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ANTIOXYDANT AND INSECTICIDAL ACTIVITY OF ALGERIAN *MYRTUS COMMUNIS L.* EXTRACTS

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ABSTRACT

This study was designed to examine the antioxidant and insecticidal activity of the essential oils and methanolic extracts of *M.communis* grown in three different areas of Algeria. Myrtle leaf was the valuable organ for the essential oil production representing a yield between 0.33 and 0.41 % (w/w). Leaves methanol extract was analyzed in terms of the dosage in total phenolic and flavonoids contents. Gallic acid equivalent representing total phenolic constituents of methanolic extracts were 78.79, 75 and 83.03 mg GAE/g; and Quercetin equivalent representing total flavonoids were 21.61, 16.81 and 17.42 mg QE/g of myrtle from Tizi Ouzou, Hamam Melouan and Tablat respectively. Antioxidant activity of the essential oil and the methanolic extract from different myrtles were evaluated by using DPPH radical scavenging, methanolic extracts of different myrtle showed better antioxidant activity than essential oils. The samples were tested for their insecticidal activity against adults of *S.oryzae (L.)* and *T. confusum (Duv)*, using direct contact application and fumigation methods.

KEYWORDS: Myrtus communis, Essential Oil, Methanol Extract, Antioxidant and Insecticidal Activity

INTRODUCTION

Myrtle (*Myrtus communis L.*) is a diploid (2n = 2x = 22) shrub typical of the Mediterranean area. (González-Varo et al, 2009) Common myrtle belongs to the Myrtaceae family, which comprises approx. 145 genera and over 5500 species. (Snow et al, 2011) .This shrub with leathery hermaphrodite, persistent and it has longevity of over 300 years. Generally, it is found at an altitude not exceeding 800 m. It grows equally well on limestone or silica, and easily adapts to many soils. It is fairly resistant to cold and readily acclimatizes to hot weather. It is a shrub, one to three meters tall, with bright green leaves and white flowers during the blossoming season (June to July). The fruit is spherical in shape, dark blue to black in color, edible but astringent, and grows in autumn (Jeanmonod et al, 2007). Commonly called myrtle, its name goes back to Greek myrtos probably loaned from Semitic, it is known not only as an aromatic plant, but also for its therapeutic importance. It is known for its essential oil extracted from leaves and mature fruits, rich in linear, cyclic, and bicyclic monoterpenes (Savikin-Fodulovic et al, 2000). Different parts of the plant find various uses in the food industry, such as for flavouring meat and sauces, and in the cosmetic industry. In Algeria, the wild plant known as Al-Rihan or Chilmoune, grows very well in various areas. It is mainly spread around the Mediterranean, including the Middle East and many other countries in Southern Europe. It is also found in Asia, New Zealand, America, Southern Russia and Australia

(Barboni et al, 2010), (Ozek et al. 2000). Contrary to synthetic insecticidal agents and food additives which can cause a number of adverse effects, there are botanical pesticides which are effective, environment-friendly, easily biodegradable and also inexpensive. (Dharmagadda et al 2005). According to De Mendonca et al 2005

M. communis has the potential of being acute ovicidal, fumigant, insect growth regulatory and insecticidal against various insect species. Furthermore, essential oils have a broad spectrum of bioactivity because of the presence of several active ingredients that act through several modes (Liu, C.H, 2006). (Repellent et al.1973) and their lipophilic nature facilitates them to interfere with basic metabolic, biochemical, physiological and behavioral functions of insects. (Zoubiri ET al 2010).

The main goal of the present study is to evaluate the antioxidant and insecticidal activity of the essential oil and methanolic extract of *M. communis L*, grown in Tablat (Medea), Timizar (Tizi Ouzou) and Hamame Melouan (Blida), the total phenolic content with Folin-Ciocalteu of the methanolic plant extracts were studied, then insecticidal activities against adults of *S. oryzae (L.)* and *T. confusum (Duv.)* was evaluated.

The rice weevil, *S. oryzae* (L.) (Coleoptera: Curculionidae), is one of the most destructive pests of stored cereals and processed cereal products worldwide. It is classed as a primary pest, one which can easily infest sound cereal seeds. (Hill D.S, 1990)

T. confusum du Val (Coleoptera: Tenebrionidae), the confused flour beetle, is one of the most important insect pests of the milling industry. This species is extremely tolerant of low humidity levels (Howe, 1965) and is classified as a secondary pest given that it can develop easily in mechanically damaged grain, grain already infested by other pests, or processed grain products, especially flour (Hill, 1990). This species, like many others, has also developed resistance to some residual insecticides and phosphine (Zettler, 1991) (Arthur et al, 1992).

MATERIALS AND METHODS

Plant Material

Myrtle aerial parts were collected at the stage of ripe berries in December 2012 from three different regions (North of Algeria).The sampling was done by a randomized collection of 10–15 shrubs in an area of about 200 m^2. Myrtle leaves were isolated manually from the aerial parts in our laboratory. Botanical identification of this species was carried out by Prof. H. Abdelkrim (Botanic department of superior national school agronomic).

Insect

Adults of *T. confusum* and *S. oryzae* were mass produced in glass jars (250 ml) covered with a piece of fine cloth. The cultures were maintained in the laboratory without exposure to any insecticide on corn grain at 28 ± 1°C, 70-75% r. h and a 16:8 light: dark photoperiod.

Contact Toxicity on Filter Paper

The contact activities of three essential oils and their methanol extracts of myrtle against adults of *S. oryzae* and *T. confusum* were determined firstly in impregnated-paper assays.

What man filter paper discs (9 cm diameter) were treated with 1 ml of different concentrations of *M. communis* extract and essential oil, whereas controls received the same volume of absolute solvent (acetone). The solvent was allowed to evaporate completely at room temperature. Each filter paper disc was then placed on a glass Petri dish

(9 cm diameter) and 20 unsexed *S. oryzae* adults (0-14 days old) and *T. confusum* adults (adults of mixed age and sex were used in the test) were introduced into the center of each Petri dish before wrapping with its lid (3 replications/ concentration). The number of dead insects was recorded after each 24h (1, 2, 3, 4, 5, 6 and 7 days).

In a separate experiment, susceptibility of *S. oryzae* and *T. confusum* adults to the fumigant action of test compounds was investigated. A cotton stem treated with quantity of essential oil was placed in the bottom lid of a plastic cylinder (3.67 cm diameter * 6.5cm). This prevented direct contact of the weevils with the test compounds then which the lid was sealed. After 10 minutes, 20 adults of each species were placed in each plastic cylinder then covered with a lid. Mortality counts were initially corrected as suggested by Abbott (1925) (Abbott, 1925) and the different 50% and 90% lethal doses were calculated using probit analysis in which probit transformed percentage mortalities were regressed on log dose. (Finney, 1971)

Extraction and Isolation of the Essential Oils

For each sample of the myrtle, 100 g. of dried and ground plant material were separately subjected to hydrodistillation for 4 hours using a Clevenger-type apparatus according to the European Pharmacopoeia. The oils were dried over anhydrous sodium sulfate (Na_2SO_4) and conserved at 4°C in sealed brown flasks.

Preparation of the Methanol Extract

The air-dried and finely ground sample constituted by leaves of *M.communis* was extracted by using the methanol 99 %. The sample weighing about 30 g was extracted in a Soxhlet with methanol for 7 h, which was then concentrated by using a rotary evaporator under vacuum at 64°C, lyophilised and kept in the dark at +4°C until tested.

Total Phenolic Content

Total phenolic contents from leaves methanol extract were assayed spectrophotometrically using the Folin-Ciocalteu method, with gallic acid as standard. (Singleton et al, 1999)

Briefly, 0.25 ml of extract dissolved in methanol was mixed with 1.25 ml of Folin-Ciocalteu solution.

The mixture was shaken and allowed to stand for 3 min, before adding 1 ml of solution of sodium carbonate (Na_2CO_3) at a concentration of 75 g/l. After 30 min of incubation in the dark at room temperature, the absorbance was measured at 765 nm. Total phenolic contents of leaf were expressed as mg gallic acid equivalents per gram (mg GAE/g) through the calibration curve with gallic acid. The calibration curve range was 5-30 µg/ml (R^2 = 0.997). All samples were performed in triplicates.

Total Flavonoid Content

The total flavonoid content of the sample was determined by the trichloride aluminum ($AlCl_3$) Method (**Lamaison et al, 1990),** with quercetin as standard. Thus, 1 ml of extract dissolved in ethanol was added to 1 ml of solution of trichloride aluminum (2 % w/v).

After 1 h of incubation at room temperature, the absorbance was red at 420 nm. Total flavonoid contents of leaf were expressed as milligrams of quercetin equivalent (QE) per gram extract. The calibration curve range was 5-30 µg/ml. The experiment was carried out in triplicate and average value recorded.

Antioxidant Activity (DPPH assay)

The DPPH (2, 2-diphenyl-1-Picrylhydrazyl) free radical scavenging assay was carried out for the evaluation of the antioxidant activity. The essential oils were dissolved in ethanol and various concentrations (200, 400, 800, 1200, 1600, 2000 mg/l) of each oil were used. The concentrations what relate the methnol extracts were (10, 20, 50, 100 and 200 mg/l). BHT (Butylated hydroxytoluene) was acted as positive control at the same concentrations of the oils. Briefly l. 25 µl of various concentrations of the samples of oils in ethanol were added to aliquots (975 µl) of a 60 µM ethanol solution of DPPH but concerning the extracts we used a methanol. The mixture was shaken vigorously and left standing at room temperature for 30 min. The absorbance of the resulting solution was then measured at 517 nm. The ability to scavenge the DPPH radical was calculated using the following equation:

% Inhibition = (Abs blank -Abs sample / Abs blank) × 100

Where Abs blank is the absorption of the blank sample (t = 0 min) and Abs sample is the absorption of the tested sample or substance solution (t = 30 min).

RESULTS AND DISCUSSIONS

The oils yields of *M. communis* leaves from Tizi Ouzou, Tablat and Hamam Melouan were, on the basis of dry weight, 0.33, 0.41 and 0.35 % (v/w), respectively. The methanolic extract yields, phenolic and flavonoid contents were showed in Table 1.

Table 1: Yield, Polyphenols and Flavonoids Contents of Methanol Extracts

Sample	Yield (%)	Phenolic Contents mg GAE/g	Flavonoids Contents mg QE/g
M.communis of T. Ouzou	17.34	78.79±2.96	21.61±0.23
M.communis of H.Melouan	15.8	75±2.23	16.81±0.99
M.communis of Tablat	16.47	83.03±1.89	17.42±1.5

Contact Activity with Treated Filter Paper

The toxicity of methanol extracts and essential oils to *S. oryzae* and *T. confusum* adults exposed to direct contact with the myrtle extracts is recorded in Table 2 and 3. Significant differences in toxicity of test extracts to the adults were observed. Methanol extract from *M. communis* acted causing 50% mortality within 5 day after treatment. The results of probit analysis showed that *T. confusum* was comparatively more resistant (LD50: 0.44- 0.5- 0.58 mg/cm^2) to the toxic effect of myrtle extract of Tizi Ouzou, Hamam Melouan and Tablat respectively than S. oryzae (LD50: 0.42- 0.33- 0.42 mg/cm^2).

On the other hand, the myrtle oil was more toxic than the methanol extract. We noted that 50% of mortality was recorded within 3 day only after treatment. Comparison of LD_{50} values for the different oils against both insect species showed that there was no significant difference between essential oils from different areas but in general, *S. oryzae* was more sensitive than *T. confusum* (table 3).The mode of action of the essential oils or their constituents, as insecticides is not clearly known. However, due to observed repellent, antifeedant and growth regulation effects, it is evident that essential oils affect insect physiology in diverse ways. Essential oils and their constituents affect biochemical processes, which specifically disrupt the endocrinologic balance of insects (Rattan, 2010).

Table 2: LD_{50} and LD_{90} Calculated for Mortality within 5 Days of Exposure of *S. oryzae* and *T . confusum* on Filter Paper Impregnated Methanol Extracts

Pest species	Phytochemical	LD_{50} (mg/cm^2)	LD_{90} (mg/cm^2)
S. oryzae	M.communis of T. Ouzou	0.42	2.67
	M.communis of H.Melouan	0.33	2.98
	M.communis of Tablat	0.42	2.58
T. confusum	M.communis of T. Ouzou	0.44	2.75
	M.communis of H.Melouan	0.5	2.08
	M.communis of Tablat	0.58	5.61

Table 3: LD_{50} and LD_{90} Calculated for Mortality within 3 Days of Exposure of S. Oryzae and T. confusum on Filter Paper Impregnated Essential Oils

Pest Species	Phytochemical	LD_{50} (ml/cm^2)	LD_{90} (ml/cm^2)
S. oryzae	M.communis of T. Ouzou	0.1	1.48
	M.communis of H.Melouan	0.1	1.38
	M.communis of Tablat	0.13	1.4
T. confusum	M.communis of T. Ouzou	0.24	0.99
	M.communis of H.Melouan	0.18	0.38
	M.communis of Tablat	0.2	0.54

Fumigant Activity

The observed fumigant activity at dose of 0.116 µl/cm^3 shows that essential oils are sources of biologically active vapors that are potentially efficient insecticides, the results of probit analysis showed that *T. confusum* was comparatively more resistant (TL50: 148- 105- 142 h) to the toxic effect of myrtle oil of Tizi Ouzou, Tablat and Hamam Melouan respectively than *S.oryzae* (TL50: 81- 96- 124 h). However there was significant difference between essential oils from different areas, the insecticidal activity of the myrtle from Tizi Ouzou and Hamam Melouan was lower than that of the myrtle from Tablat against *T. confusum* . Similar results have been reported for the toxicity of each essential oil against *S.oryzae* and oil from Tablat (TL50:81h) was always significantly more effective than the others (Figure 1).

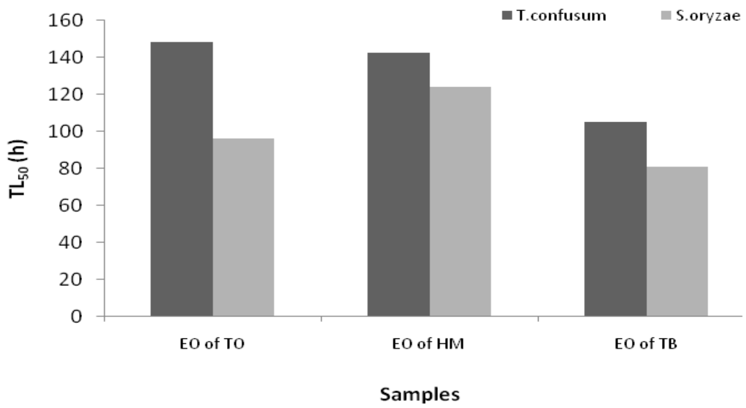

Figure 1: TL_{50} of Fumigant Activities of Myrtle Essential Oils (EO) from Different Areas against *S. Oryzea* and *T. Confusum* at Dose of 0.116 µl/Cm^3

*TO:Tizi Ouzou.

*TB: Tablat.

*HM: Hamam Melouan.

DPPH and Reducing Power

In essence, the antioxidants react with the stable free radical 2, 2-diphenyl-1-picrylhydrazyl (deep violet color) and convert it, by their hydrogen donating ability to 2, 2-diphenyl-2- picrylhydrazine with discoloration. The degree of discoloration indicates the free radical scavenging potentials of the sample/antioxidant. Free radical-scavenging capacities of all samples and positive control (BHT), measured by DPPH assay are shown in

Figure 2 According to these findings the oils of *M. communis* extracted from different areas were less effective than synthetic standard (BHT) and methanolic extracts which reached a maximum of 7.79- 8.02 and 11.23% of essential oil from Tizi Ouzou, Tablat and Hamam Melouan respectively for a concentration of 2000 mg/l.

The methanolic extracts of these three samples of myrtle exhibited very good radical-scavenging activities compared to essential oils and BHT. Thus, for the concentration of 5 mg/l it showed approximately double antioxidant index with synthetic antioxidant BHT (33.62-27.08 and 27.52 %) of methanolic extract from

Tizi ouzou, Tablat and Hamam Melouan respectively.

The IC50 (concentration required to inhibit 50 % of DPPH radicals) of methanol extract from Tizi ouzou, Tablat, Hamam Melouan and BHT were found to be 10.79±0.48, 9.15±0.1, 10.42±0.1 and 27.99±0.66 mg/l respectively, lower IC_{50} value indicated higher antioxidant activity. For essential oils, IC50 could not be calculated due to their low inhibition activity which remained below 12 % at 2000 mg/l concentration.

The ability of the leaf extracts of *M. communis* L. to scavenge free radicals could be attributed to an overall participation of their active constituents, such as phenolic acids and flavonoids (table 1).

As demonstrated in several studies, the antioxidant activity of the phenolic compounds were attributed to its redox properties, which allow them to act as reducing agents, hydrogen donators, singlet oxygen quenchers and metal chelators (Rice-Evans et al.1995)

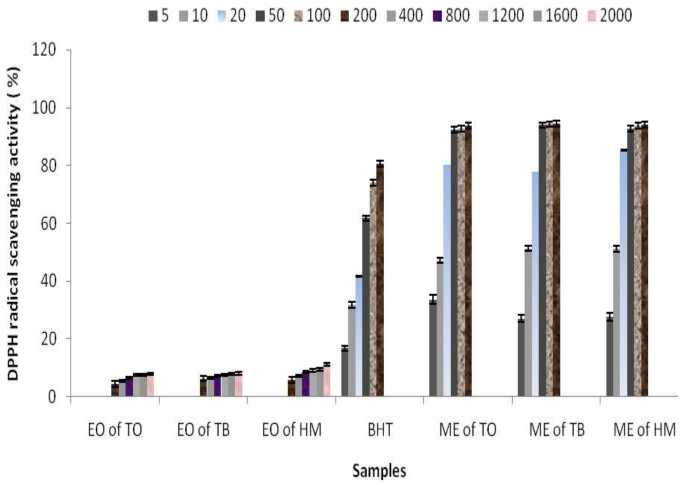

Figure 2: Free Radical-Scavenging Capacities of BHT and *M. communis* Essential Oils (EO) and Methanol Extracts (ME) from Different Areas Measured in DPPH Assay

*TO: Tizi Ouzou.

*TB: Tablat.

*HM: Hamam Melouan.

CONCLUSIONS

Myrtle is one of the most common her b which has been known for its medicinal and aromotherapeutic properties since ancient times. The findings of this study show that the reducing power of the all essential oils was lower than that of methanolic extracts which exhibited good levels of antioxidant activity. The antioxidant activity of plants is mainly contributed by the active compounds of phenolic fraction present in them.

These findings prove the possible presence of biologically active compounds in leaves that could be used for the formulation of supplements and/or ingredients for the food industry, inorder to replace synthetic carcinogenous, and thus restricted antioxidants.

All samples of essential oils reduced significantly the percentage of *T. confusum* and *S. oryzea* adults, these results indicate that the insecticidal mode of action of may be largely attributable to fumigant action; they may be toxic by penetrating the insect body via the respiratory system.

The study has also shown that there was no significant difference between essential oils from different areas but in general, *S. oryzae* was more sensitive than *T. confusum*

Furthermore, this study provides the first data on the insecticidal activity of the extracts of the Algerian *M. communis*.

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