

Antibacterial Activity And Acute Toxicity Effect of Flavonoids Extracted From *Mentha longifolia*

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Abstract: The methanolic and ethanolic extracts of *Mentha longifolia* were tested for their antimicrobial activity against some clinical bacteria. Then, a phytochemical screening was realized for the ethanolic extract and the acute toxicity was measured for the identified flavonoids present in it. The results showed that the ethanolic extract was the most active against the tested bacteria. The phytochemical screening of this extract indicated that it contained mainly five flavonoids identified as luteolin-7-O-glycoside, luteolin-7,3'-O-diglycoside, apigenin, quercetin-3-O-glycoside and kaempferol-3-O-glycoside. Among these molecules, the quercetin-3-O-glucoside had the highest antibacterial activity, but the synergism between the apigenin, the quercetin-3-O-glycoside and the kaempferol-3-O-glycoside gave the best minimal inhibitory concentration for each tested species. The quercetin-3-O-glucoside had also the best acute toxicity effect on mice. The substitution type of flavonoids had an impact on the antibacterial activity but not on the acute toxicity effect.

Key words: *Mentha longifolia* • Extracts • Flavonoids • Antimicrobial • Acute toxicity

INTRODUCTION

Several plants were widespread for their many therapeutic and pharmaceutical virtues, especially anti-infectious and preventive ones against different diseases. These benefits provide from their big content on bioactive compounds [1-3]. In this work, we had tried to determinate the antibacterial activity of ethanolic and methanolic extracts of *Mentha longifolia*. This activity had been selected because of its great medicinal relevance [4,5]. Then, we had determinate the principal bioactive compounds present in the most active extract. Finally, the antibacterial activity and the acute toxicity effect were measured for the identified molecules.

MATERIAL AND METHODS

The plant came from the area of El Aouana, Jijel (Algeria) and the bacteria used came from the microbiological and infectious laboratories of Mohammed Ben-Yahia's hospital (Jijel, Algeria). 0.2 ml of each bacterial solution was taken then put in 20 ml of sterile nutritive broth and incubated at 37°C during 3 to 5 hours in order to standardize the cultures at 10⁶ cfu / ml.

Preparation of the Extracts: The plant material (20 g of powdered material for each extraction) was extracted with 600 ml of aqueous methanol (50%) by using a Soxhlet apparatus for 8 h and with 600 ml aqueous ethanol (80%) by maceration for 2 h. The extraction with ethanol was repeated thrice. The obtained methanolic and ethanolic extracts were filtered and evaporated by using a rotary evaporator and freeze dryer, respectively to give the crude dried extract.

The Antibacterial Activity: The diffusion on agar method was used [6]. This activity was carried out on Muller-Hinton medium (Pasteur Institute, Algiers, Algeria) with an incubation at 37°C during 12 H.

The Phytochemical Screening: We carried out a separation of the compounds present in the ethanolic extract according to their nature and their structure by partitions between solvents: The first partition was carried out with petroleum ether in order to get out the non phenolic compounds. This phase was then thrown away. The second partition was done with diethylic ether to extract the phenolic acids and the aglycones. The third was carried out with the ethyl acetate which gets up the

mono-substituted flavonoids. So the remaining aqueous phase contained only flavonoids linked to two or more compounds.

For each phase, a phytochemical screening was carried out using chemical methods and thin layer chromatography (TLC) [7]. Then, for the flavonoid present in sufficient quantities, we carried out an identification using a series of spectral analyses [8-11]. In the case of the complete identification of the substituted flavonoids, an acid hydrolysis was carried out by heating using HCl in a bath water during 30 min. The aglycone was recovered by partition with diethylic ether and was identified by UV-visible spectrophotometry and Co-TLC with control substances. The remaining aqueous phase, containing the substitutions separated from the aglycones, was then concentrated and subjected to Co-TLC on silica gel with the solvent system: acetone/ water (9/1). The revelation was carried out by malonate of aniline and heating at 100% in the incubator.

Acute Toxicity Test: The mice (13, 5-15, 0g) were obtained from the Central Animal House, University Mentouri (Constantine, Algeria). The animals were ventilated and maintained at room temperature of about 27°C and were starved for 12 h prior to testing. The animals were divided into groups containing five mice each. Each group was orally administered with 1,2,3,4,5,6,7 and 9 g/kg of the considered extracted flavonoid. The last group was given distilled water (8 ml/kg) and was considered as a test control. Symptoms of toxicity and mortality were observed for 24 h after the administration [12].

RESULTS

The results showed that the ethanolic extract was more active than the methanolic one (Table 1). In fact, the ethanolic extracted was able to inhibit the growth of *S. aureus*, *B. cereus* and *B. subtilis*. But, it did not give any inhibition against *E. coli* and *P. aeruginosa*.

The phytochemical analysis of the separate compounds showed that the flavonic phases contained mainly phenolic acids (UV-visible spectra with only one characteristic peak around 209 nm) and flavonoids (with two characteristic peaks: the first around 310 and 385 nm and the second around 250 and 280 nm) [10]. Only five flavonoids were present in sufficient quantities allowing their identification. The spectral analysis gave the following observations: The first compound was a luteolin mono-substituted. Its acid hydrolysis released glucose and luteolin. It was thus a luteolin-7-O-glucoside. For the second compound, it released two glucoses and a luteolin. It was thus a luteolin-7,3'-O-diglucoside. The third compound was an aglycone apigenin. The forth and fifth compounds were successively quercetin and kaempferol mono-substituted. Their acid hydrolysis released glucose and quercetin for the fourth compound and glucose and kaempferol for the fifth. So, they were successively quercetin-3-O-glucoside and kaempferol-3-O-glucoside.

These five flavonoids identified gave an inhibition of all the species with MICs varying between 0,095 mg/ml and 0,025 mg/ml. Only the luteolin-7-O-glucoside and the luteolin-7,3'-O-diglucoside did not have an action on the growth of *E. coli* and *P. aeruginosa*.

Table 1: MICs values recorded by each extract (mg/ml)

	<i>E. coli</i>	<i>S. aureus</i>	<i>B. cereus</i>	<i>P. aeruginosa</i>	<i>B. subtilis</i>
Ethanolic extract	-	0,180	0,160	-	0,090
Methanolic extract	-	-	0,250	-	0,170

Table 2: MICs values recorded for the identified flavonoids (mg/ml)

	Luteolin-7-O-glucoside	Luteolin-7,3'-O-diglucoside	Apigenin	Quercetin3-O-glycoside	Kaempfero-3-O-glucoside	Synergism
<i>E. coli</i>	-	-	0,090	0,080	0,095	0,050
<i>S. aureus</i>	0,070	0,075	0,065	0,065	0,065	0,070
<i>B. cereus</i>	0,090	0,095	0,080	0,050	0,080	0,045
<i>P. aeruginosa</i>	-	-	0,080	0,060	0,085	0,040
<i>B. subtilis</i>	0,050	0,060	0,030	0,025	0,030	0,010

Table 3: Results of the acute toxicity effect expressed in percentages

Doses Flavonoids	1 g/kg	2 g/kg	3 g/kg	4 g/kg	5 g/kg	6 g/kg	7 g/kg	8 g/kg	9 g/kg	LD
Quercetin3-O-glycoside	0	0	0	0	20	40	60	80	100	5
Apigenin	0	0	0	20	40	60	80	100	100	4
Luteolin-7-O-glucoside	0	0	0	20	40	60	80	100	100	4
Luteolin-7,3'-O-diglucoside	0	0	0	20	40	60	80	100	100	2
Kaempfero-3-O-glucoside	0	0	20	40	60	80	100	100	100	2

Quercetin-3-O-glycoside inhibited the growth of the five species and gave for each one the lowest MIC. But, the synergism between the three most active identified flavonoids gave a best antimicrobial activity with 0,050 mg/ml for *E. coli*, 0,070 for *S. aureus*, 0,045 mg/ml for *B. cereus*, 0,040 mg/ml for *P. aeruginosa* and 0,010 mg/ml for *B. subtilis* (Table 2).

For the acute toxicity effect, it was observed that the quercetin-3-O-glucoside had the lowest values with an LD of 5 mg/g. The apigenin and the to substituted luteolins had the same effect with an LD of 4 mg/g. The kaempferol-3-O-glucoside had given an LD of mg/g and was thus considered as the most toxic compound among the tested flavonoids (Table 3).

The results also showed that the substitution degree of the luteolin had no impact in the acute toxicity effect.

DISCUSSION

The highest antibacterial effect of the ethanolic extract of *M. longifolia* may be due to its high content on flavonoids. In fact these compounds are known for their strong antimicrobial activity [2,13]. The TLC revealed the presence of phenol acids and flavonoids in great quantity in *M. longifolia* which indicated that this plant could have a great interest in various industries: therapeutic, pharmaceutical and agricultural.

The obtained results confirmed the benefits given by the medicinal plants. In fact, some flavonoids present in them demonstrated a big antibacterial activity and a very low acute toxicity effect.

This investigation also reported the role of the flavonoid substitutions in the tested activities. In fact, the results showed clearly that the substitution degree of the luteolin influenced the antibacterial activity but not the acute toxicity effect.

The quercetin-3-O-glycoside was the most interesting flavonoid. It showed an important antibacterial activity against all the tested bacteria and a very low acute toxicity effect which encouraged its use. Some previous work had already reported these results [14-16].

It was also interesting to note that the best MICs values were given by the synergism of the apigenin, the quercetin-3-O-glycoside and the kaempferol-3-O-glycoside. However, *B. subtilis* was the most sensitive species for all the tested extracts. The lowest MIC recorded for this species was of 0,010 mg/ml. This could indicate that weak concentrations in these extracts were sufficient to limit and even to eliminate completely the infections caused by this microorganism.

CONCLUSION

The flavonoids of *M. longifolia* allowed the inhibition of some microorganisms that may be causal agents of human urinary, intestinal and respiratory tract infections; indicating that they could be used to cure these diseases. Among the identified flavonoid, the quercetin-3-O-glycoside showed the best antibacterial activity and acute toxicity effect which encouraged its use.

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