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Article

Antimicrobial and antibiofilm activities of phenolic compounds extracted from *Populus nigra* and *Populus alba* buds (Algeria)

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The interest of this work is the discovery of new antimicrobial agents of plant origin to inhibit the formation of microbial biofilms. The present research was conducted to identify and quantify the phenolic compounds extracted from *Populus nigra* and *Populus alba* buds harvested in the area of Tizi-Ouzou (Algeria), and to evaluate their antimicrobial and antibiofilm activity. High performance liquid chromatography (HPLC) was performed to identify the phenolic compounds in the ethyl acetate fraction of *P. nigra* and the methanolic extracts of *P. nigra* and *P. alba*. The antimicrobial activity of the crude extracts and the fractions of these two species was tested against 11 microorganisms, using the disk diffusion method, while the antibiofilm effect of certain extracts was carried out in a 96-well microplate and on a biomaterial (catheter). HPLC analysis revealed the presence of 10 bioactive compounds. The main phenolic compounds identified in the three extracts were p-coumaric acid, ellagic acid, and Kaempferol. This study was able to demonstrate that the extracts of *P. nigra* and *P. alba* buds have interesting antimicrobial properties, with diameters ranging from 6.6 to 21.3 mm. In addition, extracts of *P. nigra* exhibited antibiofilm effects greater than 70%. Our results provide evidence for the antimicrobial and antibiofilm potential of bud extracts from both poplar species. Thus, these results will pave the way for further research on these two plants.

Keywords: *Populus nigra*. *Populus alba.* HPLC. Polyphenols. Antimicrobial activity. Antibiofilm activity.

INTRODUCTION

Biofilms are a community of microorganisms incorporated in a matrix of organic polymers that adhere to a surface (Carpentier, Cerf, 1993; Costerton *et al*., 1999; Mah, O'Toole, 2001). The organization of microbial communities of biofilms into functional consortium and the nature of the surface are specified (Costerton *et al*., 1995). These bacteria can adhere to inert or living surfaces, and interface with one another. It is estimated that more than 99% all of the microorganisms find themselves in a wide range of ecosystems in biofilms, making bacteria stronger and more efficient in facing the hostile environment in which they develop, compared to bacteria evolving under planktonic shape (Costerton *et al*., 1987; Lewis, 2001; Donald, Costerton, 2002). Bacterial organization into biofilms presents a major source of sanitary, industrial, and ecological problems (Gilbert *et al*., 2002). It is important to note that once that a biofilm is formed, the bacteria are protected against immune defence systems and treatments, whether physical or chemical, such as disinfectants, detergents, and antibiotics. Currently, several questions have been raised regarding the safety and effectiveness of chemicals used in medicine. For more than twenty years, many determinants of resistance have been described with the appearance of increasingly resistant microbial strains (Alekshun, Levy, 2007). The regular and inappropriate use of antibiotics has led to the strong adaptation of bacterial strains and the selection of multi-resistant strains; this has consequences for human health (Kokkiligadda *et al*., 2013; Pasca *et al*., 2017). In the face of this phenomenon, jeopardized by the emergence of multi-resistant germs, interest in medicinal plants has developed because of their therapeutic properties and natural compounds that have been shown to exhibit antimicrobial and antibiofilm activities.

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The aim of the present work was to evaluate the antimicrobial and antibiofilm effects of *Populus nigra* and *Populus alba* extracts. Some studies have demonstrated the antibacterial and antifungal effects of *P. nigra* extracts. However, the activity of *P. alba* extracts has not yet been evaluted. In addition, no report regarding the antibiofilm activity of these two species is available.

MATERIAL AND METHODS

Plant material

The buds of *P. nigra* and *P. alba* were harvested in February 2015 in the region of Tizi-Ouzou, Algeria. The botanical identification of plant material was carried out by a botanist from the Department of Biology, University Mouloud Mammeri, Tizi-Ouzou (Algeria). The plant material collected was dried in the shade at an atmospheric humidity of 40% for 15 days, and then crushed into powder for analysis.

Preparation of plant extracts

The polyphenols of the buds of the two *Poplar* species were extracted by maceration as described by Romani *et al*. (2006) and Benhammou *et al*. (2008), with some modifications. Crushed plant material (5 g) was macerated in 100 mL of an organic solvent of different polarity (methanol, ethanol, ethyl acetate). After 24 h, the samples were filtered through a filter paper (Whatman No.1). Then, the filtrates were gathered and evaporated to dryness using a rotavapor (Buchi R-200) at 40°C.

For fractionation, the powder was macerated with acetone/distilled water (35/15, V/V). The aqueous phase obtained following removal of the solvent (acetone) was then separated sequentially with hexane, dichloromethane, ethyl acetate, and n-butanol. The dichloromethane (DF), ethyl acetate (AF), n-butanol (BF), and aqueous (AqF) fractions were collected separately and dried using a rotavapor. The recovered extracts were stored at 4 °C until further examination (Derbel *et al*., 2010).

Determination of phenolic compounds by HPLC

The identification of phenolic compounds was performed using HPLC. The HPLC system consists of a vacuum degasser, an autosampler, and a binary pump with a maximum pressure of 400 bar (Agilent 1260, Agilent Technologies, Germany) equipped with a reversed phase C18 analytical column of 4.6 × 100 mm and 3.5µm particle size (Zorbax Eclipse XDB C18). The DAD detector was set to a scanning range of 200–400 nm. Column temperature was maintained at 25 °C. The injected sample volume was 2 µl and the flow-rate of the mobile phase was 0.4 mL/min. Mobile phase B was milli-Q water with 0.1% formic acid and mobile phase A was methanol. The optimized gradient elution was as follows: 0–5 min, 10–20% A; 5-10 min, 20-30% A; 10-15 min, 30-50% A; 15-20 min, 50-70% A; 20-25 min, 70-90% A; 25-30 min, 90–50% A; 30-35 min, return to initial conditions.

For quantitative analysis, a calibration curve was obtained by plotting the peak area against different concentrations for each identified compound at 280 nm. The obtained curves for all identified compounds showed a good linearity with an average of $r^2 = 0.99$.

DETERMINATION OF ANTIMICROBIAL ACTIVITY

Microbial strains

The antimicrobial activity of extracts of *P. nigra* and *P. alba* was tested against 11 microorganisms including nine bacteria; six Gram-positive bacteria, namely *Staphylococcus aureus* ATCC 29213, *S. aureus* ATCC 6538, *S. aureus* resistant to methicillin ATCC 43300, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* ATCC 6633, and *Listeria innocua* Clip 74915; and three Gram-negative bacteria, including *Escherichia coli* ATCC 9029, *E. coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853. For both yeasts, we used *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* ATCC 9763. The bacterial and fungal strains were obtained from the Research and Development Center (CRD SAIDAL) in El-Harrach (Algeria) and the Laboratory of Biomathematics Biophysics Biochemistry and Scientometry (BBBS) of the University of Bejaia (Algeria).

Agar diffusion test

Pre-culture of the microbial strains was prepared by selection and culture of one colony of each species on an agar surface to reach exponential growth phase. From this culture, a quantity of the bacterial strain was suspended in sterile physiological water.

The optical densities were then adjusted using a UV spectrophotometer (Optizen pop, Koria) at a wavelength of 625 nm ($\lambda = 0.08–0.1$, corresponding to 10^8 colony forming units [CFU] mL^{-1}) for bacteria and 530 nm ($\lambda = 0.12–0.15$, corresponding to $1–5 \times 10^6$ CFU mL^{-1}) for the yeasts. The standardized inoculum (10^6 CFU mL^{-1}

for yeasts and bacteria) was streaked with a swab on Mueller–Hinton agar (Condapanadise, Spain) for bacteria or Mueller–Hinton agar supplemented with 2% glucose and 0.5 µg mL^{-1} methylene blue for yeasts (Testore *et al*., 2004; Epsinel-Ingroff, 2007). Then, 6 mm diameter filter paper discs impregnated with 10 µL of solubilized extract in dimethyl sulphoxide (DMSO; 1 mg/disc) were deposited on the surface of the previously inoculated agar using sterile forceps. The petri dishes were left for 1 h at room temperature for pre-diffusion of the substances, before being incubated at 37 °C for 18–24 h for bacteria (Adesokan, Akanji, Yakubu, 2007), and at 30 °C for 48 h for the yeasts. Control disks were included in the tests. The disks were impregnated with DMSO (Biochem, India) and antibiotics (gentamicin). The experiment was performed in triplicate. Antibacterial activity was determined by measuring the diameter of the inhibition zones around the disks (Doughari, Pukuma, De, 2007). The effect of the extracts was evaluated according to the criterion of Tekwu, Pieme, and Beng (2012). Thus, a substance is said to be inactive if the diameter of the zone of inhibition is less than 7 mm, while it is said weakly active if the diameter is between 7 and 10 mm. On the other hand, it is deemed moderately active when the diameter is greater than 10 mm and less than 15 mm. It is significantly active when the diameter is greater than 16 mm.

DETERMINATION OF ANTIBIOFILM ACTIVITY

Antibiofilm activity in a 96-well microplate

The anti-adherent properties of the methanolic and ethanolic extracts of *P. nigra* and *P. alba* on *S. aureus* ATCC 6535 and methicillin-resistant *S. aureus* (MRSA) were evaluated as described by Saising *et al*. (2012) with some modifications. The sterile microtiter plates were prepared by dispensing 100 µL of Brin Heart Infusion Broth (BHI) with 2% glucose (p v^{-1}) into each well. From the mother solution of extracts tested at a concentration of 20 mg mL^{-1}, 100 µL was added to the second row of the microplate. Seven serial dilutions were then made using a micropipette, thus 100 µL of overnight cultures (37 °C) of the tested strains diluted to 10^6 CFU mL^{-1} in fresh BHI with 2% glucose was transferred to each well. The cultures were added to the wells in triplicate, and a growth control (cells + broth), media control (single broth), and white control (broth + extract) were included. Following incubation at 37 °C for 24 h without shaking, the culture supernatant was discarded. Then, each well was washed twice with phosphate-buffered saline (PBS) to remove loosely associated cells. The plates were dried in the oven at 60 °C for 30 min. After drying, the surface-fixed cells were stained with 200 µL of 0.1% violet crystal. Quantitative evaluation of biofilm cells was estimated by solubilisation of the dye with 200 µL of 96% ethanol, and the optical density (OD) was determined at 570 nm using a UV spectrophotometer (Optizen pop). The percent inhibition of the biofilm was calculated using the following formula: [(OD growth control – OD sample) / OD growth control] × 100 (Chaieb *et al*., 2011).

Antibiofilm activity on the biomaterial

In vitro experiments evaluated the ability of the extracts of *P. nigra* and *P. alba* to inhibit biofilm formation on catheter tubes. Briefly, sterile pieces of a 1 cm intravenous infusion tube were incubated with 100 µL of bacterial suspension and 100 µl of extract at different concentrations (20–0.156 mg mL^{-1}) in a microplate. After incubating the plates at 37 °C for 24 h, the catheter pieces were washed twice with PBS, dried, and stained with 200 µL of 0.1% crystalline violet. Biofilm biomass was determined by the crystal violet colorimetric method as described above.

RESULTS AND DISCUSSION

HPLC separation of the phenolic compounds from the methanolic extract of *P. nigra* and *P. alba* and the AF of *P. nigra* is presented in Figure 1, and the concentrations are shown in Table I. The separation detected the presence of p-coumaric acid (Tr = 20 min, peak 1), myricitrin (Tr = 20.7 min, peak 2), luteolin-7-O-glucoside (Tr = 21.1 min, peak 3), rosmarinic acid (Tr = 21.7 min, peak 4), ellagic acid (Tr = 22.2 min, peak 5), quercetin (Tr = 23.7min, peak 6), naringenin (Tr = 24.2 min, peak 7), luteolin (Tr = 24.8 min, peak 8), kaempferol (Tr = 25.5 min, peak 9), and apigenin (Tr = 25.8 min, peak 10) in the extracts tested.

The HPLC fingerprinting of *P. nigra* and *P. alba* extracts revealed a difference in the phenolic composition of the samples. Several compounds identified in the AF, namely luteolin, luteolin 7-*O*-glucoside, naringenin, and apigenin, were not found in the other extracts. Rosmarinic acid and quercetin were only detected in the methanolic extract of *P. nigra*. Chromatographic analysis also showed that there is a difference in all peak intensities. This suggests the existence of a different content of phenolic compounds in the tested samples: all extracts contained ellagic acid, kaempherol, and p-coumaric acid, although

TABLE I - Identification of phenolic compounds in *P. nigra* and *P. alba* buds extracts

peaks	Compounds	Retention time (min)	Quantity (mg g^{-1} of residue)		
			AF.Pn	ME.Pn	ME. Pa
1	*p*-coumaric acid	20	2.47	1.73	0.50
2	Myricitrin	20.7	13.42	nd	nd
3	Luteolin7-*O*-glucoside	21.1	39.86	nd	nd
4	rosmarinic acid	21.7	nd	14.46	nd
5	ellagic acid	22.2	1.79	1.98	1.79
6	Quercetin	23.7	nd	2.07	nd
7	Naringenin	24.2	nd	nd	nd
8	Luteolin	24.8	10.81	nd	nd
9	Kaempferol	25.5	6.20	5.17	0.54
10	Apigenin	25.8	1.12	nd	nd

Legend: AF.Pn = Ethyl acetate fraction of *Populus nigra*; ME.Pn = methanolic extract of *Populus nigra*; ME.Pa = methanolic extract of *Populus alba*; nd= non detected

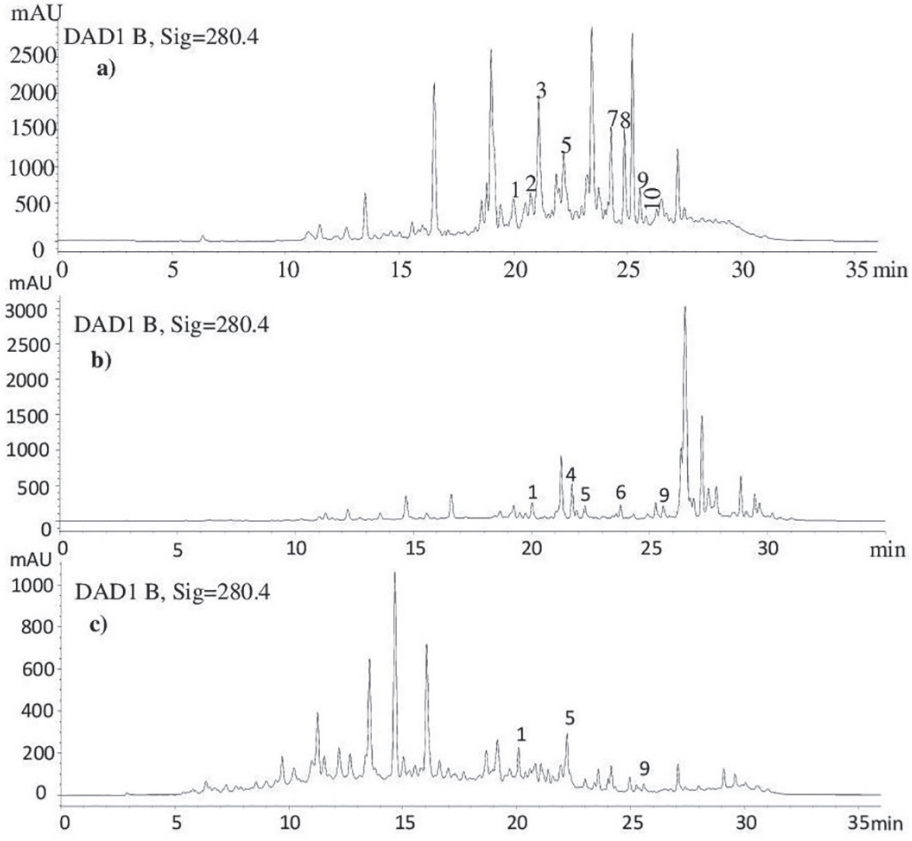

FIGURE 1 - Chromatographic profiles acquired at 280nm of poplar extracts. (a) Ethyl acetate fraction of *P. nigra* (AF.Pn). (b) methanolic extract of *P. nigra* (ME.Pn). (c) methanolic extract of *P. alba* (ME.Pa). Peaks numbers are referred to those reported in Table I.

the highest concentration was observed for the AF. The work of Falcao *et al*. (2010) also showed the presence of p-coumaric acid and apigenin in poplar-type propolis. Some peaks of the obtained chromatograms were not determined because of the unavailability of certain standards.

The *in vitro* antimicrobial activity of 13 extracts against 11 reference microbial strains tested in this present study was qualitatively evaluated by the presence or absence of inhibition zone (IZ) diameter. The crude extracts of *P. alba* showed moderate activity against Gram-positive bacteria, including *S. aureus* ATCC 29213, *S. aureus* ATCC 6538, MRSA, *E. frendii*, and *L. Innocua* (Table II). Low activity was recorded against Gram-negative bacteria, namely *E. coli* ATCC 25922, *E. coli* ATCC 8739, and *P. aeruginosa*, while no effect was observed with *C. albicans* and *S. cerevisiae.*

It should be noted that the fractions stemming from white poplar buds have a moderate inhibitory effect on the growth of Gram-positive bacteria. In fact, the highest inhibition was recorded for the AF against *S. aureus* ATCC 6538 and *L. innocua* with an IZ diameter of 18.33 and 19 mm, respectively.

With respect to the organic extracts of black poplar (ME, EE, and AE), the S. aureus ATCC 29213 and MRSA strains express a high sensitivity toward these extracts with the following IZ diameters: 21.3 and 17.83, respectively, for the ME, 17.5 and 16.33, respectively, for the EE, and 17 and 17.83 , respectively, for the AE. Low sensitivity was observed for *E. coli* ATCC 25922, *E. coli* ATCC 25922, *E. coli* ATCC 8739, and *P. aeruginosa.*

For the fractions derived from the methanolic extract of *P. nigra*, we noticed that the AF and DF exerted greater activity against *S. aureus* ATCC 29213, SARM, and *L. innocua* with an IZ diameter of 20, 18.3, and 17.66 mm, respectively, for the AF, and of 18, 19.5, and 16 mm, respectively, for the DF. However, the AqF gave results that varied from 6.6 to 8.16mm in diameter, which reflects little to no activity.

According to the current results, the fractions and crude extracts of the buds of the two species studied present antimicrobial activity against all of the microorganisms studied. Thus, the IZ diameters were between 6.6 and 21.3 mm. In addition, *S. aureus* ATCC 29213, *S. aureus* ATCC 6538, and *B. subtilis* have been considered the most sensitive. These results are in agreement with those of Vardar-Ünlü *et al*. (2008).

Compared to gentamicin, the methanolic and ethanolic extracts have a greater effect on *E. faecalis,* and in the case of *S. aureus* ATCC 6538, it was the methanolic extract of *P. nigra* and the AF of *P. alba* that were more effective.

In general, the results show that *P. nigra* extracts possess a potent antibacterial activity compared to *P. alba*

TABLE II - Antimicrobial activity of differents extracts through diffusion method on a solid medium

	Extracts (1mg/disk)	Inhibition zone diameter (mm)*									
		Gram positive bacteria					Gram negative bacteria			Levures	
		Ef	*Sa1*	*Sa2*	SARM	*Bs*	*Li*	*Ec1*	*Ec2*	*Ps*	*Ca*	*Sc*
Populus nigra (Pn)	ME	11.33	21,3	16.16	17.83	15,33	9.33	7.66	8,66	7.50	11	9
	EE	11.66	17.5	15	16.33	12	8.83	7	8	7.33	12	10.66
	AE	11	17	14.83	17.66	13	11.66	7	7.6	7	13.66	10.33
	DF	9	18	12	19,5	12.33	16	8	7.5	8.33	12.33	10.33
	BF	10	10,33	12.5	14	15.33	11	7	6	6.5	7.8	8.33
	AF	10.33	20	14	18.33	16	17.66	7.66	8.5	7	9.66	8
	AqF	6.6	8,16	10.5	7	9	8	Na	Na	Na	Na	Na
Populus alba (Pa)	ME	12	16.33	14.16	14	12.66	16.33	8.66	9.6	9.66	Na	Na
	EE	12.66	13	13.16	11.33	Na	12.33	8	9	9	Na	Na
	DF	7.33	11	11	9.66	8.66	11	8	Na	7.33	8.33	8
	BF	11.33	13.33	13.33	10.63	12	15.6	Na	10.66	7.16	Na	Na
	AF	8.66	12.66	18.33	13.5	11.33	19	9.66	9	8.33	Na	8
	AqF	9	9	11.66	8	7	8.5	Na	Na	7	Na	Na
G (15µg/disk)		10	21	15	18	Nt	29	20	15	9	18	16

Legend: "*"=The diameter of the inhibition zone around the filter paper discs (6 mm); Na = No activity; Nt = No tested; ME = Methanolic extract; EE = Ethanolic extract; AE = Ethyl acetate extract; DF = Dichloromethane fraction; BF = n-butanol fraction; AF = Ethyl acetate fraction; AqF = Aqueous fraction, G = Gentamicin. Sa1= *S. aureus* ATCC 29213; Sa2 = *S. aureus* ATCC 6538; Ec1= *E. coli* ATCC 8739; Ec2 = *E. coli* ATCC 25922.

extracts. The AF had a significant effect, and the DF exerts an antimicrobial effect too restricted on all microorganisms tested. This shows that the bioactive compounds are less soluble in this solvent. These results are consistent with those of Abu-shanab *et al*. (2004), Owolabi, Omogbai, and Obasuyi (2007), and Valarmathy *et al*. (2010). These studies reported a sensitivity of bacterial strains to organic extracts compared with the aqueous extract.

Analysis of the experimental data revealed that organic extracts were more effective against Gram-positive than Gram-negative bacteria. Gram-negative bacteria are highly resistant, and this resistance is likely related to the nature of their outer membranes, which are impervious to lipophilic compounds (Djenane *et al*., 2012). Gram-positive bacteria are more sensitive and less protected against polyphenolic agents because they only have an outer layer of peptidoglycans, which can only prevent the diffusion of molecules whose molar mass is greater than 50 000 D (Hogan, Kolter, 2002; Abirami, Gomathinayagam, Panneerselvam, 2012). Smith-Palmer, Stewart, and Fyfe (1998), Marino, Bersani, and Comi (1999) and Inouye, Takizwa, and Yamaguchi (2001) obtained similar results to those of the present study, supporting the hypothesis that Gram-positive bacteria are more sensitive to plant extracts.

In a recent study, Vardar-Ünlü, Silici, Ünlü (2008) reported that the antimicrobial activity of an extract is likely due to the presence of synergy among various phenolic components.

Biofilms are the dominant microbial lifestyle, and are present in different environments, notably clinical, industrial, and food treatment environments, and drinking water distribution networks (Baker, Banfield, 2003; Kavanaugh, Ribbeck, 2012; Oral *et al*., 2010). It is important to mention that the microbial organization in biofilms is important for their virulence. This organization provides enhanced resistance to antimicrobial agents (Flowers *et al*., 1989), which leads to serious problems for human health (Costerton *et al*., 1999).

In this study, for the first time, the antibiofilm activity of methanolic and ethanolic extracts of *P. nigra* and *P. alba* against two bacteria, namely MRSA and *S. aureus* ATCC 6538, were studied. These bacteria have a great ability to form biofilms (Figure 2). The effect of the AF and ME prepared from the buds of these two species on the inhibition of the formation of biofilm *B. substitus* on a venous catheter was also examined.

Extracts of *P. nigra* and *P. alba* have shown efficient antibiofilm activity against the tested strains (Figure 3). Inhibition percentages greater than 70% were recorded for *P. nigra* extracts and more than 50% for *P. alba* extracts. Nevertheless, none of the extracts could inhibit biofilm formation completely.

Among the plant extracts tested, only the *P. nigra* extract has showed strong anti-adhesion activity, with an inhibition value of 88.64% for MRSA, while the methanolic extract of *P. alba* gave appreciable results in the inhibition of bacterial biofilms, with rates of 63.68% and 58.18% for MRSA and *S. aureus* ATCC 6538, respectively. Thus, MRSA is more sensitive than *S. aureus* ATCC 6538.

Evaluation of the inhibition potential of plant extracts against cell attachments to catheter tubes (Table III) showed that *P. nigra* extracts present a greater effect than *P. alba* extracts on inhibition of *B. subtilis* biofilm formation. The ethyl acetate fraction of *P. nigra*was effective at a concentration of 0.814 mg mL^{-1}, followed by the methanolic extract of *P. nigra* at 1.344 mg mL^{-1}, and the methanolic extract of *P. alba* with 10.174 mg mL^{-1}. The AF of *P. alba* was 11.541 mg mL^{-1}.

P. nigra extracts were revealed to effectively inhibit biofilm formation of *S. aureus* ATCC 6538, MRSA, and *B. subtilis* (Table III, Figures 3 and 4). According to our observations, the antibiofilm potential against these three strains may be related to the presence of one or more bioactive compounds with pronounced antibiofilm properties. In fact, flavonoids, quercetin, kaempferol, naringenin, and apigenin are generally considered to be the compounds most responsible for this activity (Vikram *et al*., 2010; Cho *et al*., 2015). In addition, preventative action of p-coumaric on the formation of biofilms of *E. coli* in urinary catheter fragments was observed at a concentration of 0.25% and 0.5% (Kot *et al*., 2015).

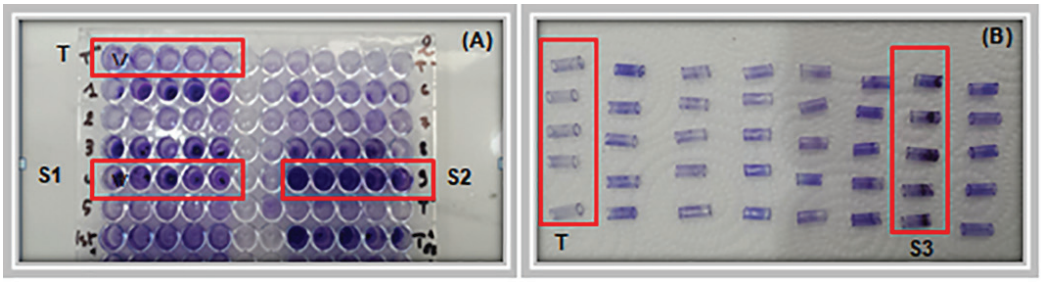

FIGURE 2 - Adhesion test of bacterial strains on a 96-wells microplate (A) and on a catheter (B).

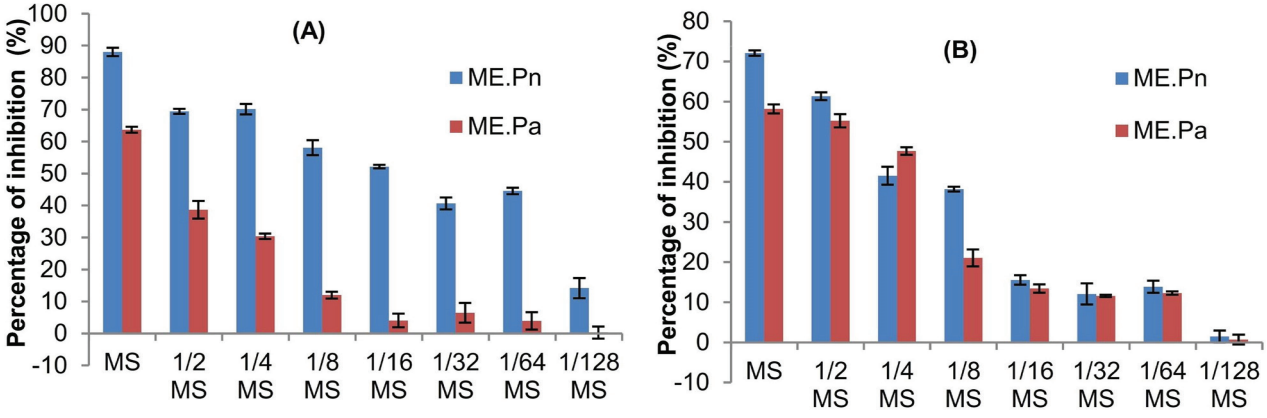

FIGURE 3 - The effect of *P. Nigra* methanolic extract (ME. Pn) and *P. Alba* methanolic extract (ME. Pa) on the attachment of methicillin resistant *S. aureus* (MRSA) ATCC 43300 (A) and of *S. aureus* ATCC 6538 (B), expressed as percentage inhibition.

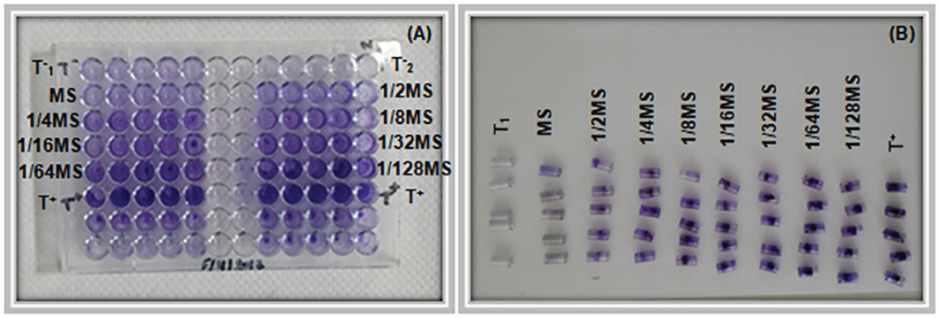

FIGURE 4 - Antibiofilm effect of methanolic extract of *P. nigra* (ME.Pn) on MRSA, in 96-well microplate (A), and ethyl acetate fraction of *P. nigra* (AF.Pn) on *B. subtilis*, in venous catheter (B).

TABLE III - Biofilm inhibitory concentration of ethyl acetate fraction and methanol extract of *P. nigra* and *P. alba*.

strain	ME.Pn BIC_{50}[a]	AF.Pn BIC_{50}	ME.Pa BIC_{50}	AF.Pa BIC_{50}
Bacillus subtilis	1.344±0.28	0.814±0.81	10.174±1.84	11.541±0.19

Legend: BIC_{50} = biofilm inhibitory concentration required to reduce biofilm coverage by 50%. [a] Data provided in mg mL^{-1}.

The results obtained in this study have revealed a promising pathway in the inhibition of biofilms by polyphenolic extracts and for the reduction of microbial colonization on epithelial surfaces and mucous membranes, which can lead to infections (Bavington, Page, 2005). Inhibition of the attachment of planktonic microbial cells to a substrate is easier than that of an already formed biofilm, due to a lack of penetration, inactivation of the drug, subpopulations of persistent strains, or the variable physiological state of microorganisms in the biofilm (Kamal *et al*., 1991; Oie *et al*., 1996; Ceri *et al*., 1999; Donlan, 2000; Cerca *et al*., 2005).

CONCLUSIONS

This study of *P. nigra* and *P. alba* buds has shown that they are a good source of biologically active compounds, which can be used in therapeutic and pharmaceutical fields due to their rich and varied composition of secondary metabolites. Moreover, extracts of these plants can serve as effective antimicrobial agents. Although the literature provides information on the use of Populus extracts for an antimicrobial effect. However, this study is one of few that addresses the antibiofilm activity of *P. nigra* and *P. alba* extracts. Thus, the inhibition of biofilm formation by the extracts revealed in this study is significant. Further complementary studies will be necessary to isolate and identify the bioactive molecules responsible for the antibiofilm activity of each extract. In addition, evaluation of antibiofilm activity *in vivo* will be important to determine the mechanism by which phenolic compounds affect biofilm formation.

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