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Research Article

# Antioxidant and Antibacterial Activities of Methanolic and Alkaloids Extracts of *Ruta montana* L.

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**ABSTRACT**

This study was aimed at investigating the phytochemical constituents, antioxidant and antibacterial activities of *Ruta montana* L (*Rutaceae*) aerial parts. Antioxidant properties were assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and reducing power assay. Antibacterial activity of the extract was assessed by agar diffusion method and four strains bacteria were used for this purpose. Qualitative phytochemical analysis showed the presence of alkaloids, tannins, coumarins, sterols and triterpens with absence of anthocyans and saponins. This richness confirmed by extraction yield 6.46%, 5.52% and 0.27% for methanol leaf, methanol seed and alkaloid extracts respectively. This extracts have shown an effective free radical scavenging activity with low $IC_{50}$= 38.2 µg/ml was observed for methanol leaf extract and $IC_{50}$=43 and 95.61 µg/ml for methanol seed extract and alkaloid extract respectively but less than the standard. These extracts have shown also a high power to reduce iron. The extracts are tested to them antibacterial activity on four bacterial strains, where the most important inhibition was recorded with the alkaloid extract of *R. montana* L. on *B. cereus* and *S. aurus*.



## INTRODUCTION

The region of Beni Aziz-Setif, North-East of Algeria, is known by its rich flora used by the local population. Their potential plant resources and the valorization of their species have only been partially studied. *Ruta montana* L is used in digestive disorders and helminthiasis, it's traditionally known for its abortive and anti-fever effects [1 - 3], and is known by its antifungal properties [4, 5], anti-inflammatory drug, disinfectant, antipyretic and pest-destroying [6]. In fact, their therapeutic properties are due to the presence of thousands of secondary metabolites in particular essential oils and alkaloids [7].

In this paper we report the phytochemical screening and aims to evaluate the antioxidant, antibacterial activities of different extracts from *Ruta montana* L belonging to the family of Rutaceae.

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## MATERIALS AND METHODS

### Vegetal Material

The aerial parts of *Ruta montana* L were collected in Septembre 2017 from Beni Aziz mountain (Northeast of Algeria), identified by Dr. Kirouani (Laboratory of Zoology - Department of Biology- University of Mohammed El Bachir El Ibrahimi-Bordj Bou Arreridj-Algeria) (Fig.1). The aerial parts of the plant were dried at room temperature before extraction.

**Figure 1:** Plant *Ruta montana L.* (Beni Aziz Region –Setif- Algeria)

**Phytochemical Screening**

The phytochemical screening of the aerial parts of *R. montana* L was conducted for the determination of alkaloids, anthocyanes, tannins, coumarins, saponins, sterols and triterpenes.

**1. Detection of Tannins**

2 ml of aqueous decoct to 5% was added to a few drops of ferric chloride solution to 5%, were mixed with ethanol. The appearance of a green or brown-green color indicates the presence of tannins [8, 9].

**2. Detection of Alkaloids**

10 g from the vegetable powder were mixed with 50 ml of sulfuric acid diluted to 1/10, then stand 24h at room temperature. After filtration, the filtrate was extracted by distillated water in such manner to obtain 50 ml of filtrate. The filtrate was dividing into two equal parts. The first was treated with a few drops of Mayer's reagent and the second with Wagner's reagent. Positive observation: turbidity or precipitation [8].

**3. Detection of Sterols and Triterpenes**

Maceration of 5 g of vegetable powder with ether at 5% during 24h, after evaporation, 0.5 ml of acetic anhydride were added and then 0.5 ml of chloroform. Then 1 ml of concentrated sulfuric acid is added (Liebermann-Burchard reaction). At the contact zone of the two liquids a brownish red ring was formed denoting the presence of sterols and triterpenes [9].

**4. Detection of Coumarins**

In a test tube were placed 1 g powder of aerial parts, covered by a soaked filter paper with diluted NaOH (10%) placed in a boiling water during few minutes the filter paper is then examined under Ultra-Violet light. A yellow fluorescence indicated the presence of coumarins [8].

**5. Detection of Anthocyanes**

An infusion of 5 g of vegetable powder in 100 ml of boiling water distilled during 15 minutes then filtrated, 2 ml of infused were added to 2 ml of HCl (2N) and mixed with some drops of ammonia, the test was negative if no color develops [8] .

**6. Detection of Saponins**

Two grams of powdered sample was boiled in 80 ml of distilled water and then filtered and shaken vigorously for about 5 min. Formation of foam after shaking was taken as a confirmation for the presence of saponins [10, 11].

**Extraction**

**1. Extraction by Maceration**

Fifty grams of sample dry powder (leaves and seeds) were extracted by maceration in 250 ml of methanol and stand for 3 days in room temperature (22°C) and filtered with Whatman filter paper. The filtrate was evaporated at low temperature under reduced pressure in a rotavapor. At last, dried extracts were then stored at 4°C in until use.

**2. Extraction by Soxhlet**

The dry aerial parts were powdered and 50 g of powder were defatted with petroleum ether under reflux conditions, then defatted dry powder were witted with 20 ml of $NH_4OH$ (0.5N) for 24 hours at room temperature then was extracted using a soxhlet apparatus with 250 ml of $CH_2Cl_2$ during 5h at 35°C. The organic extract was shaken three times with 150 ml of sulfuric acid (0.1N). The acid extract were treated with few milliliters of $NH_4OH$ (0.5N) to pH 9 to liberate the free alkaloids which were separated by extraction with 150 ml $(C_2H_5)_2O$ for 3 times, then dried with anhydrous sodium sulfate and concentrated under reduced pressure to obtain crude alkaloids , according to the method described by Bruneton 1999 [12].

**Antioxidant Activity**

**1. Determination of DPPH Radical Scavenging Activity**

The antioxidant activity of the each extracts or standard was determined in terms of hydrogen-donating or radical-scavenging ability, using the stable radical 1.1-diphenyl-2-picrylhydrazyl (DPPH) according to the method of Kirby and Schmidt 1997. Briefly, 1.5 ml of various concentrations (0.06-1.0 $mg.ml^{-1}$) of methanol extract was added to 1 ml of DPPH radical solution in methanol 4% (w/v). The mixture was shaken vigorously and kept at room temperature in the dark for 30 minutes. The absorbance of the samples and control solutions were measured at 517 nm. The inhibition of free radical DPPH was calculated in percent (I%) as follows:

$$\mathbf{I\%} = 100 \times (\text{A control-A sample})/\text{A control}$$

Where A control is the absorbance of the control reaction (containing all reagents except the test compound), A sample is the absorbance of the test compound. The ascorbic acid was used as a standard [13].

**2. Determination (Reducing Power Assay)**

The reducing powers (RP) were determined according to the method of Oyaizu 1986. 0.5 ml of each extract was mixed with 1.25 ml of 200 mM of sodium phosphate buffer (pH 6.6) and 1.25 ml of 1% potassium ferricyanide [$K_3Fe(CN)_6$] and the mixture was incubated at 50°C for 20 min. Then, 1.25 ml of 10% trichloroacetic acid was added, and the mixture was centrifuged at 1500 rpm/min for 10 min. The upper layer (1.25 ml) was mixed with 1.25 ml of distilled water and 0.25 ml of 0.1% ferric chloride ($FeCl_3$). Finally, the absorbance was measured at 700 nm, while the ascorbic acid was used as a standard [14].

**Antibacterial Activity**

**1. Bacterial Strains**

The antibacterial activity of the different *R. montana* L extracts were tested against four strains of bacteria, two strains are positive-Gram: *Staphylococcus aureus* and *Bacillus cereus* and two other strains are negative-Gram: *Escherichia coli* and *Salmonella typhimuruim*. These strains were obtained from Laboratory of Microbiology, Department of Biology (University Ferhat Abbas - Setif1 - Algeria).

**2. Agar Diffusion Method**

The antimicrobial activity was carried out according to the method described by Kacem et al 2015 and Hammami et al 2015. Different *R. montana* L extracts were dissolved in dimethylsulfoxide (DMSO) and sterilized by filtration. The filter paper discs (6 mm in diameter) were individually impregnated with 10µl of each extract and then placed into the agar plates which had previously been inoculated with the tested microorganisms. The inoculated plates were kept, firstly for 2 h at 4°C, and then incubated for 24 h at 37°C [15, 16]. The diameters of the inhibition zones (mm) were measured including the diameter of discs and the MIC (Minimal Inhibitory Concentration) values of each compound, defined as the lowest concentration that completely inhibits the visible bacterial growth [17].

All the tests were performed in triplicate. Amoxicilline, Ceftazolin and cefazolin served as positive controls.

**Statistical Analysis**

The data were expressed as Mean ± Standard Deviation (SD) of three measurements for the analytical determination and differences at $P < 0.05$ were considered statistically significant.

**RESULTS AND DISCUSSION**

**1. Phytochemical Screening**

The phytochemical screening of the aerial parts of the plant revealed the presence of tannins, alkaloids, coumarins, sterols, triterpens and absence of anthocyans and saponins (Table 1).

**Table 1:** Phytochemical analysis of aerial parts of *Ruta montana* L.

| Chemical groups | Tanins | Alkaloids | Anthocyans | Sterols or triterpens | Coumarins | saponins |
|---|---|---|---|---|---|---|
| Results | + | + | - | + | + | - |

(+) present, (-) absent

These results are completely according to the works of Daoudi et al 2016 with *R.montana* L and *R.chalepensis* L, so Belkassam et al 2011 with same plant confirmed the presence of all chemical groups while the saponins present as traces [18, 4] . Mansouri et al 2005 and Benziane 2007 with *R.montana* L; were gave same results except the presence of saponins [19, 8].

The chemicals groups detected in our study confirmed the works of Vasudevan and Luckner 1968 and others [20 - 23] with *Ruta montana* L. and also proved by studies on *Ruta graveolens* L [24]. The methanol extracts (leaves and seeds) yield 6.46% and 5.52% respectively, a very low yield which could be due to the difference between species and region; so the alkaloids extract yield 0.27%.

**Anti-oxidant Activity**

**DPPH Radical-Scavenging Activity**

The DPPH free radical method is able to measure the antiradical power of antioxidants. DPPH scavenging activity is usually presented by $IC_{50}$ value, defined as the concentration of the antioxidant needed to scavenge 50% of DPPH present in the test solution. Therefore, extract concentrations providing 50% inhibition ($IC_{50}$) were calculated using the data plotted in Fig. 2A. Lower $IC_{50}$ value reflects better DPPH radical-scavenging activity, where the methanol leaf extract showed the highest antioxidant activity ($IC_{50}$ 38.2 µg/ml) compared to methanol seeds extract and alkaloids extract, which gave $IC_{50}$ 43 µg/ml and 95.61 µg/ml respectively, but not stronger than the standard ascorbic acid ($IC_{50}$

4.34 µg/ml). So the methanol extracts are more actives than the alkaloids extract, which is due to the complexity of crude extracts with phenols substances and the synergic between them for a best antioxidant activity [25].

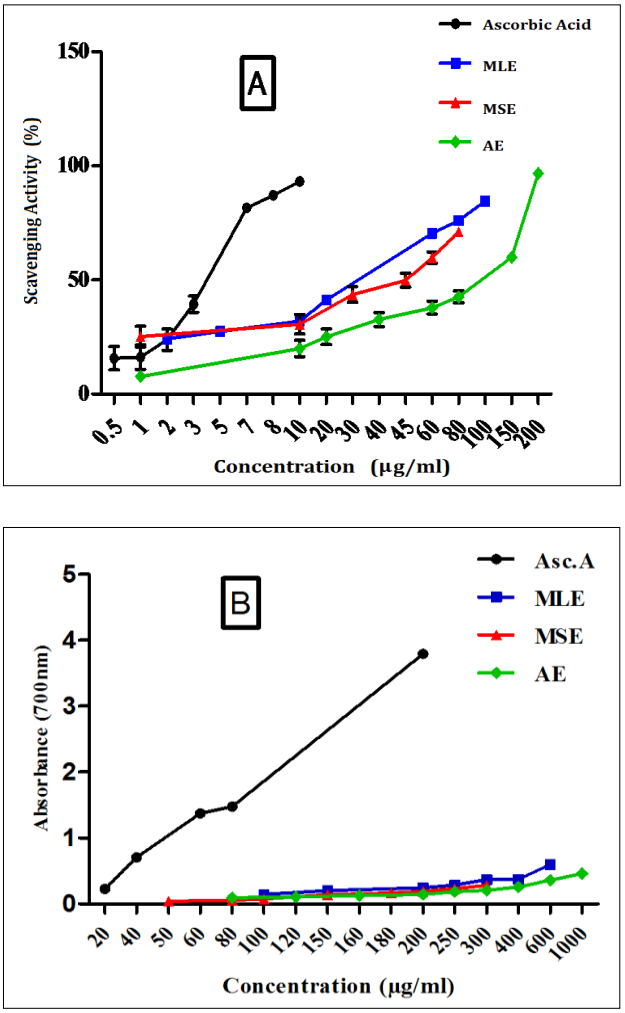


**Figure 2:** Antioxidant activity of *Ruta montana* L, methanol leaves extracts (MLE), methanol seeds extracts (MSE) and alkaloids extracts (AE). **A:** DPPH-scavenging activity, **B:** reducing power. Ascorbic acid was used as positive control.

The phytochemical screening test showed the presence of tannin and coumarins in the plant, this is likely to be responsible for the free radical scavenging effects observed, where the phenols have the ability to donate electron/hydrogen that results in converting highly reactive free radicals to non-reactive stable molecules [26].

The strong free radical scavenging activity in methanol leaf extract with $IC_{50}$ 38.2 µg/ml might be due to the high quantity of phenols and flavonoids present. This indicates that the methanol leaf extract of *R.montana* L possess a good potential source for natural antioxidants to prevent free radical oxidative damage.

The results obtained by Khlifi et al 2013 with *R. chalpensis* L revealed an ($IC_{50}$ 70.01 mg/ml of aerial parts). *R.montana* L. showed stronger DPPH- radical scavenging activity than *R.chalepensis* L and *R.graveolens* L according to Fekhfekh et al 2012, where the aqueous and ethanol extracts were more active than essential oils [27, 28].

## Reducing Power Activity

As shown in Fig. 2B the reducing power of methanol leaves, seeds and alkaloids extracts as compared with ascorbic acid as standard, it was found that the reducing power of the extracts increased with the increase of their concentrations. All extracts presented clearly low antioxidant activity compared with the reference vitamin C; where for last one, the total reducing power was 80µg/ml. The crudes extracts, which contained the highest amount of total phenolics, showed a higher reducing power than alkaloids extracts, these results are same with those obtained by Fekhfekh et al 2012, where the aqueous and ethanol extract showed a much more reducing power then essential oil of *R.chalpensis* L [28].

## Antibacterial Activity

The antibacterial activity of the various *Ruta montana* L extracts was assessed against positive-Gram bacteria (*B. cereus* and *S.aurus*) and negative-Gram bacteria (*E. coli* and *S. typhimurium*). Table 2 showed that all extracts of *Ruta montana* L. prevented the growth of the tested microorganisms with an inhibition zone medium diameter increasing proportionally with the concentrations of the tested samples. The obtained inhibition on bacteria strains varied from 36.66 to 6.5, 35.83 to 6.5 and 33 to 6.5 mm for alkaloid, methanol leaves and methanol seeds extracts respectively.

The *Ruta montana* extracts showed strong antibacterial activity (inhibition zone >20 mm), moderate activity (inhibition zone between 12–20 mm) and no inhibition (zone <12 mm). According to the width of the inhibition zone diameter, all extracts had the highest antibacterial activity against all bacteria with 34.5±1.5 - 22.5±0.5, 34.66±0.57 - 19.16±0.28 and 32.5±1.32 - 18.66±0.57 mm for alkaloid, methanol seeds and methanol leaves extracts respectively at 200 mg/ml. In addition, at 6.25 mg/ml all extracts had no antibacterial activity

against all bacteria with 11.16±1.04 - 7.33±0.57, 8.16±0.28 - 7.16±0.76 and 10.83±1.15 -7.33±0.28 mm for AE, MSE and MLE respectively.

The alkaloids extract was found to be the most effective than methanol extracts against all bacteria tested with a highest inhibition zone recorded with *B. cereus* and *S. aurus* (36.66±1.52 and 31.83±1.04 mm respectively) at 400 mg/ml and a considerable activity with same concentration on *E. coli* and *S. typhirumium* (28.33±0.57 and 24.5±0.5 mm respectively).

So the methanol extract exhibited the strongest inhibition zone on *B. cereus* and *S.typhirumium* (35-27.33mm) while *E. coli* and *S. aurus* showed a modest antibacterial activity (23.16-21.33 mm).

Tested bacteria were more sensitive to *Ruta montana* L. extracts than references antibiotics (Amoxicillin, Ceftazolin and Cefazolin).

According to França Orlanda and Nasciment 2015, the classification for this activity is suggested; defining strong MIC essential oils can hold up to 0.5 mg/ml, moderate for MIC 0.6–1.5 mg/ml and low for MIC above 1.5 mg/ml. Although the *R. montana* L extracts presents a low MIC for all bacteria at 3.12 mg/ml, for all analyzed bacteria classified as extracts showed low activity [29].

The lower antibacterial activity of alkaloid extract against *S. aurus* from 100 to 3.12 mg/ml could be due to the higher resistance of positive-Gram microorganisms to those compounds.

**Table 2:** Antibacterial activity of methanol and alkaloids extracts of *Ruta montana* L.

| | ***Bacillus cereus*** | | | ***Salmonella typhimurium*** | | |
|---|---|---|---|---|---|---|
| | **Iz ± SD** | | | **Iz ± SD** | | |
| **mg/ml (10µl/disc)** | **MLE** | **MSE** | **AE** | **MLE** | **MSE** | **AE** |
| **400** | 33±1 | 35.83±0.28 | 36.66±1.52 | 28.83±0.76 | 27.33±0.28 | 24.5±0.5 |
| **200** | 32.5±1.32 | 34.66±0.57 | 34.5±1.5 | 21.66±2.51 | 21.66±0.57 | 22.5±0.5 |
| **100** | 28.33±0.57 | 21.66±1.52 | 31.16±0.76 | 18±1 | 17.5±0.5 | 20.66±0.57 |
| **50** | 23±1 | 19.5±0.5 | 27.5±0.5 | 17±0.5 | 15.±0.16 | 17.83±0.76 |
| **25** | 20.16±1.04 | 17.16±0.28 | 23.16±1.75 | 12.5±0.5 | 11.33±0.28 | 14.16±0.76 |
| **12.5** | 18.16±0.76 | 13±1.32 | 15.5±1.32 | 8.83±0.28 | 9.16±0.28 | 11.83±0.28 |
| **6.25** | 10.33±1.52 | 8.16±0.28 | 11.16±1.04 | 7.33±0.28 | 7.83±0.28 | 9±0.5 |
| **3.12** | 6.66±0.28 | 6.5±0.00 | 6.5±0.00 | 6.5±0.00 | 6.5±0.00 | 6.5±0.00 |
| **MCI** | 3.12 mg/ml | | | 3.12 mg/ml | | |
| **AX** | 13.5 | | | 17 | | |
| **CAZ** | 12.3 | | | 16 | | |
| **CZ** | 22.5 | | | 20 | | |
| | ***Echerichia coli*** | | | ***Staphylococcus aurus*** | | |
| | **Iz ± SD** | | | **Iz ± SD** | | |
| **mg/ml (10µl/disc)** | **MLE** | **MSE** | **AE** | **MLE** | **MSE** | **AE** |
| **400** | 21.33±1.52 | 23.16±0.28 | 28.33±0.57 | 22.66±1.52 | 22.33±0.28 | 31.83±1.04 |
| **200** | 18.66±0.57 | 20.5±0.5 | 24.5±0.5 | 19.66±0.57 | 19.16±0.28 | 25±1 |
| **100** | 16.83±0.76 | 16.83±0.28 | 22±0.5 | 17.83±0.76 | 17.83±0.76 | 14.5±0.5 |
| **50** | 14.5±0.5 | 15.5±0.5 | 18.83±0.28 | 16±1 | 15.16±0.28 | 12±1 |
| **25** | 12.16±0.76 | 10.66±0.57 | 15.5±0.86 | 12.5±0.5 | 12.5±0.5 | 9.16±0.76 |
| **12.5** | 8.66±0.57 | 8.83±0.28 | 11.5±0.5 | 11.83±0.76 | 8.83±0.28 | 8.66±0.28 |
| **6.25** | 7.33±0.28 | 7.16±0.76 | 8.83±0.28 | 10.83±1.15 | 7.83±0.76 | 7.33±0.57 |
| **3.12** | 6.5±0.00 | 6.5±0.00 | 6.5±0.00 | 6.5±0.00 | 6.5±0.00 | 6.5±0.00 |
| **MCI** | 3.12 mg/ml | | | 3.12 mg/ml | | |
| **AX** | 11.9 | | | 15 | | |
| **CAZ** | 11 | | | 10 | | |
| **CZ** | 23.5 | | | 15 | | |

Iz: inhibition zones (mm). MIC: minimum inhibitory concentration (mg/ml)
MLE: methanol leaves extract, MSE: methanol seeds extract, AE: alkaloids extract, AX: Amoxicillin, CAZ: Ceftazolin and CZ: Cefazolin

Several study, which investigate the action of essential oil of *R. graveolens* L, *R. chalepensis* L and *R. montana* L against pathogenic microorganisms, agree that essential oils are more effective against positive-Gram bacteria than against negative-Gram bacteria [18, 29, 4] .

The works of Daoudi et al 2016 with crudes extracts of *R. montana* L and *R. chalepensis* L at 100 mg/ml showed that *R.montana* L extracts had no inhibition against tested bacteria and *S.aurus* more sensitive than other bacteria strains to crudes *R. chalepensis* L extracts, while the essential oils from *R.montana* L exhibited high antibacterial activity against *S.aurus* (32.66±1.15) most important than *R. chalepensis* antibacterial activity (17.33±1.53) and no inhibition against *E. coli* and *B. cereus* while *S. aurus* showed that are more sensitive against the alkaloid extract than other tested bacteria[18].

## CONCLUSION

This study; phytochemical screening, comparative antioxidant and antibacterial activities between methanol leaves, seeds and alkaloids extracts of *Ruta montana* L were carried to deepen the research which has been a little developed until now. The phytochemical screening carried revealed the richness of our plant in secondary metabolites, where we found the presence of tannins and coumarins which gave a strong antioxidant activity to the methanol leaves extract, while alkaloids known by theirs toxicities, which express an antibacterial activity against all micro-organisms tested in this present work, sterols and triterpens while the saponins and anthocyans are absent. The results of this work can be exploited for use by pharmaceutical industry.

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