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ANTIMICROBIAL ACTIVITY OF LEAVES OF *RUBUS ULMIFOLIUS* SCHOTT AGAINST SOME PATHOGENIC BACTERIA AND FUNGI AND EVALUATION OF THE ACUTE TOXICITY

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ABSTRACT

The aqueous, methanolic and phenolic extracts of Rubus ulmifolius leaves were studied and tested for their antimicrobial and antifungi activity against some clinical bacteria .Than the acute toxicity was evaluated. The results showed that the phenolic extract was the most active of staphylococcus aureus with a maximum zone of inhibition 7-12mm. All the other bacteria and fungi showed no significant susceptibility. Among, the acute oral toxicity investigation of aqueous extract shows no mortality and any evidence of adverse effects have been observed in mice following acute oral administration at the highest dose of 1,2g/ kg.

KEYWORDS: ***Rubus ulmifolius,*** **antimicrobial activity, phenolic extract and toxicity**

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INTRODUCTION

In recent years, pathogenic microorganisms have developed multiple drug resistance. Therefore, there is a need to develop alternative antimicrobial drugs from various sources such as medicinal plants [1].*Rubus ulmifolius* is a plant used in traditional medicine against the diarhoea, sore throat, and as healing, antibacterial, antiviral [2] and antiprofilative cancer cells [3, 4, 5] and also for its beneficial effects [6, 7, 8,9,10]. It forms the majority of hedgerows, the vast majority of bushes agricultural areas and abandoned fallow. It is distributed in the Euromediterranean regions and Asia [11], in Algeria it is found in Great and Small Kabylie and around Annaba especially in wet forests [12]. Advances in research on herbal medicine, which is currently experiencing a remarkable renaissance, have resulted in new plants, offer hope for treating many diseases. Currently, medicinal and aromatic plants remain the inexhaustible resources which can replace antibiotics. The aim of this study is to evaluate several extracts against several bacterial strains responsible for many human diseases and acute toxicity in mice.

MATERIALS AND METHODS

(i) Collection and extraction of plant sample

The fresh sample of *Rubus ulmifolius (R ulmifolius*) collected in Chrea at 1200km on altitude brought back immediately to the laboratory in a sterile plastic bags. The plants were authenticated R ulmifolius by Professor M. Abdelkrim of the Department of Botany in Scientific High School of Agriculture Sciences. The leaves were rinsed thoroughly under running tap water and the clean samples were then dried in an oven at 45°C for 4-7 days until they were completely dried before grinding them into powder form[14,15].The phenolic extraction (EE) was performed according to the protocol of Durling et al. (2007) [13]. The *R ulmifolius* extract was prepared as follows: the powder was suspended in an alcohol solvent (70g in 300ml of ethanol associated with 50 ml of demineralized water). The preparation placed in a water bath at 40 ° C for 3 hours in the dark and then filtered with Watman paper n°3. The methanolic extract (ME) was prepared with soxhlet (50g of dried powder in 500 ml of methanol), the ME extract was concentrated in a rotary evaporator under reduced pressure[14]. The Aqueous extract (AE) was prepared in a way that, as much as possible, mimicked the method traditional herbal practitioners use to extract their plant medicines. The dried leaves were suspended in distilled water (10 g dried leaves in 100ml of water) and the mixture boiled for 30 min. The decoction obtained was cooled and filtered[15].

(ii) Determination of Antimicrobial Activity and acute toxicity

The quantitative evaluation of the antimicrobial activity focused on eight strains from the microbiology laboratory of the hospital Boufarik. Some of these organisms were isolated by cytobacteriological urine analysis (table 1). We used the Mueller Hinton agar medium that allows the growth of many bacteria, and contains no inhibitor or antibiotic that may influence the results. Medium pH was between 6 and 7.4mm. The thickness of the agar is 4 mm and the temperature of incubation at 37 ° C for 24 hours and Strains collected and inoculated on Sabouraud medium poured into Petri dishes and incubated at 27 ° C for 7 days. Healthy Albinos mice (36) Mus Musculus of either sex were procured from N.M.R.I (Naval Medica Research Institut) Swiss imported by frensh origin imported by IFFA –CREDO (Lyon) delivered by pharmacological laboratory of Antibiotical Complex (SAIDAL, Medea) in Algeria, weighing 18–24 g, were divided in groups of 6. The animals were housed 6 per plastic cage, and the photoperiod (light on from 06:00 to 18:00 h), air changes and room temperature (24 ± 1 ◦C) were controlled. All animals had free access to tap water and food, except for a short fasting period before oral administration of single doses of the *R ulmifolius* extract. The extract was dissolved/suspended in distilled water and administered by gavage at doses of 0,2; 0, 4, ;0,6; 0,8; 1 and 1,2 g/kg. The general behavior of mice was observed continuously for 1 h after the treatment and then intermittently for 4

h, and thereafter over a period of 24 h[16]. The mice were further observed for up to 14 days following treatment for any signs of toxicity and deaths, and the latency of death[13]. The LD50 value was determined according to the method of Litchfield and Wilcoxon (1949)[17].

Table 1

ATTC bacterail strains	Clinical isolate bacteria strains
Pseudomonas aeruginosa 27853	/
Escherichia coli 25922	urine
Staphylococcus aureus 25923	pus
Candida albicans 2091	/
Aspergillus niger 16404	/
Klebsiella pneumoniae	urine
Salmonella typhimurium	bloodculture

(iii) Screening of antimicrobial activity

We used the Mueller Hinton Agar medium that allows the growth of many bacteria, and contains no inhibitor or antibiotic that may influence the results. The thickness of the agar is 4 mm and the temperature of incubation at 37 ° C for 24 hours and Strains collected and inoculated on Sabouraud medium poured into Petri dishes and incubator at a temperature of 27 ° C for 7 days.Introduce 1 ml of bacterial suspension to which 9 ml of saline is added and from the mother solution to dilutions of 10^{-1} to $10^{10-3.}$ Evaluation of antibacterial activity was performed by the agar diffusion method called disk (6 mm) diffusion method [18.]For reading the results: The biological activity is manifested by the appearance of a halo of inhibition of microbial growth around the disk containing the extract to be tested. The reading is done by measuring the diameter of inhibition observed. The result of this activity is expressed as the diameter of the zone of inhibition and can be symbolized by crosses [19, 20,21]are considered respectively as strain:

Resistant (-): D <8 mm.
Sensitive (+): 9 mm ≤ D ≤ 14 mm.
Very sensitive (+ +): 15 mm ≤ D ≤ 19 mm.
Extremely sensitive (+ + +) D ≥ 20 mm.

(iv) Statistical analysis

Results are expressed as the mean ± S.D. of three independent experiments (n=3) with EXEL 2007.

RESULTS

The results shown in the table 2 the antimicrobial activity of the aqueous, methanolic and phenolic extract of *R ulmifolius* measured by the method of diffusion in a solid medium. Strains *Staphylococcus aureus* (Pus) and (ATTC) showed extreme sensitivity against the aqueous extract at 100% with diameters ranging from (7 and 12mm). Note that testing of the methanol extract of the *R ulmifolius* exhibit antimicrobial activity with different degrees only *Staphylococcus aureus*, it is zero below 1/100 and 1/1000, this translates into a resistance. Whereas with a concentration of 1/10, the action of the extract increases and activity varies between sensitive and very sensitive with diameters ranging from 7 to 10 mm, peaking with 100% concentration where the germs becomes extremely sensitive with diameters ranging from 17 to 19 mm. This assay shows the abilities of the phenolic extract a resistance of all strains tested with the exception with *Staphylococcus aureus*, which shows the high sensitivity between extremely sensitive at 100% of concentration with diameters ranging from 18 to 22 mm, and very responsive and sensitive to the concentration at1/10 with diameters ranging from 7 to 11 mm. The activity is zero in the remaining concentrations. *Staphylococcus aureus* sensitivity is proportional to the concentration of the extract.

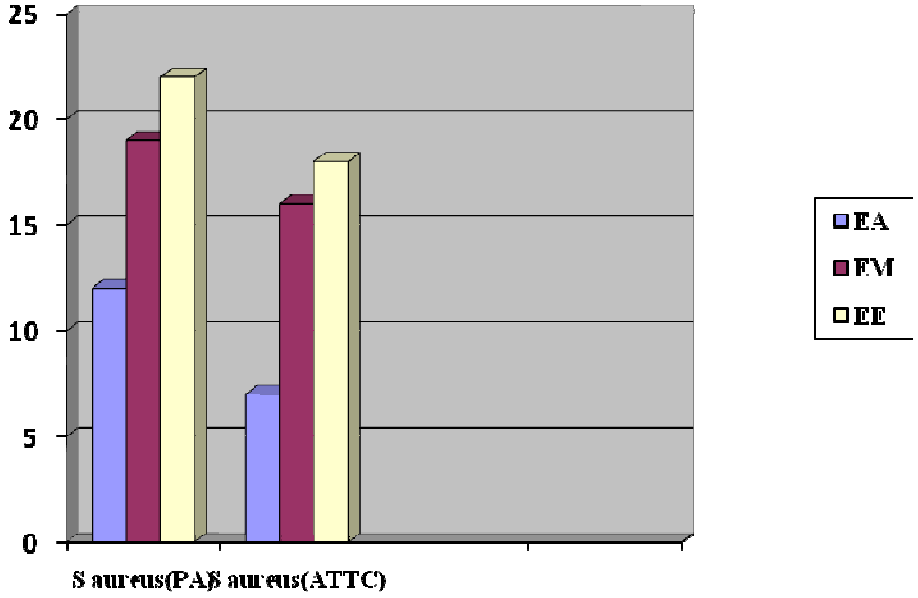
Table 2

Name of extract		Microorganism and zone of inhibition (mm)							
		Ec1	Ec2	Sa1	Sa2	Kp	St	Ca	An
	100%	0	0	12±0	7±1	0	0	0	0
Aqueous	1/10	0	0	0	0	0	0	0	0
	1/100	0	0	0	0	0	0	0	0
	1/1000	0	0	0	0	0	0	0	0
	100%	0	0	19±0	17±0	0	0	0	0
Methanolic	1/10	0	0	7±0,053	10±0	0	0	0	0
	1/100	0	0	0	0	0	0	0	0
	1/1000	0	0	0	0	0	0	0	0
	100%	0	0	22±2	7±0	0	0	0	0
Polyphenolic	1/10	0	0	18,33±0,58	11±0	0	0	0	0
	1/100	0	0	0	0	0	0	0	0
	1/1000	0	0	0	0	0	0	0	0

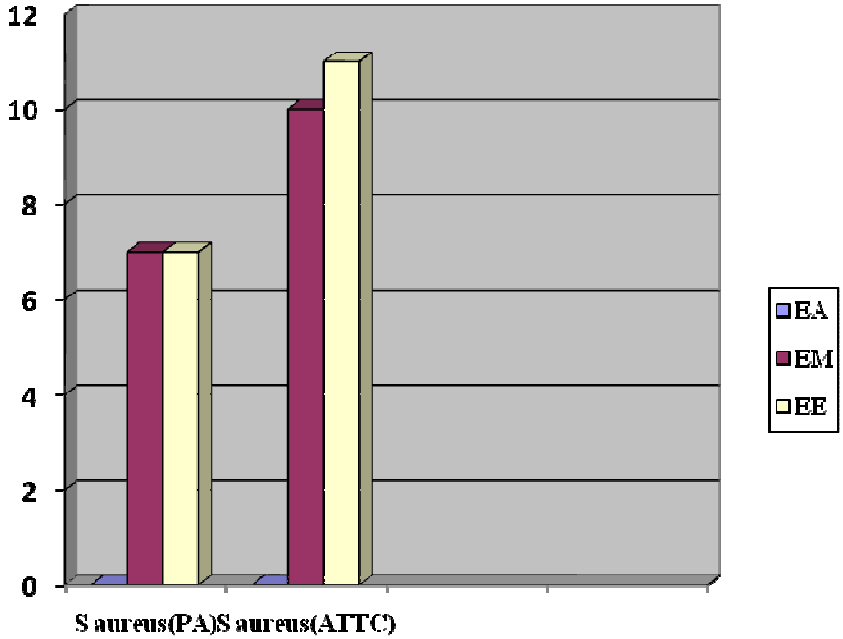
Ec1 : Eshericchia coli from urine ***Ec2 : Esherichia coli ATTC*** ***Sa1 : Staphylococcus aureus from pus***
Sa2 : Staphylococcus aureus ATTC ***Kp : Klebseilla pneumoneae*** ***St : Salmonella typhimurium***
Ca : Candida albicans ***An : Aspergillus niger***

The results for the activity of the extracts against *Staphylococcus aureus* are reported in graph 1 and 2.

Graph 1
Diameter of inhibition zones (mm) of susceptible strains (100% concentration)



Graph 2
Diameter of inhibition zones (mm) susceptible strains (Concentration 1/100)



For the 100% concentration, in terms of the inhibition zones, values ranged from 7 to 12 mm for the aqueous extract of 16 to 19 mm for the methanol extract and finally the highest were assigned to the polyphonic extract with diameters ranging from 18 to 22 mm. The values were zero for the aqueous extract and ranged from 7 to 10 mm for the methanol extract and 7 to 11 mm for polyphenolic extract. Additionally, three samples were effective on a single bacterial strain Staphylococcus aureus, while other strains were resistant The study of antibacterial activity is based on measuring the diameters of the inhibition halos of each crude extract (AE, EE, ME) from *R ulmifolius*, the strains studied with the dilution showed the greatest sensitivity in the trial with a variance of concentrations to determine the minimum inhibitory dilution, in this case 1/10. Based on these findings no toxic effect was observed up to at the highest dose of 1,2g/ kg body weight treated via oral route over a period of 14 days.

DISCUSSION

The antimicrobial activity of *R ulmifolius* extracts were evaluated by a paper disc diffusion method against tested bacteria and fungi. Incubation of all the tested micro-organisms at different dilutions of phenolic and methanolic extract showed positive test to only bacterial strains namely *S. aureus.* The results showed that the phenolic extract was the most active of staphylococcus aureus. Others bacteria did not show susceptibility and showed resistance against the different extract of the plant. Our results agree with those of Panizzini et al,. 2002[2], who showed that the high antimicrobial activity on the same plant, R ulmifolius against *Staphylococcus aureus.* In fact, it has been shown that the mode of action of phenolic compounds depends on the concentration of the tested extracts [22]. There were reports on the uptake of antimicrobial compounds presence in the plant extract which affect greatly on the content in the cell wall of microorganisms[23]. The antimicrobial properties exhibited by the extracts may be associated with the presence of tannins, saponins, cardiacglycosides found in phytochemical screening in *R ulmifolius*. A large number of flavonoids have been reported to possess antimicrobial properties [24, 25, 26, 27] hydroxylation. Therefore, that is no clear predictability regarding the degree of

hydroxylation and the toxicity of the phenolic compounds towards microorganisms[28]

Other authors have suggested that the antibacterial activity of phenolic compounds is probably due to their ability to combine with extracellular soluble proteins and therefore the bacterial cell walls[29]. The medicinal plants are known to provide a rich source of botanical antibiosis and many of them have been used because of their medicinal values in treating various ailments in human.Therefore, such plants should be investigated tobetter understand their properties, safety and efficacy. Furthermore, in most of the indigenous system of medicines, medicinal plants are used in their crude form, since there are many reports stated that the active substance of medicinal plants are unstable in nature when fractionated and they work well synergistically[30].

CONCLUSION

This study showed that the aerial part of *Rubus ulmifolius* possesses active pharmacological principles that contribute to the antimicrobial properties. The present investigation also suggests that the study plant can be utilized as effective and safe natural source for antimicrobial agents and in other hand , the studies have shown that plant is non-toxic but we suggest that to investigate chronic toxicity of a high administering of a daily dose for a long period and his histological impact of different organs in rats.

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