

Chemical composition and Antimicrobial Activity of *Accacia tortilis subsp. radiana* Tar From Bechar region, Algeria.

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Abstract.

Antimicrobial activity of medicinal plants have been recognized for a long time. This study has been concerned with determining the chemical composition and antimicrobial activity of Tar extracted from *Accacia radiana subsp.radiana* wood growing wild in Bechar region, (South West of Algeria). This tar was extracted by traditional method and then was analyzed by gas chromatography coupled with mass spectroscopy technique (GC-MS technique) and calculation of Kovats Retention Indices values. Exactly 69 components were recognized that components having the highest percent are as follow: Syringol (6.86%),2,6-Dihydroxy-4-methoxyacetophenone(5.94%) ,2,3-Dimethoxybenzyl alcohol (5.06%), p-Ethylguaicol (3.68%). Antimicrobial effects of this tar were tested against six strains of bacteria and five strains of fungi. All strains were inhibited (Minimal Inhibition Concentration)at the concentration of 0.1mg/ml, *Escherichia coli* ATCC 25922 and *Enterococcus faecalis* ATCC 29212 were the most sensitive with CMI of 0.008 mg/ml. for Fungi, the Tar shows that the best CMI(0.006 mg/ml) was obtained against *Alternaria alternata* and *Fusarium oxysporum f. sp. albedinis*.

Keywords: *Accacia tortilis subsp.radiana*, Tar, Chemical Composition, Antimicrobial activity, Bechar.

provoked interest as a source of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases and the prevention of food from

Introduction

Plants oils and extracts have been used for a wide variety of proposes for many thousands of years. Recently, the essential oils and various extracts of plants have

and gets converted into water vapor, gaseous volatiles, tarry volatiles and char(Srikanth and Kolar 2009; Shanmugavasan et al. 2011).

Vegetal tar is the end product of wood carbonisation following distillation using extreme heat. Vegetal tar has been used in medicine for more than 2000 years to treat a range of skin conditions because of its soothing and antiseptic properties. This tar is known for the presence of chemical compounds with biocide properties like antitermitic or fungicidal(Tanya et al.2009).

Vegetal Tar was used traditionally in the south of Algeria for treatment of different water storage vessels. The popular claims that tars have fungicide and bactericide properties (water treatment, Scabies...) .

Biological activities of vegetal tar 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 and Hildebrand 2003; Terfaya et al. 2019; Mezouari et al.2019; Bendjima et al. 2020).

Acacia radiana is a cosmopolitan genus containing in excess of 1350 species that together with the African and the Middle

the toxic effects of oxidants Infectious (Siti Amirah et al. 2012; Tissouras et al. 2013).

Phytopathogenic diseases are the most important diseases in the world that impose a lot of financial burden to human communities. Artificial antibiotics although could have played a significant role in the treatment of infectious diseases problems that had appeared in relation with the manifestation of microbial resistance against antibiotics resulted in inclination to use medicinal plants more than ever (Ayfer 2003).

Wood represents an abundant renewable source of readily available energy on combustion. On the other hand, thermal decomposition reactions in the absence of oxygen enable the recovery of solid and liquid fuels as well as valuable fine chemical by-products (Carlos Amen-Chen et al. 1997).

Wood pyrolysis under atmospheric pressure with long vapour residence time is called carbonization. Pyrolysis as an extraction method of phytochemicals from plant parts for medicinal applications is less explored. Pyrolysis is the subjection of organic compounds to very high temperature and study its resulting decomposition. Biomass pyrolysis is defined as the process during which the biomass undergoes thermal degradation

normalization) (1986) have determined the specific density at 20°C. Refractive index (RI) was measured with a refractometer, at 20°C. The methods used for the determination of acid index are also used with the standards AFNOR (1986).

Gas chromatography-mass spectrometry (GC-MS)

The analysis of the Tar was performed using a Hewlett-Packard Agilent 5973 network GC System, equipped with a DB-5 capillary column and a HP 6890 mass selective detector in the electron impact mode. Helium was the carrier gas at 1 ml/min. Injector and MS transfer line temperatures were at 250 °C and 260 °C respectively. Column temperature was set at 40 °C for 1 min, then programmed from 50 °C to 250 °C at a rate of 5°C/min, and finally held isothermally for 20 min. For GC-MS detection an electron ionization system was used with ionization energy of 70 eV. The identification of the compounds was based on their Kovats indices (KI) and on gas chromatography couples to mass spectrometry.

Antimicrobial activity

Microbial strains

Eastern monotypic genus (Grouzis 2003; Mezouari et al.2019), it is a short desert tree with an impressive umbrella shape, in the family of *Fabaceae* belonging to the tribe *Acacieae* and considered as an autochthonous taxon particularly valuable in the regions of Africa (Abdou et al.2020). This study focuses on chemical composition and antimicrobial activity of *Accacia raddiana* Tar.

Materials and Methods

Sampling

Plant Material and extraction tar procedure

Aerial parts (wood) of *Acacia raddiana* were collected from Bechar area (southwest of Algeria) in September 2017. Collected woods were cut and dried at room Temperature. The extraction was conducted according to the method of the distillation per descensum with some modifications. Trunks were cut in small pieces and placed in a special dispositive for 5 hours (Terfaya et al.2019). The distilled tar was stored at ambient temperature until analysis.

Physico-chemical Characterization of *Acacia raddiana* Tar

Methods conform to AFNOR (Association française de

The bioassay used for antimicrobial activity of Tar in question was the disc – diffusion method (Bekhchi et al. 2008; Bourkhiss et al.2007; Makhloufi et al.2011, Makhloufi et al.2012).The diameters of the inhibition zones were measured in millimeters. Control assay discs were also used; all tests were performed in triplicate.

Dilution method (MIC)

Different concentrations of Tar were prepared according to the method reported by Remmal et al (1993), and Farah et al (2001). Quantities of this dilution are added to test tubes containing PDA and Muller Hinton Agar. Antimicrobial tests were tested by dilution method according to methods cited by Kaloustian et al ., (2008), Mezouari et al.,(2019), Terfaya et al (2019). 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.

Antimicrobial activity of *Acacia raddiana*

Tar was determined by paper disc diffusion and dilution methods against five selected fungi: *Aspergillus niger*, *A.flavus* , (Isolated from dates), *Alternaria sp*, *Fusarium sp* isolated from soil and *Fusarium oxysporum f. sp. albedinis* isolated from infected palm trees, and against the following pathogen Bacteria: *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* CIP 106818, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC27853 ,*Bacillus cereus* ATCC 11778 , *Enterococcus faecalis* ATCC 29212 and *Listeria monocytogenes* ATCC19115.

Disc diffusion method

First, the mentioned micro-organisms were cultured on culture environment of Moeller Hinton (for bacteria) and sobered dextrose Ager for fungi in order to obtain a fresh culture to fresh or to prepare after 24 hours at 37 °C for bacteria and 48 hours at 25 °C for fungi .

Results and discussion

The raw tar of *Acacia raddiana* wood was a dark, viscous liquid with an acidic smell. Table 1 shows some Physical and chemical properties of the studies tar.

Table 1 Physicochemical index of *Acacia raddiana*. tar

Yield	pH	Refractive Index	Specific Density at 20 °C	Rate of Matter Dries
0.9%	5.2	1.676	1.154	62%

al.1994; Benlarbi et al. 2014; Terfaya et al. 2017; Bendjima et al.2020). This difference in tar content is related to several factors, such as the geographical area of collection, climate, stage of development and the season.

The chemical composition of *Acacia raddiana* Tar was investigated using GC-MS technique. The constituents are listed in Figure 1. Exactly 61 compounds were identified. According to the Table 2, the components having the most percent include: Syringol (6.86%), 2,6-Dihydroxy-4-methoxyacetophenone (16.97%), 2,3-Dimethoxybenzyl alcohol (5.06%), p-Ethylguaiaicol (3.68%).

Statistical Analysis

Collected data were subjected to statistical analysis, using ANOVA (* P <0.05) according to the Newman-Keuls test.

The average yield of Tar extracted from *Acacia raddiana* in the range of 0.90%. This result is relatively higher to that obtained for the same species (0.803%) in the north of Bechar (Benlarbi 2018). However, the tar of the current study was higher than that given by different species (*Juniperus oxycedrus*) in the region of Tlemcen whose yield was 0.659%, *Olea europaea sylvestris* (0,803%) and *Juniperus phoenicea* (0.542 %) in the region of Bechar(Terfaya and Makhloufi 2015).

The Physicochemical parameters were found to be some times lower and some times higher than other tars reported in the the literature (Scott 1984; Maggi et

Table 2 Chemical composition of *Acacia raddiana* Tar

N°	TRX	KI	Composé	%
1	5.17	832	2-Cyclopenten-1-one	0.06
2	5.58	835	Furfuryl alcohol	0.22
3	5.73	860	Ethylbenzene	0.03
4	5.9	869	p-Xylene	0.03
5	6.7	907	2-methyl-2-cyclopenten-1-one	0.12
6	6.86	913	Acetylfuran	0.23
7	8.18	964	3-Methyl-2-cyclopentenone	0.31
8	8.44	975	Methyl 2-furoate	0.07
9	8.65	983	Phenol	0.5
10	8.92	993	Furfuryl acetate	0.15
11	9.07	999	2,3-Dimethyl-2-cyclopenten-1-one	0.12
12	9.97	1031	3-methylcyclopenten-1,2-dione	0.76
13	10.22	1040	3-methyl-cyclohex-2-en-1-one	0.35

14	10.66	1056	o-Cresol	0.54
15	10.88	1064	3,4,5-trimethyl-2-cyclopenten-1-one	0.35
16	11	1068	Acetophenone	0.04
17	11.28	1078	p-Cresol	1.34
18	11.7	1093	o-Guaiacol	2.93
19	11.82	1097	Methyl benzote	0.29
20	12.04	1105	(2E, 4 ^E) Octadienal	0.2
21	12.14	1108	2,6-Dimethylphenol	0.23
22	12.55	1123	Ethyl-2-hydroxy-2-cyclopenten-1-one	0.65
23	13.02	1139	p-Ethylphenol	0.20
24	13.21	1146	Veratrole	0.21
25	13.33	1150	2,4-Dimethylphenol	0.71
26	13.92	1171	2,5-Dimethylphenol	0.73
27	14.19	1181	2-Methoxy-3-methylphenol	0.59
28	14.41	1188	Isocresol	0.36
29	14.72	1199	p-Cresol	2.6

30	15.04	1211	Catechol	1.85
31	15.36	1222	p-Guaiacol	0.32
32	15.55	1229	m-Cumenol	0.27
33	15.67	1234	2-Ethyl-6-methylphenol	0.37
34	15.83	1239	Homoveratrole	0.33
35	15.96	1244	5-Ethyl-2-methylphenol	0.12
36	16.06	1248	2,4-Dimethoxyphenol	0.3
37	16.63	1268	3-Methoxycatechol	1.87
38	17.1	1286	p-Ethylguaiacol	3.68
39	17.16	1288	Indanone	0.45
40	17.54	1301		0.96
41	18.63	1343	3-Methylcatechol	0.49
42	19.07	1360	Syringol	6.86
43	19.14	1363	Eugenol	0.85
44	19.27	1368	p-dimethylacetal anisaldehyde	0.54
45	19.4	1373	Dihydro Eugenol	1.44

46	19.67	1383	Ethyl nonanoate	0.39
47	20.25	14.05	3,4,5-Trimethoxytoluène	0.44
48	20.34	1409	Isovanillin	0.28
49	20.43	1412	ci-isoeugenol	0.83
50	21.5	1455	2,3-Dimethoxybenzyl alcohol	5.06
51	21.57	1458	(E)- isoeugenol	1.69
52	22.18	1482	2,4,6-Trimethoxytoluene	0.51
53	22.5	1495	Acetovanillone	0.74
54	23.44	1534	2,6-Dihydroxy-4-methoxyacetophenone	5.94
55	23.56	1539	B- Thujaplicinol	0.77
56	23.9	1554	4-Ethoxy-3-methoxybenzyl alcohol	1.01
57	24.88	1595	3-(Allyloxl)-4-methoxybenzaldehyde	0.5
58	25.19	1608	2,6-Dimethoxy-4-allylphenol	1.32
59	25.37	1616	2,6- Dimethoxy-4-propylphenol	3.00
60	26.28	1657	2,5-Dimethoxyterephthelaldehyde	1.00
61	26.55	1669	Syringaldehyde	1.13

62	26.93	1686	Coniferyl aldehyde	0.3
63	27.24	1708	Ferulic acid	2.32
64	27.65	1718	2,2-Biphenyldiol	0.97
65	28.15	1742	Acetosyringone	0.68
66	28.94	1779	Propiosyringone	1.43
67	36.92	2098	Heneicosane	0.26
68	38.63	2295	Tricosane	0.22
69	46.95	2795	Octacosane	0.27
Total				65.61

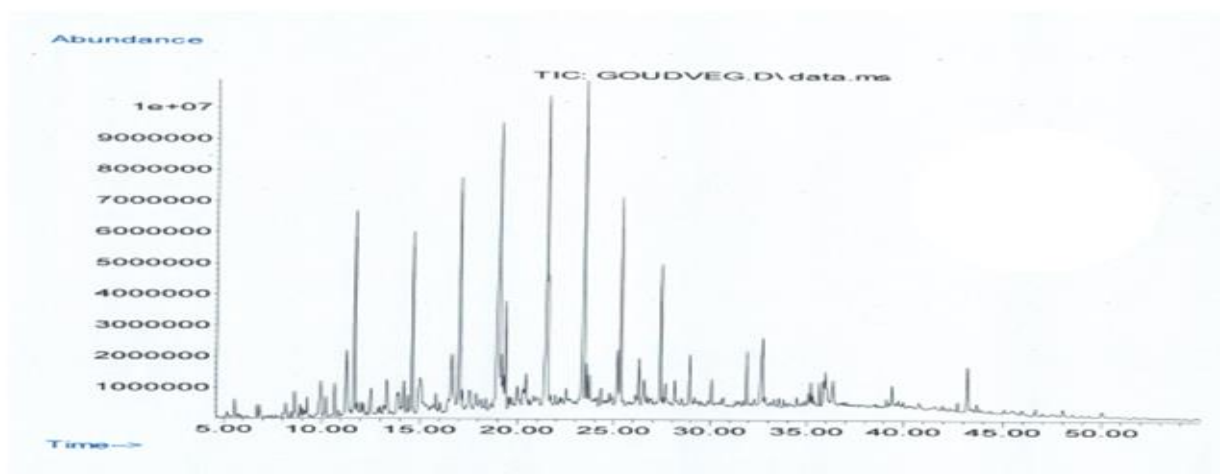


Figure 1 GC-SM of the constituents of *Acacia raddiana* Tar

19mm (including the diameter of the disc-6 mm) with the highest inhibition zone values observed against *Klebsiella pneumoniae* (19mm) and *E.coli* (18mm). These values are lower than those found with Ampicillin (46mm). For molds, the diameters of inhibition zone ranged from 20 to 40 mm. The highest inhibition zone values observed against *Fusarium oxysporum f. sp albedinis*

Antimicrobial Activity

Antimicrobial effects of the studied Tar were examined according to the Agar disc diffusion method. According to these results, the studied Tar has a great antibacterial activity against the investigated strains. The growth inhibition zones measured by disc diffusion method are presented in Figure 2 .The diameters of growth inhibition zone ranged from 12 to

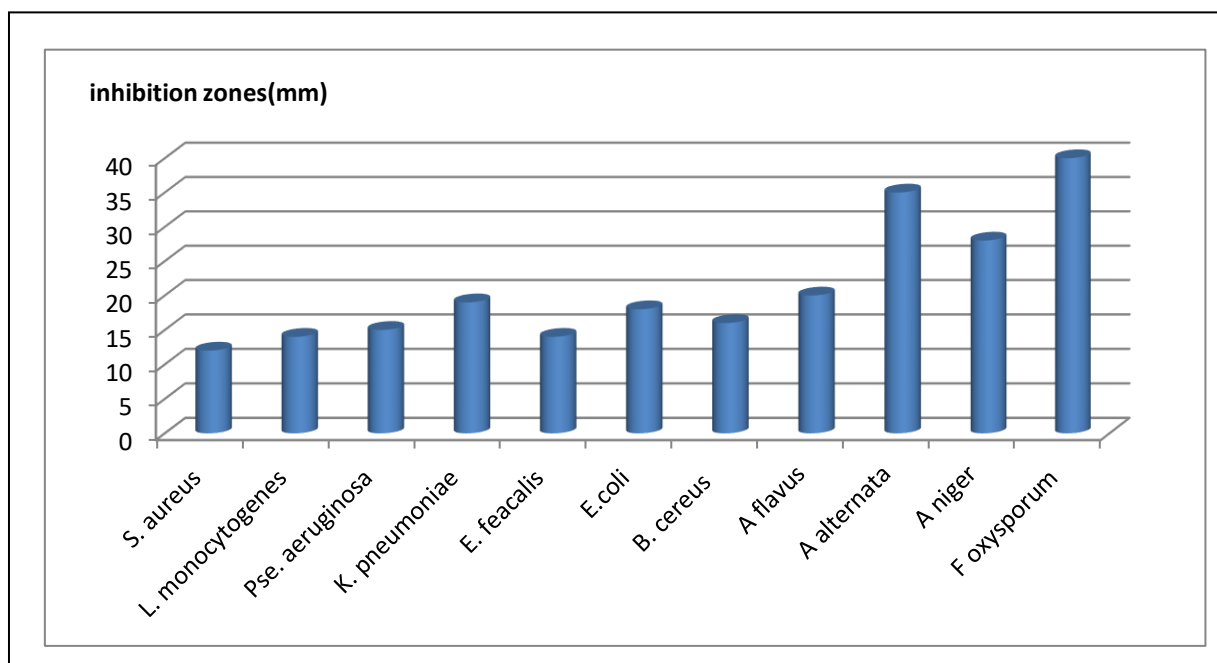


Figure 2 Antimicrobial activity of *Acacia raddiana* Tar by disc diffusion assay

tested at a concentration of 0.008 mg/ml. *Fusarium oxysporum* f. sp. *albedinis* and *Alternaria alternata* had lowest MICs of (0.006mg/ml). For bacterial strains, all germs are inhibited at 0.1mg/ml. Table 3 shows MICs. *Escherichia coli* ATCC 25922 and *Enterococcus faecalis* ATCC 29212 had lowest MICs of (0.06mg/ml).

Antimicrobial effects of the studied Tar were examined according to the method of Agar dilution (Figure 3). The tar of *Acacia raddiana* has in vitro, a good inhibitory activity against all tested microorganisms. However, the microorganisms studied did not show the same sensitivity against the tar. Tested tar inhibited both fungal strains

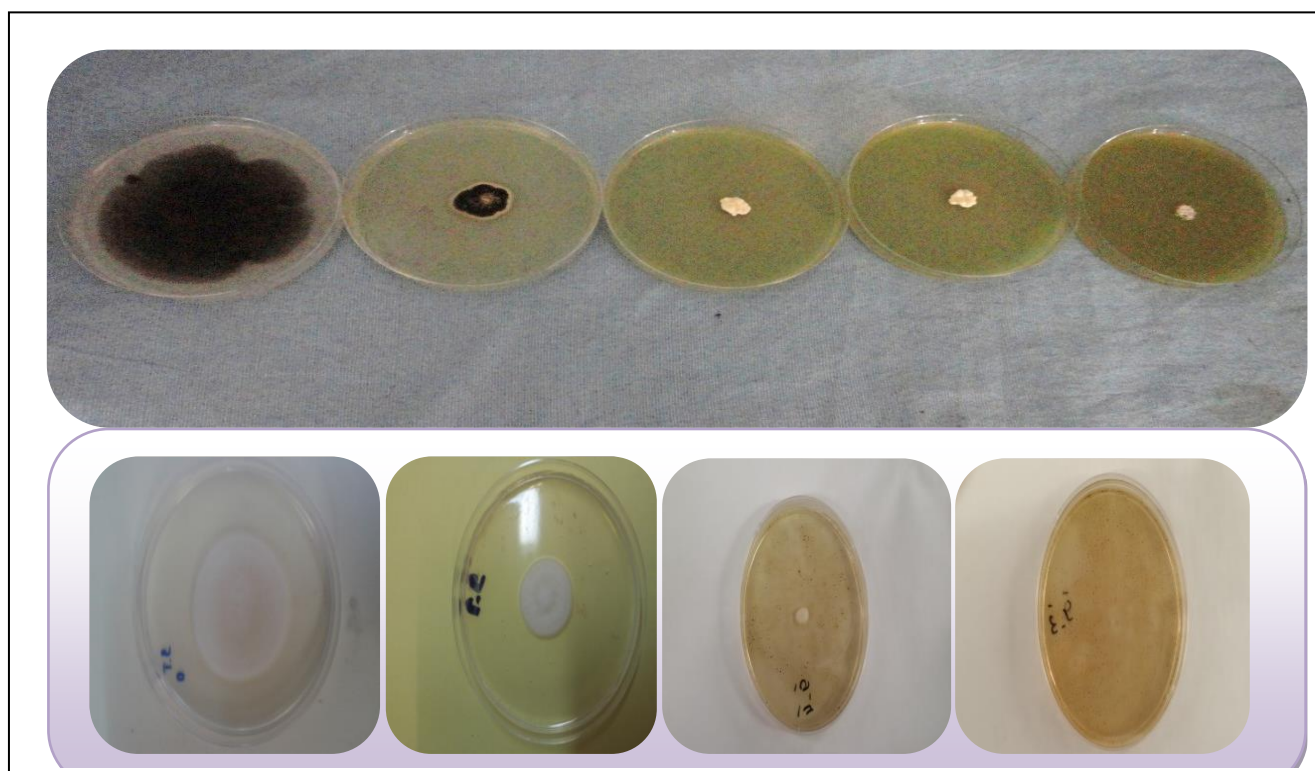


Figure.3 Effect of *Acacia raddiana* tar with various concentrations on *Aspergillus niger* and *Fusarium oxysporum* f. sp. *albedinis* (Source: original. 2019)

Table. 3 MIC of *Acacia raddiana* tar against Bacteria and molds

Concentration (mg/ml) Strains	0.004	0.006	0.008	0.02	0.04	0.06	0.08	0.1

<i>Staphylococcus aureus</i> ATCC 25923	+	+	+	+	+	+	+	-
<i>Listeria monocytogenes</i> ATCC19115	+	+	+	+	+	+	+	-
<i>Pseudomonas aeruginosa</i> ATCC27853	+	+	+	+	+	+	+	-
<i>Klebsiella pneumoniae</i> CIP 106818	+	+	+	+	+	+	+	-
<i>Enterococcus faecalis</i> ATCC 29212	+	+	+	+	+	-	-	-
<i>Escherichia coli</i> ATCC 25922	+	+	+	+	+	-	-	-
<i>Bacillus cereus</i> ATCC 11778	+	+	+	+	+	+	-	-
<i>Aspergillus flavus</i>	+	+	-	-	-	-	-	-
<i>Alternaria alternata</i>	+	-	-	-	-	-	-	-
<i>Aspergillus niger</i>	+	+	-	-	-	-	-	-
<i>Fusarium oxysporum f. sp. albedinis</i>	+	-	-	-	-	-	-	-

Acacia raddiana tar was reported in the literature for woods and bark (Maggi and Delmon1994; Mingjin et al.1996 ; Dinesh et al. 2008). In addition, some authors that certain compound of tar such as 2,6-

(-) inhibition, (+) growth

The chemical compositions of the studied tar revealed that they had compositions similar to those of other *tars*. Indeed, although the chemical compositions of

the possible presence of biologically active compounds in tar that could be used for the formulation of supplements and/or ingredients in pharmaceutical and cosmetic industry.

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Dihydroxy-4-methoxyacetophenone, p-Ethylguaiaicol, 2-Methhoxy-3-methylphenol had excellent antioxidant and antimicrobial properties (Dongrui et al.2017; Digambar et al. 2019).

Acacia raddiana tar exhibited MIC values against all tested microorganism. Similary, Tar extracted from *Juniperus oxycedrus*, *Olea europaea sylvestris* and *Juniperus phoenicea* showed broad spectrum inhibitory against bacteria and molds tested(Benlarbi et al. 2014;Terfaya and Makhoulfi 2015 ; Terfaya et al. 2017 ; Benlarbi 2018; Terfaya et al.2019; Mezouari et al. 2019; Bendjima et al. 2020). For that reason, Today the efficacy of medical tar preparations is well established in psoriasis, (atopic) dermatitis, dandruff, seborrheic dermatitis, and pruritus. Antifungal activity of shampoos with as low as 0.5% coal tar has been described (Arnold 1997).

Conclusion

Vegetal Tar extracted from *Acacia raddiana* had a good antimicrobial activity. The strong antifungal activity of *Acacia raddiana* against 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. These findings prove

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