We observe that mortality and toxicity in different extract treatments are higher in *A. fabae* larvae compared to adults, demonstrating that larvae are more sensitive to insecticides than adults. The low mortality and toxicity in adults may be associated with their limited capacity for absorption and penetration of the extracts through the cuticle. The structure of the cuticle and the degree of sclerotization in larvae can influence the level of toxicity, as the integument serves as the primary barrier of defence against xenobiotics (Alberti and Coons, 1999; Tunaz and Uygun, 2004).

The main results of our study indicate the presence of insecticidal potential and toxicity in the leaves and stem bark of the plant, which vary from one extract to another based on the quantity of secondary metabolites. For the leaves, the MeOH extract is the most effective and the most toxic, followed by the aqueous extract, while the lowest activity was observed in the *N*-hexane and DCM extracts (Tables 3, 4, and 5). Regarding the stem bark, the MeOH extract showed higher mortality and toxicity, followed by the aqueous and *N*-hexane extracts, while the DCM extract exhibited the lowest values (Tables 6, 7, and 8).

The ANOVA test reported a statistically highly significant difference ($P < 0.001$: ***) between the various concentrations of leaf and stem bark extracts of the plant in terms of mortality rates for larvae and adults (Tables 3 and 6). This means that the extracts have a highly significant concentration-dependent effect on mortality rates in both *A. fabae* larvae and adults. The findings of Rahman et al. (2007), and Imran et al. (2021) suggest that mortality rates are directly proportional to the extract concentration, indicating that higher treatment concentrations lead to increased mortality rates.

Regarding the tested leaf extracts, the MeOH extract exhibited the highest mortality rates in both larvae and adults compared to other extracts, with larval mortality rates of 89.411 ± 9.66% at 60 mg/ml and 68.235 ± 7.89% at 30 mg/ml, and adult mortality rates of 81.538 ± 10.32% at 60 mg/ml and 67.692 ± 5.16% at 30 mg/ml (Table 3). The LD_{50} also indicated strong toxicity of the MeOH extract, with a value of 17.747 mg/ml against larvae and 21.819 mg/ml against adults (Tables 4 and 5).

Similarly, the MeOH extract from the stem bark, which is the most interesting, caused mortality rates of 89.411 ± 9.66% and 68.235± 7.89% in larvae, and 81.538 ± 6.31% and 67.692 ± 5.1% in adults at 60 and 30 mg/ml, respectively (Table 6). These effects are similar to those observed with the MeOH leaf extract. The LD_{50} also indicates high toxicity, with a value of 12.377 mg/ml against larvae and 21.312 mg/ml against adults (Tables 7 and 8).

Furthermore, the aqueous leaf extract and the aqueous and *N*-hexane stem bark extracts exhibit nearly similar insecticidal activity in larvae, resulting in mortality rates of 71.764 ± 9.66%, 75.294 ± 9.66% and 75.294 ± 9.66% at 60 mg/ml, and 43.529 ±7.89%, 50.588 ± 7.89%, and 54.117 ± 9.66% at 30 mg/ml, respectively (Tables 3 and 6). These extracts also demonstrated comparable contact toxicity against larvae, with LD_{50} values of 28.542, 27.178, and 27.599 mg/ml (Tables 4 and 7). On the other hand, mortality rates and toxicity values for adults are lower than those for larvae, represented as 67.692 ± 5.16%, 60.769 ± 10.32% and 53.846 ± 8.15% at 60 mg/ml, and 42.307 ± 14.13%, 37.692 ± 10.32% and 35.384 ± 6.31% at 30 mg/ml, respectively (Tables 3 and 6). The LD_{50} values for adults are 35.109, 43.467, and 55.722 mg/ml (Tables 5 and 8).

However, the extracts that exhibited low insecticidal activity and toxicity against *A. fabae* larvae compared to other extracts are the *N*-hexane and DCM extracts from the leaves, as well as the DCM extract from the stem bark, with mortality rates of 61.176 ± 7.89%, 61.176 ± 7.89%, and 54.117 ± 15.78% at 60 mg/ml; and 40 ± 9.66%, 54.117 ± 9.66%, and 25.882 ± 7.89% at 30 mg/ml, respectively (Tables 3 and 6); and toxicity values of (LD_{50} = 41.5, 35,763, and 65,356 mg/m, respectively) (Tables 4 and 7). Regarding adults, mortality and toxicity induced by *N*-hexane and DCM leaf extracts, as well as the DCM stem bark extract, were lower than those observed in larvae, with mortality rates successively of 53.846 ±8.15%, 51.540 ±5.15%, and 51.538 ±12.63% at 60 mg/ml, and 28.461 ±5.16%, 35.387 ±6.31%, and 21.538 ±12.63% at 30 mg/ml (Tables 3 and 6), and toxicity of (LD_{50} = 60.774, 56.527, and 70.237 mg/ml, respectively) (Tables 5 and 8).

Table 3. The mean mortality rate of *Aphis fabae* larvae and adults exposed to different concentrations of leaves extracts after 48 hours of exposure.

Concentration (mg/ml)	Mean Percentage Mortality (Mean (SD))							
	N-hexane extract		DCM extract		MeOH extract		Water extract	
	Larvae	Adults	Larvae	Adults	Larvae	Adults	Larvae	Adults
60	61.176 (7.89) a	53.846 (8.15) a	61.176 (7.89) a	51.540 (5.15) a	89.411 (9.66) a	81.538 (10.32) a	71.764 (9.66) a	67.692 (5.16) a
30	40 (9.66) b	28.461 (5.16) b	54.117 (9.66) a	35.387 (6.31) b	68.235 (7.89) b	67.692 (5.16) b	43.529 (7.89) b	42.307 (14.13) b
15	25.882 (7.89) bc	14.615 (6.31) c	18.823 (9.66) b	14.618 (6.31) c	36.470 (9.66) c	33.076 (5.16) c	36.470 (9.66) bc	26.153 (10.32) bc
7.5	22.352 (9.66) c	12.307 (6.31) c	15.294 (7.89) b	10.003 (5.15) c	25.882 (14.76) c	14.615 (6.31) d	22.352 (9.66) c	12.307 (6.31) c

Mortality is expressed as the mean ± SD (n=5). Statistical significance was calculated by one-way ANOVA test (P < 0,001: ***). Different letters in the same column indicate significant

differences between different concentrations of each extract for larvae and adults, determined by Tukey's test.

Table 4. Contact toxicity of leaves different extracts against *Aphis fabae* larvae after 48 hours of exposure.

Extract	LD_{50} [a]	95% FL[a]	Slope ± SE	$\chi 2$ (df)	*P*-value
***N*-hexane**	41.5	31.626 - 62.926	1.191 ± 0.199	2.591 (2)	0.274
DCM	35.763	-	1.648 ± 0.209	7.390 (2)	0.025
MeOH	17.747	15.207- 20.530	2.135 ± 0.219	3.98 (2)	0.137
Water	28.542	23.065 - 36.930	1.39 ± 0.2	3.478 (2)	0.176

[a] Dose (mg/ml)

Table 5. Contact toxicity of leaves different extracts against *Aphis fabae* adults after 48 hours of exposure.

Extract	LD_{50} [a]	95% FL[a]	Slope ± SE	$\chi 2$ (df)	*P*-value
***N*-hexane**	60.774	46.115 - 93.904	1.480 ± 0.218	3.805 (2)	0.149
DCM	56.527	43.960 - 82.783	1.570 ± 0.221	1.423 (2)	0.491
MeOH	21.819	18.962 - 25.132	2.263 ± 0.221	1.979 (2)	0.372
Water	35.109	29.310 - 44.018	1.769 ± 0.214	0.506 (2)	0.776

[a] Dose (mg/ml)

Table 6. The mean mortality rate of *Aphis fabae* larvae and adults exposed to different concentrations of stem bark extracts after 48 hours of exposure.

Concentration (mg/ml)	Mean Percentage Mortality (Mean (SD))							
	***N*-hexane extract**		**DCM extract**		**MeOH extract**		**Water extract**	
	Larvae	**Adults**	**Larvae**	**Adults**	**Larvae**	**Adults**	**Larvae**	**Adults**
60	75.294 (9.66) a	53.846 (8.15) a	54.117 (15.78) a	51.538 (12.63) a	89.411 (9.66) a	81.538 (6.31) a	75.294 (9.66) a	60.769 (10.32) a
30	54.117 (9.66) b	35.384 (6.31) b	25.882 (7.89) b	21.538 (12.63) b	68.235 (7.89) b	58.461 (10.32) b	50.588 (7.89) b	37.692 (10.32) b
15	25.882 (7.89) c	21.538 (5.16) c	18.823 (9.66) b	12.307 (6.31) b	57.647 (9.66) b	37.692 (6.31) c	29.411 (12.47) c	21.538 (12.63) bc
7.5	15.294 (7.89) c	16.923 (9.65) c	15.294 (7.89) b	10.000 (5.16) b	36.470 (9.66) c	21.538 (12.63) c	18.823 (9.66) c	12.307 (6.31) c

Mortality is expressed as the mean ± SD (n=5). Statistical significance was calculated by one-way ANOVA test ($P < 0{,}001$: ***). Different letters in the same column indicate significant differences between different concentrations of each extract for larvae and adults, determined by Tukey's test.

Table 7. Contact toxicity of different stem bark extracts against *Aphis fabae* larvae after 48 hours of exposure.

Extract	LD_{50} [a]	95% FL [a]	Slope ± SE	$\chi 2$ (df)	*P*-value
***N*-hexane**	27.599	23.612 - 32.841	1.975 ± 0.214	1.294 (2)	0.425
DCM	65.356	47.09 - 114.792	1.261 ± 0.210	5.504 (2)	0.064
MeOH	12.377	9.609 - 15.046	1.635 ± 0.211	2.201 (2)	0.333
Water	27.178	22.884 - 33.000	1.760 ± 0.208	1.476 (2)	0.478

[a] Dose (mg/ml)

Table 8. Contact toxicity of different stem bark extracts against *Aphis fabae* adults after 48 hours of exposure.

Extract	LD_{50} [a]	95% FL [a]	Slope ± SE	$\chi 2$ (df)	*P*-value
***N*-hexane**	55.722	40.89 - 93.786	1.212 ± 0.205	1.275 (2)	2.529
DCM	70.237	-	1.549 ± 0.227	6.023 (2)	0.049
MeOH	21.312	18.02 - 25.219	1.850 ± 0.209	0.633 (2)	0.729
Water	43.467	35.095 - 58.508	1.611 ± 0.213	0.605 (2)	0.739

[a] Dose (mg/ml)

The insecticidal activity of the extracts is attributed to the presence of various active chemical compounds. Based on our results regarding the quantity of bioactive compounds in the most effective extracts (MeOH extracts from leaves and stem bark), we can attribute their insecticidal activity to the significant presence of phenolic compounds, saponins, and possibly alkaloids.

In general, secondary metabolites produced by plants present a wide range of activities. Their actions can disrupt physiological and cellular processes responsible for maintaining homeostasis, leading to changes in various organs and tissues, ultimately leading to death. (Chowański et al., 2016). Flavonoids, alkaloids, terpenes, sterols, phenols, tannins, waxes, and fats, among others, are components that defend against invertebrate pests and microbial pathogens (Gottlieb, 1990; Wink and Schimmer, 2018). Furthermore, various studies have considered plant phenolic compounds one of the most important defences against insects (Barbehenn and Martin, 1994; Barbehenn et al., 1996; Henn, 1997).

According to Simmonds (2001), flavonoids exhibit insecticidal effects. Gallo et al. (2006) demonstrated that quercetins extracted from *Vitex polygama* are highly active substances, resulting in mortality rates of 78% and 85% against *Spodoptera frugiperda* larvae.

Previous research has shown that some types of quercetin can strongly block mitochondrial ATPase (Lang and Racker, 1974), the mixed-function oxidases (MFOs) dependent on cytochrome P-450 (Mitchell et al., 1993), and glutathione-S-transferases in *S. frugiperda* larvae (Yu and Abo-Elghar, 2000).

Concerning saponins, numerous researchers have demonstrated that plant-derived saponins possess significant insecticidal and cytotoxic potential due to their toxic nature against a wide range of insect pests such as aphids, beetles, weevils, leafhoppers, caterpillars, moths, etc. Saponins significantly affect the behavior, metabolism, and physiology of aphids, thereby reducing their populations on plants (Singh and Kaur, 2018). Regarding alkaloids, natural alkaloids from different chemical classes have been shown to have insecticidal effects (Zhu et al., 2012; Kathuria et al., 2013; Chowański et al., 2016).

4. Conclusion

The results of this study have shown that the leaves and stem bark of *Z. spina-christi* are rich sources of important bioactive phytochemical substances. Our findings allow us to deduce that the mortality and toxicity of insects to MeOH extracts depend on the amount of secondary metabolites (polyphenols, flavonoids, tannins, and saponins) they contain. *Z. spina-christi* offers interesting insecticidal potential that could be further studied by isolating and identifying the active substances in the MeOH extracts, studying their impact on the physiology, growth, and reproduction of aphids, and possibly seeking methods to valorize this extract as a bioinsecticide. In general, this work constitutes an interesting first step in developing Algerian extracts of *Z. spina-christi* as natural, effective, and affordable alternatives to replace the current chemical pesticides.

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