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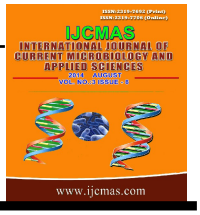


Original Research Article

Biological activities of *Olea europea sylvestris* Tar, growing wild in South west of Algeria

L.Benlarbi[1*], A.Makhloufi[1], B. Tarfaya[1], M. Belahcene[3], A.Moussaoui[1], N. Makhloufi[1], H. Bensafi[1], A. Boulanouar[1], and L. Mebarki[2]

[1]Laboratory of valorization of vegetal Resource and Food Security in Semi Arid Areas, South West of Algeria, BP 417, University of Bechar, Algeria
[2]Laboratoire des productions, valorisations végétales et microbiennes Université des sciences et de la technologie d'Oran Mohamed Boudiaf Algeria
3- Faculté SNV Centre Universitaire d'Ain Temouchent, Algeria
**Corresponding author*

Keywords

Tar, *Olea europea sylvestris*, Antimicrobial activity, antioxidant activity, South west of Algeria.

A B S T R A C T

Biological activities of medicinal plants have been recognized for a long time. In the present study, antioxidant and antimicrobial properties of *Olea europea sylvestris* Tar, *were* investigated for their antimicrobial activities against six strains of Fungi and six strains of bacteria. Its sensitiveness (Minimal Inhibition Concentration) to mentioned micro-organisms in the following: *Klebsiella pneumoniae* (0.032 mg/ml), *Staphylococcus aureus* (0.05 mg/ml), *Pseudomonas aeruginosa*. *Enterococcus faecalis*, *Escherichia coli*, *Listeria monocytogenes (0.1mg/ml).* For fungi , and according to these results, the tar has great antifungal activity against all the investigated strains. The growth inhibition rate ranged from 0.006 to 0.1mg/ml with the highest inhibition values observed against *Fusarium oxysporum* f.sp *albedinis* (1) (0.006 mg/ml). The antioxidant capacity of the tar was evaluated using hydrogen peroxide scavenging, 1,1-diphenyl-2-picrylhydrazyl (DPPH), showed potent antioxidant ability ($EC_{50=}$(EC50= 1.45±0.16 mg ml^{-1}) compared to the ascorbic acid used as positive control($EC_{50=}$2.19±0.12 mg ml^{-1}) .

Introduction

The use of tar is reserved mainly for patients with chronic stable plaque psoriasis, scalp psoriasis, atopic dermatitis, and seborrheic dermatitis .Essential oils and extracts from aromatic plants have long been used for a wide variety of medicinal and domestic purposes. Antimicrobial properties medicinal plants such as *Matricaria pubescens* (Desf.),*Rosmarinus officinalis* L and *Artimisia herba alba* against food-related microorganisms as well as their applications in food system have been investigated and reviewed (Gachkar et al.,2007; Atik bekkara et al.,2007; Chanthaphon *et al*., 2008; ; Makhloufi et al., 2011;Makhloufi et al., 2012).

Olea europea sylvestris has been the subject

of recent research .In the pharmaceutical industries, it has excellent antioxidant and antimicrobial properties, due to certain compounds (carnosol, carnosic acid, ursolic acid, betulinic acid, and the rosmaridiphenol rosmanol) (Thoresen *et al*., 2003).

Olea europea sylvestris. is a shrub belonging to *Oleaceae* family, in the mediterranean area, including Algeria, which lies on the arid slopes and hills (Dellile, 2007).In the west south of Algeria, it is quite frequent in the mountains of Saharan Atlas.

Materials and Methods

Olea europea sylvestris specimens were collected from Bechar mountain during December2012, and January 2013. These biomasses were dried for fifteen days in the dark at ambient laboratory temperature (20-28°C).

Distillation of Tar

The wood of the aerial parts were cutted before the operation, and then, 1 kg of cutted wood was submitted to distillation for 3 to 4 hours using a special dispositive**.** The distilled tar was stored at ambient temperature until it was used**.**

Antimicrobial activity

Microbial strains

The antimicrobial activity was evaluated by paper disc diffusion and dilution methods against six selected fungi: *Aspergillus niger*, *A. flavus(1) , Penicillium purpurogenum (Isolated from dates), Fusarium oxysporum f. sp. albedinis(1) FOA(1) , Fusarium oxysporum f. sp. albedinis(2) FOA(2), Fusarium oxysporum f. sp. albedinis(3) FOA(3) isolated from date palm from different areas in Saoura region, south west of Algeria ,and* six selected Gram-positive and Gram-negative species: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC27853, *Enterococcus feacalis* ATCC 29212, *Klebsiella pneumoniae* CIP 106818 and *Listeria monocytogenes* ATCC19115.

Disc diffusion method

The agar disc diffusion method was employed for the determination of antimicrobial activities of the tar. Briefly, the fungal cultures were grown on PDA. The mycelial mat of 7-day old culture was washed, suspended in normal saline solution. The colony forming units (CFU/ml) of suspension of the test fungus was determined and test inoculum was adjusted to 10^6 CFU/ml. These conidia were used for antifungal essay tests. Inocula (0.1ml) were applied on the surface of the PDA plate and spread by using sterile glass spreader (Bansod & Rai, 2008).

The qualitative antibacterial was performed using culture growth at 37 °C for 18 h and adjusted to approximately 10^8 colony forming unit per milliliter (CFU/ml) . The culture medium used for the bacteria was Mueller Hinton Agar (MHA) (Gachkar et al., 2007). Five hundred microliters of the inoculums were spread over plates containing MHA and a Whatman paper disc (6 mm in diameter) were impregnated with 10 µl of the undiluted oil and were placed on the inoculated plates. The plates were left for 30 min at room temperature, and incubated at 37 °C for 24 h (Shunying *et al*., 2005; Bekhchi *et al*., 2008; bourkhiss *et al*., 2007).The diameters of the inhibition zones were measured in millimeters. Control assay discs were also used; all tests were performed in triplicate (Yesil *et al*., 2007).

Dilution method (MIC)

Antimicrobial tests were performed according to the method reported by Remmal et al (1993), and Farah et al (2001). The tar is emulsified with an agar solution of 0.2% in order to disperse the compounds and improve their contact with the tested germs, then diluted to one tenth in the agar solution. Quantities of this dilution are added to test tubes containing Mueller Hinton agar for bacteria. The final concentrations of tar are from 0.01, to0.1 (mg/ml). In parallel, Control assay containing only the culture medium and agar solution at 0.2% were also used. The MIC is the lowest concentration of essential oil giving no visible growth in the naked eye (Kaloustian et al ., 2008).

For the antifungal activity (Assay for radial growth inhibition**)** Petri dishes containing PDAa media with tar at different concentrations were inoculated with fungus suspension obtained from a pure culture. The Petri dishes containing tar were inoculated in the same way. The Petri dishes were incubated at 25 °C for 7 days. The diameter of the *fungus* colony was obtained by calculating the average of two perpendicular diameters (Hassikou & al.,2002).

Scavenging activity of DPPH radical

The hydrogen atom or electron-donation ability of the BPEO was measured from the bleaching of the purple-colored ethyl acetate solution of DPPH•. This spectrophotometric assay uses stable 2, 2'-diphenyl 1-picrylhydrazyl radical (DPPH•) as a reagent. The free radical scavenging activity of CLME was measured by DPPH using the previously reported procedure (Athamena et al.,2010). Briefly, 0.1 mM solution of DPPH in methanol was prepared. Then, 1 ml of this solution was added to 3 ml of CLME solution at different concentrations (0.050 to 0.50 mg/ml). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then, the absorbance was measured at 517 nm in a spectro-photometer (Shimadzu, UV/Visible Recording). Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The radical scavenging activity was calculated as follows:

Scavenging effect (%) = [(Absorbance at 517nm of control- Absorbance at 517 of sample/ Absorbance at 517 of control)] × 100.

The ascorbic acid was used as positive controls.

Results and Discussion

Olea europea Tar is a dark brown, viscous liquid with a smoky odor and acrid, slightly aromatic taste with the yield of 1.20 % (v/w) on dry weight basis. It has a pH of 5,72 , a refractive index of 1.3554 and is soluble in the following solvents: very slightly soluble in water; soluble in ether, chloroform, amyl alcohol, ethyl acetate ,alcohol; and partly soluble in petroleum ether.

Antimicrobial Activity

Disc diffusion assay

The growth inhibition zones measured by disc diffusion method are presented in Fig 01. According to these results, the tar of *Olea europea sylvestris* has a great antimicrobial activity against most of the investigated strains. The diameters of growth inhibition zone ranged from 20 to 34mm (including the diameter of the disc-6 mm) with the highest inhibition zone values observed against *Klebsiella pneumoniae* (34mm).

Fig.1 Antibacterial activity of tar by disc diffusion assay

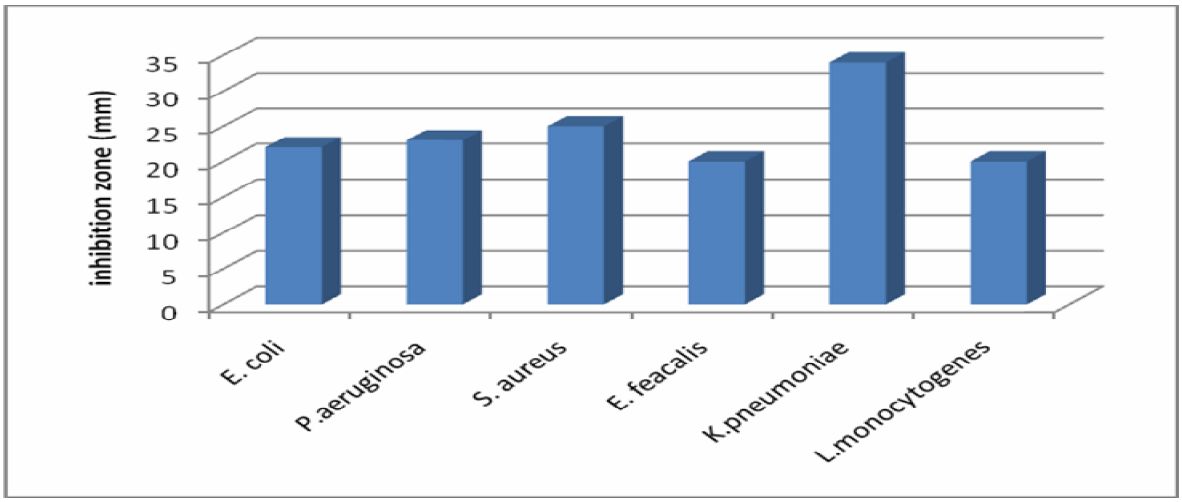


Table.1 MIC of tar (mg/ml) on bacteria strains

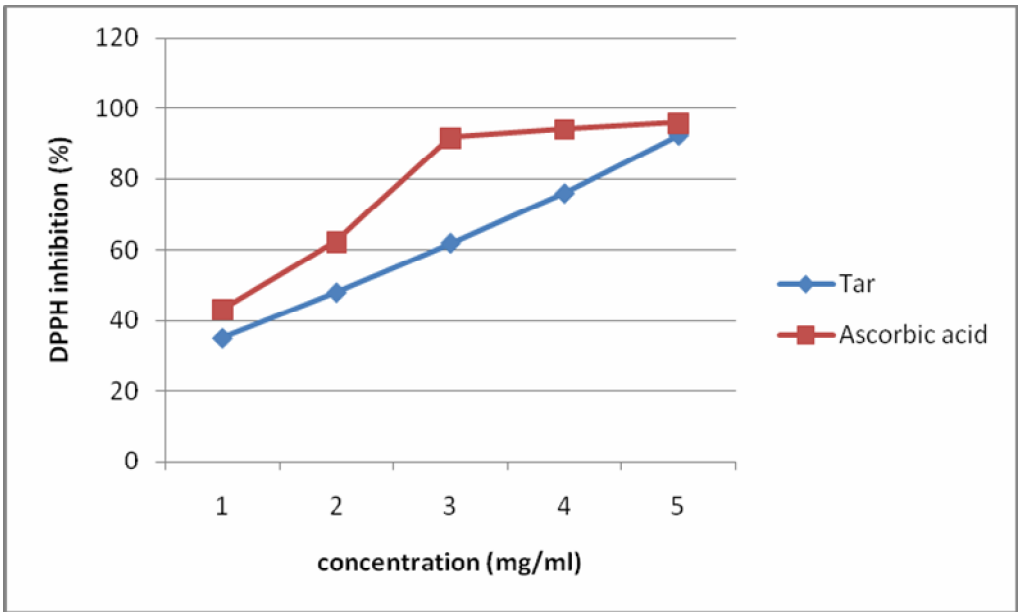
Concentration(mg/ml) / Strains	0.02	0.032	0.05	0.07	0.075	0.082	0.1
E. coli	+	+	+	+	+	+	-CMI
S.aureus	+	+	- CMI	-	-	-	-
P. aeruginosa	+	+	+	+	+	+	-CMI
L. monocytogenes	+	+	+	+	+	+	-CMI
E. faecalis	+	+	+	+	+	+	-CMI
K. pneumoniea	+	- CMI	-	-	-	-	-

Table.2 MIC of tar (mg/ml) on fungal strains

concentrations (mg/ml) / Fungi	0.006	0.008	0.01	0.032	0.049	0.065	0.082	0.1
A.niger	+	+	+	+	+	+	+	CMI
A.flavus(1)	+	+	+	+	+	+	CMI	-
P.purpurogenum	+	+	+	+	+	+	CMI	-
FOA (1)	CMI	-	-	-	-	-	-	-
FOA(2)	+	CMI	-	-	-	-	-	-
FOA(3)	+	CMI	-	-	-	-	-	-

(-):Inhibition (+): Growth

Fig.2 Antioxidant activity of tar and ascorbic acid



For the direct contact method (Table 1), the tar of *O.europea* has, in vitro, a good inhibitory activity against the tested germs. *O.europea* tar exerted an inhibitory effect against tested strains. The best MIC was observed against *K. pneumoniae* (0.032mg/ml) and *S.aureus* 0.05 mg/ml.The tar derived from *O.europea* (family Oleaceae) is used in folk medicine of north Africans countries . The tar is accessible to consumers without a prescription and is known to be used externally for skin disorders in dermatology and hair care, essentially for scalp care, eczema, scale affections, loss of hair, and psoriasis, it is also against ‘‘evil eye’’, abdominal pain and diarrhea, psychiatric disorder, cancer, fever, cephalgia, angina, weight decrease, the common cold, and hypotonia, without any scientific evidence to support these uses despite such common use (Skalli et al., 2014).

Assay for *radial* growth inhibition

The growth inhibition zones measured by disc diffusion method are presented in table. 2. According to these results, the tar has great antifungal activity against all the investigated fungi. However, the microorganisms studied did not show the same sensitivity against the tar

The growth inhibition rate ranged from 0.006 to 0.1mg/ml with the highest inhibition values observed against *FOA(1)* (0.006mg/ml).

In the literature, the tar has been shown to have many medicinal properties such as activity as antipruritic, and antimicrobial agent in vitro (Leung and Foster, 1996).

Antioxidant activity (DPPH assay)

Figure 2 show the effect of different concentrations of *Olea europea* tar and Ascorbic acid on the scavenging rate of DPPH. The kinetic behaviors of tar show that the reaction approaches an almost steady state in 30 minutes. Free radical scavenging activity of the tar depends on its concentration and there is a direct correlation between these. A lower EC50 value reflects a better protective action.

EC50 values of the tar have been compared with ascorbic acid. The free radical-scavenging activity of ascorbic acid (EC50= 1.45±0.16 mg ml^{-1}) was superior to the tar (EC50= 2.19±0.12 mg ml^{-1}).

Tar from *Olea europea sylvestris* had a good biological activity. The strong antifungal activity of *O. europea sylvestris* against array of filamentous fungi strains is an indication of the broad spectrum antifungal potential of the tar. This could make the tar a promising group of natural compounds for development of safer antimicrobial agents.

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