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Original Research Article

Antibacterial effects of *Prunus cerasus* and *Chamaemelum nobile* against drug resistant strains induced urinary disorders

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Abstract: *Prunus cerasus* leaves (PCL) and *Chamaemelum nobile* stalks (CNS) aqueous extracts have antimicrobial, antioxidant and pharmacological properties. This study aimed to assess PCL and CNS antibacterial effects on both pathogenic strains (*Escherichia coli* and *Staphylococcus aureus*). Extraction methods were used for this experiments including maceration, decoction and infusion of aerial plant parts (leaves and stalks). Different concentrations of the aerial plant part aqueous extracts were tested and have shown their antimicrobial activity by spreading method from a well on Mueller-Hinton agar.PCL aqueous extract was active on both bacteria strains with different minimal inhibitory concentrations (MIC) as follow: $125 < MIC < 375 \times 10^6$ µg / ml for S. aureus and $250 < MIC < 500 \times 10^6$ µg / ml for E. coli. The strain of E. *coli* was resistant to both antibiotics used namely AMC and E15 whereas S. *aureus* was resistant to both antibiotics OX and E15. The antimicrobial activity of PCL was greater than that of CNS aqueous extract with a broader antimicrobial spectrum at lower concentrations. *Prunus cerasus* and *Chamaemelum nobile*, as aromatic plants, could be used in alternative medicine and having striking features to prevent urinary tract infections.

Keywords: *Prunus cerasus*; *Chamaemelum nobile*; *Escherichia coli*; *Stapylococcus aureus*; urinary tract infections

INTRODUCTION

Antibiotics are widely used to inhibit a bacterial growth and the spread of infections. They subsequently are benefit for a human health. To day, studies provide in one hand data about antibiotic resistant germs and another hand the resulting risk when this drug was released in the environment (Makky et al, 2012; Ghaedi et al, 2015). Drugs have unexpected side effects such as allergy, stomach pain, diarrhea and vomiting (Santos et al, 2003). Traditional medicine rescued and partially replaced the drugs thus reducing their toxic effects. Aromatic and medicinal plants may be an alternative source of antibacterial remedy due to their bioactive compounds (Gerard et al, 2011). Urinary tract infections (involving various targets as prostate, kidney, bladder and urethra) are a common reason for consultation and prescription in current practice (Berroukche et al, 2018). The urinary tract is the second site of bacterial infection after lung system (Millller et al, 2001). Really, Antibiotic therapy encouraged the development of the multi-resistant bacteria strains. This condition has prompted the use of new processing pathways as herbal medicine (Ghaedi et al, 2015). Systematic studies, among natural pharmacological compounds, revealed their significant role in the prevention against bacterial infections and human diseases (Ghaedi et al, 2015). *Prunus cerasus* and *Chamaemelum nobile*, belonging respectively to the families of Rosaceae and Asteraceae, have various species widely cultivated in the Algerian areas. These plants were used in folk medicine in the Mediterranean countries for a long time, for the treatment of benign urinary tract inflammations, rheumatism, neuralgia, indigestion, anemia and fever (Moumen et al, 2016). Their pharmacological properties are due to bioactive compounds such as polyphenols, flavonoids, terpenes, tanins and anthraquinones (Younes et al, 2007). This study aimed to assess the preventive effects of the mentioned Algerian herbs against bacterial strains inducing urinary tract infections.

MATERIAL AND METHODS

Plant material and extraction

Prunus cerasus leaves (PCL) and *Chamaemelum nobile* stalks (CNS) were collected in the Saida and Constantine regions, located respectively

in the Western and Eastern of Algeria, during summer 2006. Identification of plants was performed by the botanical laboratory of Saida University. Amonts of powdered PCL (50 g) and CNS (50 g) were extracted with 500 mL distilled water in different phases (Maceration, decoction & infusion). PCL and CNS aqueous extracts were filtrated and stored, at 4 °C, in the dark until use.

Culture media

To obtain young bacterial culture, Chapman medium and nutrient agar were used respectively for *Staphylococcus aureus* ATCC25923 (Gram-positive) and *Escherichia coli* ATCC25922 (Gram-negative). Muller Hinton medium was used to study antibacterial activity.

Bacterial suspension

Bacterial strains were sown on petri dishes containing appropriate medium, incubated for 24 hours, to get young bacterial colonies. Inoculi were prepared by the direct inoculation of colonies in 1 mL of sterile saline solution and adjusted to the 0.5 standard of the McFarland scale (Optical density, at 625 nm, adjusted from 0.08 to 0.1), corresponding to 1.5 x 10^8 CFU/mL for the bacteria.

Antibacterial activity

Antibacterial activity tests of CNL and CNS aqueous extracts were performed with the diffusion method on agar medium (disc method). This method was used to determine the MIC (Minimum Inhibitory Concentration) of different phases of aqueous extracts (Maceration, decoction & infusion). 100 µL of bacterial suspensions were added to 0.1 mL of the aqueous extracts prepared with different dilutions in physiological water (12.5 %, 25 %, 50 %, 75 % and 100 %). Subsquently 10 µL of each inoculum were plated on the Mueller-Hinton agar culture medium (MHACM).

Five disks (6 mm diameter) of filter paper containing 20 µL of the aqueous extract of each dilution, are deposited in the MHACM. Dishes were incubated at 37 ° C, for 24 hours. Disks, soaked with antibiotics; Kanamycine (K), Tobramycine (TM), Erythromycine (E), Imipeneme (IPM), Amikacine (AN) and Oxacilline (OX), were used as positive control. Disks, with 20 µL physiological water (9 % NaCl), were used as negative control.

At the end of the test period, the diameter of the inhibition zone formed over the agar culture was measured in mm. Inhibition zones formed in the experimental dishes were compared with those of the controls. MIC was defined as the lowest product concentration that prevented visible growth of bacteria.

RESULTS

Prunus cerasus leaves (PCL) and *Chamaemelum nobile* stalks (CNS) aqueous extracts (maceration, decoction and infusion), tested on both bacterial strains namely *E coli* and *S aureus*, showed different profil of inhibitory zones. The MIC values were determined using the disc diffusion method. The results of evaluation were summarized in Tables 1-3 and presented in Figures 1-5. PCL and CNS macerations showed weak and insignificant inhibitory zones (Ø = 5-10 mm) at a lower concentration (MIC = 250 $\times 10^6$ µg /mL) against a bacterial strain *S aureus* (Table 1 and Figure 1). Regarding a bacterial strain, *E coli*, an antimicrobial activity was only observed with PCL maceration (MIC = 375 $\times 10^6$ µg /mL) even when it displayed weak inhibitory zones (Ø = 5 - 10 mm) (Table 1) (Figure 2). CNS maceration showed no inhibitory effects against *E coli* at different concentrations (Table 1). CNS crude extract macerated (500 $\times 10^6$ µg /mL), or undiluted extract, had a better antibacterial activity (Ø = 10 - 18 mm) than PCL crude extract (Ø = 5 - 10 mm) (Tab 1). Decoction PCL recorded more or less signficant antimicrobial activity against both bacterial strains studied. PCL decoction showed inhibitory effects, at MIC = 125 $\times 10^6$ µg / mL, against *S aureus* (Ø = 5 - 10 mm) and at MIC = 375 $\times 10^6$ µg / mL, against *E coli* (Ø = 5 -10 mm) (Table 2 and Figure 3). CNS decoction showed no antibacterial inhibition, at various concentrations including crude extract, against *S. aureus* strain (Table 2) whereas CNS crude extract (500 $\times 10^6$ µg / mL) revealed a weak bacterial inhibition against *E coli* (Ø = 5 - 10 mm) (Table 2 and Figure 4). PCL infusion provided a MIC (250 $\times 10^6$ µg / mL) against both bacterial strains (Ø = 5 - 10 mm) however this aqueous extract form did not have antibacterial inhibitory effects at different doses prepared (Table 3 and Figure 5).

Among the antibiotics, used in this study, it emerges two drugs Imipeneme (IPM) and Amikacine (AN) which have shown potential antimicrobial activities respectively with inhibitory zones (Ø = 22 - 17 mm) and (Ø = 17-15 mm) against both bacterial strains namely *Staphylococcus aureus* and *Escherichia coli* (Table 4). The antibacterial activities of *Prunus cerasus* leaves (PCL) and *Chamaemelum nobile* stalks (CNS), in their different physical states, were lower compared to standard antibiotics and mainly IPM and AN except for the two antibiotics Erythromycine (E) and Oxacilline (OX) which did not induce any antibacterial activity during microbiological experiments (Table 4). The solvent, physiological water (9 % NaCl), used for the dilution of crude plant extracts did not show inhibition against the tested organisms (as a negative control).

Table 1: Antibacterial activities of PCL and CNS macerations against *E coli* and *S aureus*

Bacteria strains	Concentration of maceration (× 10^6 µg / mL)									
	PCL					CNS				
	500	375	250	125	62.5	500	375	250	125	62.5
S aureus	++	++	++	-	-	+++	+++	++	-	-
E coli	+++	++	-	-	-	-	-	-	-	-

PCL: Prunus cerasus leaves; CNS: *Chamaemelum nobile* stalks

Activity (or Sensitivity): - = inacive, + = mild (Ø = 2-5 mm), ++ = weak (Ø = 5-10 mm), +++ = satisfactory (Ø = 10-18 mm), ++++ = good (Ø = 18-25 mm), + five = strong (Ø = 25-32 mm).

Table 2: Antibacterial activities of PCL and CNS decoctions against *E coli* and *S aureus*

Bacteria strains	Concentration of decoction (× 10^6 µg / mL)									
	PCL					CNS				
	500	375	250	125	62.5	500	375	250	125	62.5
S aureus	+++	+++	++	++	-	-	-	-	-	-
E coli	++	++	-	-	-	++	-	-	-	-

PCL: Prunus cerasus leaves; CNS: *Chamaemelum nobile* stalks

Activity (or Sensitivity): - = inacive, + = mild (Ø = 2-5 mm), ++ = weak (Ø = 5-10 mm), +++ = satisfactory (Ø = 10-18 mm), ++++ = good (Ø = 18-25 mm), + five = strong (Ø = 25-32 mm).

Table 3: Antibacterial activities of PCL and CNS infusions against *E coli* and *S aureus*

Bacteria strains	Concentration of infusion (× 10^6 µg / mL)									
	PCL					CNS				
	500	375	250	125	62.5	500	375	250	125	62.5
S aureus	+++	++	++	-	-	-	-	-	-	-
E coli	++++	+++	++	-	-	-	-	-	-	-

PCL: Prunus cerasus leaves; CNS: Chamaemelum nobile stalks

Activity (or Sensitivity): - = inacive, + = mild (Ø = 2-5 mm), ++ = weak (Ø = 5-10 mm), +++ = satisfactory (Ø = 10-18 mm), ++++ = good (Ø = 18-25 mm), + five = strong (Ø = 25-32 mm).

Table 4: Sensitivity and resistance of the tested bacteria strains to different antibiotics

Antibiotics						
Disk load (µg)						
Bacteria	K (30)	TM (10)	E (15)	IPM (10)	AN (30)	OX (1 or 5)
S aureus	+++	++	-	+++++	+++++	-
E coli	+++	++	-	++++	++++	-
Range MIC (µg/mL)	8 – 16	2 – 4	1 – 4	4 – 8	8 – 16	2
Ø (mm)	17 – 15	18 – 16	22 – 17	22 – 17	17 – 15	20

K = Kanamycine , TM = Tobramycine, E = Erythromycine, IPM = Imipeneme, AN = Amikacine, OX = Oxacilline

Activity (or Sensitivity): - = inacive, + = mild (Ø = 2-5 mm), ++ = weak (Ø = 5-10 mm), +++ = satisfactory (Ø = 10-18 mm), ++++ = good (Ø = 18-25 mm), + five = strong (Ø = 25-32 mm).

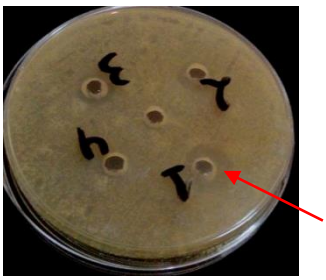
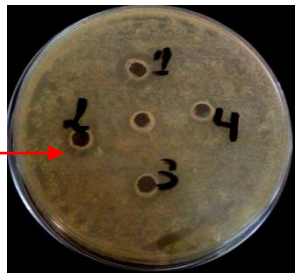


Figure 1: Inhibitory zones of PCL maceration.



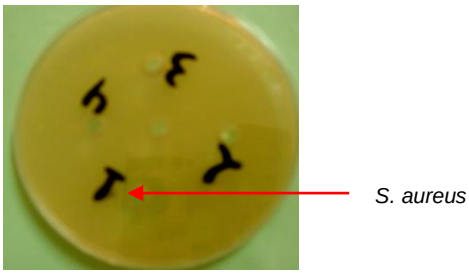


Figure 2: Inhibitory zones of CNS maceration.

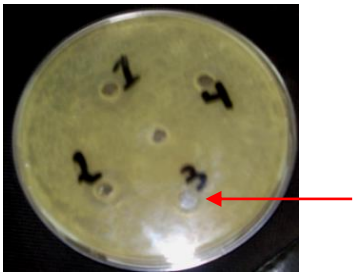


Figure 3: Inhibitory zones of PCL decoction.

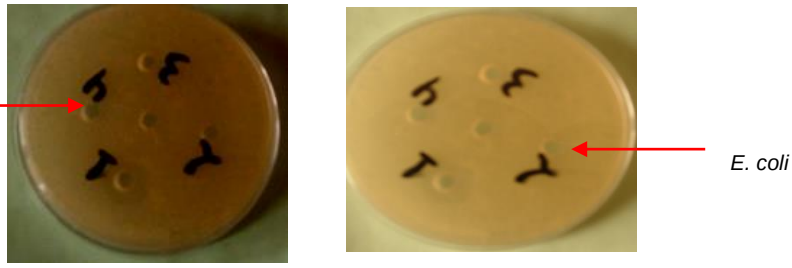


Figure 4: Inhibitory zones of CNS decoction.

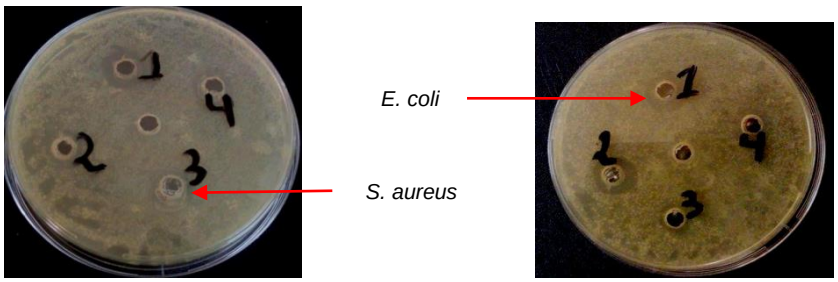


Figure 5: Inhibitory zones of PCL infusion.

DISCUSSION

This present study was performed to investigate the antimicrobial activities of aerial plant parts of two aromatic and medicinal herbs namely *Prunus cerasus* leaves (PCL) and *Chamaemelum nobile* stalks (CNS). Results show the highlights. PCL decoction showed a slight efficient antibacterial activity against the *S aureus* than PCL maceration and infusion, this was concretized by a low MIC of the PCL decoction (125 $\times 10^6$ µg / mL) compared to MICs macerated and infused PCL (250 $\times 10^6$ µg / mL). Regarding *E coli* strain, PCL infusion has better antibacterial activity than the decocted and macerated PCL, this was demonstrated by a lower MIC infusion (250 $\times 10^6$ µg/mL) than decocted and macerated PCL (375 $\times 10^6$ µg/mL). Only CNS macerate showed an antibacterial activity against *S aureus* with a MIC (250 $\times 10^6$ µg / mL) whereas the other forms, involving decoction and infusion PNS showed no antibacterial activity against both strains studied (*S aureus* and *E coli*). The bacterial strains have developed no resistance to PCL unlike CNS, in its different physical states, to which the bacteria were potentially resistant. Antibacterial inactivity, or the bacterial resistance to CNS, could be due to some experimental conditions such as the chemical nature of the solvent used, the volatility of bioactive and antioxydant components during the heating of the plant aqueous extracts, or the extraction method used or the storing process of the plant aqueous extracts. The variability of the antibacterial inhibitory activities of the plant aqueous extracts depends on certain parameters. The structure and morphology of the bacteria, for example the pigmented strains have a high resistance to the antibacterial activity of plant extracts (O'Malley et al, 2004). Gram-positive bacteria have membrane structures susceptible to the bioactive and antioxidant components of essential oils, aqueous and alcoholic extracts (Abdul Rahmane et al, 2010). Gram-negative bacteria have an outer membrane rich in lipopolysaccharides and impermeable by preventing the diffusion of hydrophobic molecules (Nokaido et al, 2003). The chemical composition of aqueous extracts could influence the antioxidant and antibacterial activities of plants towards any strain. The choice of the plant part (roots, stem, leaves, flowers, seeds and stalks) determines a specific chemical composition for each part and may have antibacterial or non-antibacterial effects. Plant extracts contain some hydrophobic compounds, with low molecular weight, such as polyphenols, phenolic acids and their derivatives. These bioactive compounds bind to the phospholipids, proteins and polysaccharides of bacterial membranes inducing their permeability, the disruption of their metabolism and death (Dorman et al, 2000; Wang et al, 2008). The results of the studies, conducted by Derwich *et al.* (2010) and Bari *et al.* (2010), confirm the data in this study on the resistance of Gram-negative bacteria to Gram-positive bacteria. According to Gulfraz *et al* (2008) and Tiwari *et al* (2009), the antimicrobial activity of aqueous extract of plant is attributed to flavonoid components (Gulfraz et al, 2008; Tiwari et al, 2009). The antibacterial activity of flavonoids is explained by the toxicity mechanism to microorganisms. Non-specific interactions such as the hydrogen bridge formation with protein and enzyme cell wall, chelation of metal ions, inhibition of bacterial metabolism and sequestration of substances necessary

for bacterial growth (Baharfar et al, 2015). The bacterial sensitivity is related to the number of free hydroxyl groups carried by the flavonoid molecules. In other words, the less hydroxylated flavonoids have an intense antibacterial activity. Amireche's work, led in 2013, suggested that flavonoids lacking free hydroxyl groups have a high affinity for cell membrane lipids (Xiao et al, 2012). The beneficial effects of PCL extracts, towards drug pathogenic resistant bacterial strains, could be related to its cyanidin and derivatives content (Saleh et al, 2017). Presented results are in line with studies performed by Blando et al, (2004). Our results showed that CNS extracts had no antibacterial activity. The secondary metabolits, found in this plant, are volatile oil consisted of acrylic acid and isobutyric acid, sesquiterpene lactones, polyacetylenes, flavonoids and other phenolics, e.g. caffeic acid (Tschan et al, 1996). This present results could be debatable for different reasons; to change the extraction methode and to use alcohol as solvent instead of water, to explore the toxicity of medicinal plants mainly in their bioactive compounds as anthocyanosides and genistein, PCL and CNS concentrations could be insufficient to trigger higher antibacterial effects and finally to investigate synergy molecular reactions of flavonoids eluciding how do this magical molecules occur in bacterial cell wall and eliminate bacterial resistance.

CONCLUSION

In literature, little has been said about antibacterial activity of both medicinal plants used in this study, namely *Prunus cerasus* and *Chamaemelum nobile*. This work is one of the few reports demonstrating a possible antimicrobial activity of *Prunus cerasus* and *Chamaemelum nobile*. Consequently, consumption of *Prunus cerasus* leaves could prevent the complications resulting of bacterial infections contracted in urinary tract. However, our results are encouraging to call futher studies to isolate and investigate the PCL and CNS chemical compounds and to determine the mechanism behind the antibacterial activity.

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