

## **Antimicrobial, Antioxidant, Cytotoxic Activities and Phytochemical Screening of Some Algerian Plants**

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### **Abstract**

The current study represents the investigation of 16 Algerian plants usually utilized. The methanolic and ethanolic extracts of these plants were tested for their antimicrobial activity (against three Gram-positive bacteria, three Gram-negative bacteria and three yeasts species), their antioxidant activity and cytotoxic activity. Then, a phytochemical screening was realized for the extracts. Our results showed that the highest antimicrobial activity was exhibited by the methanolic extracts of *Camellia sinensis*, *Allium schoenoprasum* L., *Vicia faba* L., *Citrus paradisi*, *Lippia citriodora*, *Vaccinium macrocarpon*, *Pucina granatum* and *Linum capitatum*. Also, the methanolic extracts of *Camellia sinensis*, *Allium porrum* L., *Vicia faba* L., *Vaccinium macrocarpon*, *Pucina granatum* and *Linum capitatum* showed high free radical scavenging activity. Moreover, remarkable cytotoxic activity against FL-cells was found for the methanolic extracts of *Camellia sinensis*, *Cichorium intybus* L., *Lippia citriodora* and *Pucina granatum*. The phytochemical screening demonstrated the presence of different types of compounds like tannins, flavonoids and others, which could be responsible for the obtained activities..

**Keywords:** Algerian Plants, Antimicrobial Activity, Antioxidant Activity, Aytotoxic Activity, Phytochemical Screening.

## 1. Introduction

Several plants were widespread for their many therapeutic and pharmaceutical virtues, especially antioxidant, anti-tumoral, and anti-infectious activities. A big part of the world's population still relies on the benefits of food for the treatment of common illnesses (Zhang, 2004). These benefits provide form their big content on bioactive compounds (Cheruvanky, 2004).

In our work, we had tried to determinate the antimicrobial, the antioxidant and the cytotoxicity of 16 Algerian plants usually utilized. Then, we had realized a phytochemical screening in order to determinate the principal bioactive compounds presents in their methanolic and the ethanolic extracts.

## 2. Research Method

The plants were collected from different localities of Algeria (locally produced or imported) during different periods of the year 2007 and then they were identified at the Botanical Department of University Mentouri (Constantine, Algeria). The tested microorganisms provided from the laboratory of microbiology and infectious in Mohammed Ben-Yahia's hospital (Jijel, Algeria). Each microbial solution was standardized at  $10^6$  cfu/ml. The Muller-Hinton and Sabouraud agar medium (Institute Pasteur, Algiers, Algeria) were used for testing the antibacterial activity.

### 2.1. Extraction of Plant Material

The plant materials (20 g of powdered material and 20 ml of fruit juice) were extracted with 600 ml methanol by using a Soxhlet apparatus for 8 h and with 600 ml ethanol by maceration for 2 h. 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 dried extracts were stored at 20°C in the shade until used.

### 2.2. Antibacterial Assay

The radial diffusion assay described by The National Committee for Clinical Laboratory Standards (2000) was used to determinate the antimicrobial activity of the investigated extracts.

### 2.3. Antioxidant Activity

In order to measure antioxidant activity, DPPH (2,2'-diphenyl-1-picrylhydrazyl) free radical scavenging assay was used. The method was carried out as described by Brand et al. (1995). The radical scavenging activity was calculated from the equation:

$$\text{Percentage of radical scavenging activity} = (\text{Abs control} - \text{Abs sample}) / \text{Abs control} \times 100.$$

### 2.4. Cytotoxic Assay

The cytotoxicity of the investigated extracts was measured by the neutral red uptake assay (Lindl and Bauer 1989) using FL-cells, a human amniotic epithelial cell line. Only living cells are able to manage the active uptake of neutral red. The effect of the plant extracts on the proliferation of the FL-cells was determined in 96 well tissue culture plates. Confluent monolayers were incubated with different concentrations (serial dilutions) in medium for 72 h. The 50% cell-inhibitory concentration (IC<sub>50</sub>) was determined.

### 2.5. Phytochemical Screening

This screening was carried out with the methanolic and ethanolic extracts using chemical methods and thin-layer chromatography (TLC) according to the methodology given in (Wagner and Blatt, 1996).

### 3. The Results

#### 3.1. Antibacterial Activity

The results of the antimicrobial activity showed that the plant extracts were more active against Gram-positive bacteria. On the contrary Gram-negative bacteria were more resistant. In fact, only *Escherichia coli* was inhibited by some extracts but with high MICs ranging from 0.065 mg/ml by *Pucina granatum* to 0.120 mg/ml by *Allium porrum L.* and *Apium graveolens L.* But, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* had not exhibited any inhibition; it did mean that they were resistant to our extracts or that they necessitated high concentrations (Table 1).

**Table 1:** Results of the antimicrobial activity of the investigated plants (MICs expressed in mg/ml).  
M: methanolic extract; E: ethanolic extract; *S. a*: *Staphylococcus aureus*, *B. s*: *Bacillus subtilis*; *B. c*: *Bacillus cereus*; *E. c*: *Escherichia coli*; *P. a*: *Pseudomonas aeruginosa*; *K. p*: *Klebsiella pneumoniae*; *C. a*: *Candida albicans*; *C. m*: *Candida maltosa*; *C. n*: *Cryptococcus neoformans*; L: leaves; S C: seed coats; F J: fruit juice; B: bulb, F: flowers, NT: not tested.

Plants Tested	Extracts	<i>S. a</i>	<i>B. s</i>	<i>B. c</i>	<i>E. c</i>	<i>P. a</i>	<i>K. p</i>	<i>C. a</i>	<i>C. m</i>	<i>C. n</i>
<i>Camellia sinensis</i> (L)	M	0.065	0.065	0.035	0.080	-	-	0.090	-	0.095
	E	-	-	-	-	-	-	-	-	-
<i>Allium porrum L.</i> (B)	M	0.100	0.120	0.075	0.120	-	-	-	-	-
	E	-	-	-	-	-	-	-	-	0.040
<i>Allium cepa L.</i> (B)	M	0.110	0.110	0.075	-	-	-	0.050	0.040	0.060
	E	-	-	-	-	-	-	0.060	-	0.140
<i>Carthamus tinctorius</i> (L,F)	M	0.090	0.120	0.090	0.100	-	-	0.090	-	-
	E	-	-	-	-	-	-	-	-	0.060
<i>Allium schoenoprasum L.</i> (L)	M	0.085	0.085	0.075	-	-	-	0.075	0.090	-
	E	-	-	-	-	-	-	-	-	0.110
<i>Cloeme schweinfurthii</i> (L)	M	0.110	0.010	0.075	-	-	-	-	0.140	-
	E	-	-	-	-	-	-	-	-	0.075
<i>Vicia faba L.</i> (S C)	M	0.065	0.090	0.035	0.060	-	-	0.080	0.090	0.080
	E	0.075	0.140	0.060	-	-	-	0.090	-	-
<i>Petroselinum sativum</i> (L)	M	0.120	-	0.110	-	-	-	-	-	-
	E	-	-	-	-	-	-	-	-	0.140
<i>Cichorium intybus L.</i> (L)	M	0.010	0.010	0.075	-	-	-	0.090	0.110	-
	E	-	-	-	-	-	-	-	-	0.090
<i>Citrus paradisi</i> (F)	M	0.060	0.010	0.060	0.100	-	-	0.090	0.090	-
	E	-	-	-	-	-	-	-	-	0.090
<i>Apium graveolens L.</i> (L)	M	0.075	0.100	0.090	0.120	-	-	0.090	0.090	0.050
	E	-	-	-	-	-	-	0.050	0.060	0.030
<i>Lippia citriodora</i> (L)	M	0.045	0.085	0.045	0.090	-	-	-	-	-
	E	-	-	-	0.100	-	-	-	-	-
<i>Mentha longifolia</i> (L)	M	0.100	0.120	0.100	-	-	-	0.075	0.110	0.075
	E	-	-	-	-	-	-	0.030	0.050	0.040
<i>Vaccinium macrocarpon</i> (F J)	M	0.060	0.075	0.040	-	-	-	0.025	0.090	0.030
	E	-	-	-	-	-	-	0.050	0.140	0.060
<i>Pucina granatum</i> (F J)	M	0.075	0.100	0.075	0.070	-	-	0.050	0.140	0.090
	E	0.140	-	-	0.090	-	-	0.075	-	0.090
<i>Linum capitatum</i> (L)	M	0.085	0.100	0.075	0.065	-	-	0.110	-	0.110
	E	-	-	-	0.065	-	-	-	-	-
Ampicillin (10µg/disc)		0.020	0.015	0.010	NT	NT	NT	-	-	-

It was interesting to note that the yeasts showed more sensitivity to the investigated extracts than the other antibiotic susceptible Gram-positive bacteria. Also, we had note that the methanolic extracts were more active than the ethanolic ones. The most pronounced activities, so with the lowest MICs, were shown by the methanolic extracts of *Camellia sinensis* (0.035 mg/ml against *Bacillus cereus*), *Vicia faba L.* (0.035 mg/ml against *Bacillus cereus*), *Citrus paradisi* (0.010 mg/ml against

*Bacillus subtilis*), *Lippia citriodora* (0.045 mg/ml against *Staphylococcus aureus* and *Bacillus cereus*), *Vaccinium macrocarpon* (0.025 mg/ml against *Candida albicans* and *Cryptococcus neoformans*) and *Punica granatum* (0.050 mg/ml against *Candida albicans*). The majority of the ethanolic extracts of the antibacterial active plants did not express any activity. Finally, it was remarkable that *Klebsiella pneumoniae* was not inhibited at all. All our extracts were inactive against this microorganism.

### 3.2. Antioxidant Activity

The methanolic extracts of *Camellia sinensis*, *Punica granatum*, *Allium porrum L.*, *Vicia faba L.*, *Vaccinium macrocarpon* and *Linum capitatum* showed a high effective free radical scavenging in the DPPH assay. The methanolic extracts of the two first plants had shown a very important antioxidant effect at 10 µg /ml (43% and 55% successively) whereas the ascorbic acid showed at this concentration an effect of 45% (Table 2). But, *Allium porrum L.*, *Vicia faba L.* and *Vaccinium macrocarpon* started to exhibit a high effective free radical scavenging only at 50 µg /ml (55, 50 and 54%, respectively). The ethanolic extracts of all the investigated plants were only weak active. And conformably to the antimicrobial results the ethanolic extracts showed a very low free radical scavenging effect.

**Table 2:** Results of the free radical scavenging activity, cytotoxicity against FL-cells and phytochemical screening of the investigated plants.  
M: methanolic extract; E: ethanolic extract; RSA: radical scavenging activity; L: leaves, S C: seed coats; F J: fruit juice; B: bulb, F: flowers.

Plants	Extracts	IC <sub>50</sub> µg/ml	RSA (%) 10µg/ml	RSA (%) 50µg/ml	RSA (%) 100µg/ml	RSA (%) 500µg/ml	RSA (%) 1000µg/ml	Pytochemical Screening
<i>Camellia sinensis</i> (L)	M	70	43.50	44.07	52.75	81.92	92.26	Tannins, alkaloids, anthocyanins,
	E	>1000	0.00	0.00	8.57	15.87	22.94	Phenolic acids, anthocyanins
<i>Allium porrum L.</i> (B)	M	950	9.15	55.39	92.25	95.54	95.20	Terpenoids,
	E	>1000	0.00	0.00	14.60	14.91	17.80	Flavonoids
<i>Allium cepa L.</i> (B)	M	700	6.02	17.34	15.63	98.10	94.98	Tannins, flavonoids, volatile oils
	E	>1000	0.00	0.00	0.00	0.00	6.29	Flavonoids
<i>Carthamus tinctorius</i> (L,F)	M	100	0.00	0.00	10.78	89.73	88.70	Isoflavonoids, flavonoids, coloring substances
	E	>1000	0.00	0.00	0.00	10.34	16.21	Flavonoids
<i>Allium schoenoprasum L.</i> (L,F)	M	540	0.00	0.00	18.53	74.40	99.98	Terpenoids, flavonoids, sterols, phenolic acids
	E	>1000	0.00	0.00	0.00	9.44	37.90	Flavonoids
<i>Cloeme schweinfurthii</i> (L)	M	515	2.87	0.20	10.36	64.69	84.80	Flavonoids, terpenoids
	E	>1000	0.00	0.00	0.00	0.00	2.65	Flavonoids
<i>Vicia faba L.</i> (S C)	M	650	29.98	50.72	80.84	94.29	92.45	Condensed tannins, anthocyanins
	E	>1000	0.00	0.00	10.05	22.77	31.80	Phenolic acids
<i>Petroselinum sativum</i> (L)	M	100	22.37	19.08	21.42	41.56	63.60	Terpenoids, volatile oils
	E	>1000	0.00	0.00	0.00	0.00	4.02	Flavonoids
<i>Cichorium intybus L.</i> (L)	M	15	13.78	17.13	33.51	40.10	58.69	Volatile oils, sterols
	E	>1000	0.00	0.00	0.00	12.64	17.32	Phenolic acids
<i>Citrus paradisi</i> (F)	M	100	0.00	5.62	51.00	99.00	99.62	Flavonoids, hydrolysable tannins, volatile oils
	E	>1000	0.00	0.00	0.00	0.00	13.96	Flavonoids
<i>Apium graveolens L.</i> (L)	M	625	16.12	27.45	50.32	93.90	94.34	Terpenoids, steroids, isoflavonoids
	E	>1000	0.00	0.00	0.00	0.00	23.83	Flavonoids
<i>Lippia citriodora</i> (L)	M	30	3.22	7.95	14.04	60.52	99.41	Terpenoids, volatile oils, flavonoids, phenolic acids
	E	>1000	0.00	0.00	7.78	16.38	33.80	Phenolic acids, flavonoids
<i>Mentha longifolia</i> (L)	M	820	16.90	21.47	36.19	91.76	93.12	Flavonoids, volatile oils, terpenoids, sterols
	E	>1000	0.00	2.04	12.00	16.34	24.30	Flavonoids
<i>Vaccinium macrocarpon</i> (F J)	M	>1000	26.06	54.85	92.07	93.36	95.25	Condensed tannins, anthocyanins
	E	>1000	4.30	3.51	10.24	39.90	38.80	Anthocyanins
<i>Punica granatum</i> (F J)	M	70	55.78	62.39	79.45	95.24	95.87	Condensed tannins, anthocyanins
	E	>1000	0.00	14.34	11.05	43.56	56.41	Phenolic acids
<i>Linum capitatum</i> (L)	M	>1000	40.50	46.70	53.40	89.78	93.30	Flavonoids, terpenoids, steroids, volatile oils
	E	>1000	0.00	4.02	13.00	23.51	29.00	Flavonoids
Ampicillin (10µg/disc)			45.28	96.81	96.51	97.60	96.37	

### 3.3. Cytotoxic Activity

Among the 32 extracts tested for cytotoxicity against FL-cells only the methanolic extracts of *Camellia sinensis*, *Cichorium intybus L.*, *Lippia citriodora* and *Pucina granatum* exhibited noticeable activities with IC<sub>50</sub> values below 100 µg/ml.

### 3.4. Phytochemical Screening

The phytochemical screening of methanolic extracts showed the presence of different types of active constituents, namely flavonoids, terpenoids, tannins, volatile oils, anthocyanins and phenolic acids. These compounds were present in almost all the methanolic extracts. But in the ethanolic ones, only phenolic acids, flavonoids and sometimes anthocyanins were founded. This could explain the fact that the methanolic extracts had the highest antimicrobial and antioxidant activities.

## 6. Discussion

The results of our study confirmed the importance of the investigated plants in our nutrition. The existing knowledge about some of them is in many cases very limited.

We had found that *Camellia sinensis* was the most active tested plant. Its antibacterial effect was already demonstrated by previous works (Simonetti et al., 2004; Punyarisi et al., 2005), but no work was realized on its antifungal activity against *Candida maltosa* and *Cryptococcus neoformans*, nevertheless our results showed exactly that the methanolic extract of this plant was active against *Cryptococcus neoformans* but not against *Candida maltosa*. Our phytochemical screening revealed in the methanolic extract the presence of tannins and alkaloids in big amount, which could be responsible for these noteworthy activities (Ramethinam and Rajalakshmi, 2004; Gosse et al., 2005). Thus, the estimated antimicrobial and antioxidant effects of the investigated *Camellia sinensis* are in accordance with these data.

In our study, the methanolic extract of *Vicia faba L.* had an antimicrobial and antioxidant effects in accordance with the other investigations realized on different kinds of seeds (Ray et al., 2000; Zhang, 2005). Another work had described the isolation of condensed tannins and anthocyanins from this plant (Mergham et al., 2004). Our phytochemical screening indicated the presence of these types of compounds in addition of anthocyanins and phenolic acids, which are mainly responsible for the remarkable antioxidant and antimicrobial effect of this plant.

In precedent reports about *Citrus paradisi*, the antimicrobial activity was demonstrated against several species (Cvetnic and Vladimir-Knezevic, 2004), but no report was found about the antifungal activity against *Candida maltosa* and *Cryptococcus neoformans*. The high antimicrobial activity of this plant was attributed to the essential oils, vitamin C and flavonoids that its extracts contained (CANO et al., 2008). The extracts of other species of *Citrus* namely *Citrus sinensis* and *Citrus aurantifolia*, exhibited also non negligible antibacterial effect (Madunagu et al., 1991) against some other microorganisms.

In earlier studies, it was found that *Lippia citriodora* show a strong antimicrobial activity against different types of bacteria and fungi (Oliveira et al., 2006). Unlike this result, our investigated extracts of *Lippia citriodora* exhibited activity only against Gram-positive bacteria and *Escherichia coli*. In addition, it was demonstrated that the infusion of *Lippia citriodora* has a potent superoxide radical scavenging activity and a moderate scavenging activity of hydroxyl radical (Valentao et al., 2002). The scavenging activity of DPPH radical in our screening was shown only at the highest concentration (1000 µg/ml).

For *Vaccinium macrocarpon*, precedent works had shown the antibacterial activity of this plant against several microorganisms, principally bacteria (Di Martino et al., 2005; Magarinos et al 2008), but no report was found against *Klebsiella pneumoniae*. And against *Candida albicans* the activity was not specified, we only know that the cranberry extracted limited the urinary infections caused by *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* (Sobota, 1984). In our study, it was

evident that this plant was active against *Candida albicans* and against the two other yeasts tested. Many anthocyanins and proanthocyanins isolated from this plant were responsible for the antimicrobial effect (Leitao et al., 2005).

## 6. Conclusion

In conclusion, the obtained results showed that some plants had an important antimicrobial, scavenging and cytotoxic activities at the same time like *Camellia sinensis* and *Punica granatum*. On the contrary, some others had only once or twice activity at the most, like *Cichorium intybus* L. Among the investigated plants, the two first were considered as the most interesting.

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