

Phytochemical analysis and biological activities of Tunisian *R. communis* L. aqueous extracts

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Abstract

Plant based insecticides are considered among the most economic and ecofriendly chemicals for the protection of plants and stored grains. The aim of this research was to evaluate the insecticidal and antioxidant activities of two ecotypes of Tunisian *Ricinus communis* leaves aqueous extracts. Samples collected from Mograne revealed the best total phenols (101.81 mgGAE.g⁻¹DW), and flavonoids (3.79 mgER.g⁻¹DW) contents. This aqueous extract revealed also the best antioxidant capacity (IC₅₀=310.54 µg.ml⁻¹). The insecticidal activity of *R. communis* aqueous extracts leaves were tested against *Tribolium castaneum*. Extracts were prepared and tested at three concentrations (10, 5 and 2.5%) and compared to effects of distilled water which is used as a control. The best values of LC₅₀ and LC₉₀ calculated for aqueous extracts were, respectively, 3.369 and 4.979 mg/ml, 24 h after application, for Mograne samples.

Keywords: *Ricinus communis*, insecticidal activity, antioxidant activity, aqueous extracts

Introduction

The use of natural products in pest control has been the subject of research in many countries, mainly due to the increased resistance of these organisms to synthetic pesticides. This resistance is due to the indiscriminate use of chemicals, that can cause enormous damage to humans and the environment by pervasive chemical waste (Delmonte *et al.*, 2017) [5]. In view of this situation, it is necessary to search for new alternatives for the control of agronomic pests, such as those from botanical extracts that are generally more environmentally friendly and less damaging to the health of humans as well as other non-target organisms.

Ricinus communis Linn., with the common name castor oil plant, is a member of Euphorbiaceae. is a perennial plant of 1–2 m, and, in special cases, 3–5 m in height. It is grown in tropical and subtropical regions and produces seeds over a period of 10–15 years (Bockisch, (1998) [2]. It is a soft-wooded small tree widespread throughout tropics and warm temperature regions of the world (Ilavarasan *et al.*, 2004) [8]. To date, a total of 83 compounds have been isolated from various parts of *R. communis*, including alkaloids, terpenoids, flavonoids, benzoic acid derivatives, coumarins, tocopherols, and fatty acids. The plant is proven to contain antioxidant properties, anti-inflammatory activity, cytotoxic, insecticidal, anti-diabetic activity and antibacterial activity (Shookin *et al.*, 2008 [20]; Rao *et al.*, 2013 [17]; Kuete, 2014 [10]). It has hepatoprotective effect and is used in the treatment of skin cancer (Prakash *et al.*, 2014) [15]. Its cultivation faces some challenges due to the presence of the toxic ricin, an alkaloid ricinine, and an allergenic protein polysaccharide CB-1A in castor beans. Ricin inhibits protein synthesis by inactivating ribosomes, and it is lethal when taken in any way (Maheshwari and Kovalchuk., 2016) [12].

Currently, this plant species is widely distributed worldwide and has many industrial, ornamental, and medicinal applications (Rana *et al.* 2012) [16]. Various parts of *R. communis* have been widely used in traditional medicine such as abdominal disorders, arthritis, backache, muscle

aches, bilharziasis, chronic backache and sciatica, chronic headache, constipation, rheumatism, sleeplessness, and insomnia (Marwat *et al.*, 2017) [13].

Thus, in the present work, we explore the phenolic content of aqueous extracts of *R. communis* collected from two geographical zones. Additionally, we carried out the antioxidant (using DPPH method) and the insecticidal (against *T. castaneum*) activities.

Material and methods

Plant material

R. communis was collected from two different geographic zones in Tunisia. Details of the main ecological traits of the analysed populations are presented in Table 1. Before analyses, leaves were separated and air dried at room temperature for two weeks, then ground to powder before analysis.

Table 1: Origin of plant material

Governorate	Location	Latitude	Longitude
Nabeul	Nabeul	36.4167	10° 36' 0" E
Zaghouan	Mograne	36° 25' 20"	10° 5' 25" E

Extracts preparation

The extracts were prepared by maceration in water for 24h. 10 grams of each sample were ground and mixed with 100 mL of distilled water. After filtration, each extract was stored at 4 °C prior to further analysis.

Total phenolic and flavonoid contents

The total phenolic contents (TPC) and flavonoids (TF) of each extract were quantified as described before by Jaouadi *et al.* (2019) [9].

Antioxidant activity

The evaluation of the antioxidant activity was determined using the 1,1-diphenyl-2-picryl-hydrazil radical (DPPH•), as described by Jaouadi *et al.* (2019) [9]. Results were expressed as IC₅₀ (concentration required to inhibit 50% of DPPH•).

Insecticidal activity

Insects rearing

The insecticidal efficiency was evaluated against *T. castaneum*. The pest specie was extracted from the infested wheat kept at the Laboratory of Entomology at the High School of Agriculture of Mograne.

Contact bioassay

Filter paper disc (9 cm) was placed in a Petri dish and soaked with different concentrations (10, 5 and 2.5%) of extracts and a group of 10 adults of *T. castaneum* were placed in each Petri dish. After 24 hours, the number of dead insects was recorded. All Petri dishes were stored in a climate room at 25±1°C, 60- 70% Relative Humidity, and a photoperiod of 16:8 (L:D) h. Mortality rates of different treatments were estimated and corrected using the Abbott's formula (Abott 1925)^[1].

Statistical analysis

The significance of the variation of phenolic extracts and their biological activities were determined using one-way Analysis of Variance (ANOVA) followed by Duncan's multiple range tests, using SPSS software version 26.0 for Windows. For the insecticidal activity, bioassay data were obtained using the Probit analysis to find out the median effective concentrations (LC₅₀ and LT₉₀ values).

Results and discussion

Variation of polyphenolic contents among ecotypes

In our work, the contents of phenolic compounds of *R. communis* ecotypes were variable (Table 2), with maximum levels found in plants grown in the upper semi-arid bioclimatic zone (101.81mg GAE/g⁻¹DW). As compared to previous literature data, the total amount of phenolic compounds herein found were in general lower to those revealed for *R. communis* plants grown in Hammamet, Nefza, Riadh El Andalous and Khanguet Hajej (174.25-623.7 mg GAE/g⁻¹DW) (Ghnimi *et al.*, 2014)^[6]. The variation, within bioclimates could be presumably due to genetic factors rather to environmental effect (Chetoui *et al.*, 2013)^[4]. Indeed, several studies revealed) relationships between environmental factors (soil composition, UV-radiation, temperature) and biotic stresses (pathogen and insect attack, herbivorous predators) (Rezende *et al.*, 2015^[18]; Liu *et al.*, 2016^[11]).

Table 2: Averages of total phenolic (mg GAE.g⁻¹DW) and flavonoid (mg ER.g⁻¹DW) contents according to different ecotypes.

Assays	Mograne	Nabeul
TPC (mgGAE.g ⁻¹ DW)	101.81 ^a ± 1.21	42.54 ^b ± 2.07
TF (mgER.g ⁻¹ DW)	3.79 ^b ± 0.02	4.02 ^a ± 0.0

TPC: total phenolic compounds, TF: total flavonoids. Numbers in lines followed by the same letter are not significant at p > 0.05 (Duncan's multiple range test).

Antioxidant activity

DPPH• has been used to evaluate the antioxidative activity of *R. communis* extracts. Hydrogen-donating ability of the antioxidant is responsible for its free radical-scavenging activity. The DPPH• method is based on the reduction of DPPH •solution to DPPH-H in the presence of a hydrogen-donating antioxidant (Singh *et al.*, 2009)^[21].

R. communis aqueous extracts were found to possess antioxidant activity as determined by their capacity to neutralize free radicals (Table 3). *R. communis* sample collected from Mograne exhibited the best radical scavenging activity (IC₅₀ =310.54 µg.ml⁻¹).

Table 3: Antioxidant activity of *R. communis* extracts.

DPPH	Mograne	Nabeul
IC ₅₀ (µg.ml ⁻¹)	310.54 ^b ± 6.27	550.22 ^a ± 4.03

Numbers in lines followed by the same letter are not significant at p > 0.05 (Duncan's multiple range test).

Comparing to other studies reported in the literature, our results demonstrate that, the aqueous leaves extracts of the Tunisian castor were more effective than those reported by Ilavarasan *et al.* (2005)^[8] for Indian *R. ccommunis*, where IC₅₀ values varied between 1.03 and 5.78 g/ml. The antioxidant activity of natural products, such as phenolic acids and flavanoids, is due to the presence of free hydroxyls (Cai *et al.*, 2006)^[3].

Insecticidal activity

The different concentrations of *Ricinus communis* leaf extracts (2.5%, 5% and 10%) showed effective insecticidal activity against adults of *Tribolium castaneum* in comparison to control (Figure 1). Indeed, the percent of mortality in control petri dishes were very low during the six first days of observation and they ranged between 0 and 20 % (Figure 1). Results showed that contact toxicity depended on, ecotype studied, dose used and exposure period. Indeed, adults' mortality increased with extracts concentrations and exposure time which are in agreement with the findings of Harish *et al.* (2020)^[7] who tested the efficacy of Castor, Pongamia, Eucalyptus and Neem oils against Bruchid beetle. In fact, for the two tested samples, mortality rates observed with the lowest concentration (2.5%) did not exceed 25% during fourth day after treatment. Regarding the highest concentration of *R. communis* aqueous extracts (10%) the highest mortality rate was revealed for Mograne sample (more than 80%) after eleven treatment days. However, for Nabeul extracts, mortality rates did not exceed 60%.

Pro bit analysis revealed that Mograne sample extracts showed the best insecticidal activity against *T. castaneum* with LC₅₀ and LC₉₀ values of 3.369 mg/ml and 4.479 mg/ml, respectively (Table 2).

Table 4: LC₅₀ and LC₉₀ (mg/ml) values of *R. communis* aqueous extracts against *T. castaneum*, after 24h of treatment.

Samples	LC ₅₀	LC ₉₀
Nabeul	4.422	6.872
Mograne	3.369	4.979

The disparity of toxicity among the samples might be due to differences in their chemical structures. Several studies revealed insecticidal activity of *Euphorbiaceae* family, especially *Ricinus* species (Rana *et al.*, 2012)^[16]. In line with that, Obeng-Ofori and Freeman (2001)^[14] showed the effectiveness of leaf and seed powders and slurries of several plant species, including *R. communis* and *S. nigrum*, in protecting stored wheat and maize grains against damage caused by *S. oryzae* and *P. truncatus* (Horn).

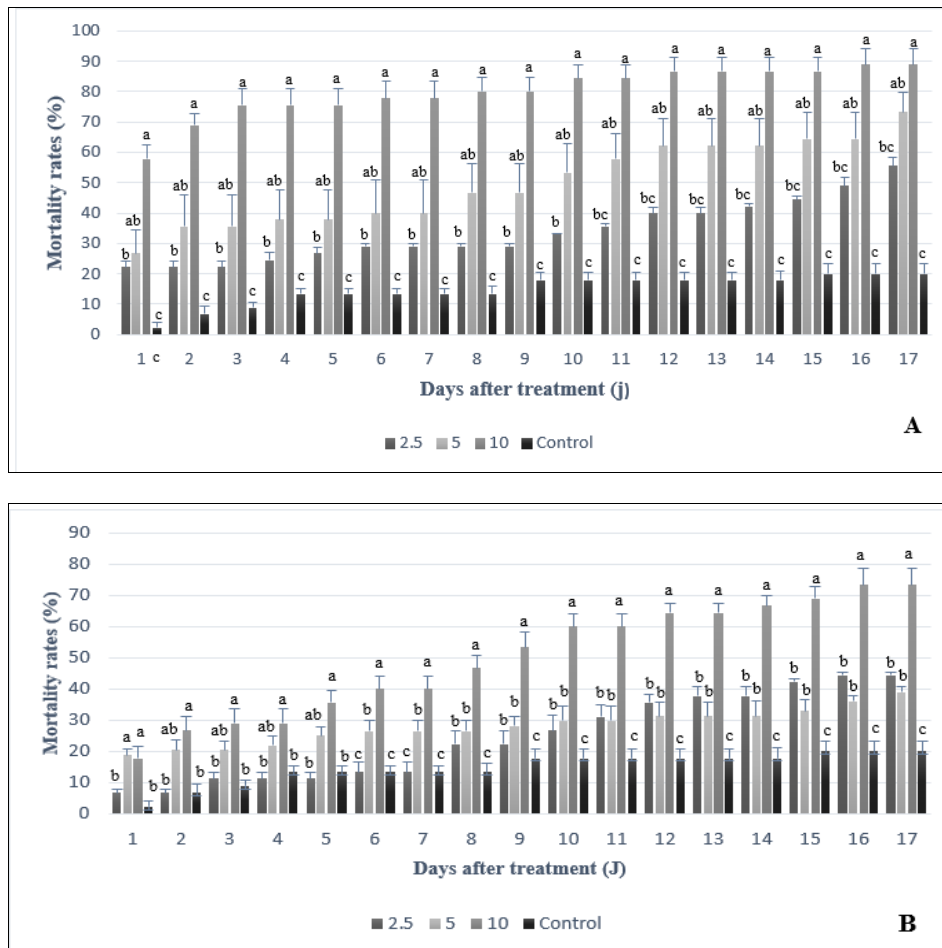


Fig 1: Insecticidal effect of *R. communis* Mograne (A) and Nabeul (B) extracts (Values followed by the same letters are not significant at $p > 0.05$ (Duncan's multiple range test).

Shaaya *et al.*, (2019)^[19] suggested good potential for the use of materials derived from *R. communis* and as toxicant agents in storage pest management systems, particularly for resource poor farmers in developing countries. The insecticidal activity of this plant materials might be attributed to its major components of protein ricins and alkaloid ricinine which are lethal at very low concentrations.

Conclusion

This work was carried out in order to evaluate the antioxidant and insecticidal activities of two *R. communis* ecotypes. The best biological activities was attributed to Mograne extract, suggesting the future use as an alternative for pest control, with minimal negative impact on health and the environment.

Conflicts of interest

The authors declare no conflict of interest.

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