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Research Article

PHYTOCHEMICAL STUDY AND ANTIBACTERIAL ACTIVITY OF DIFFERENT EXTRACTS OF *Pistacia lentiscus L* COLLECTED FROM DAHRA REGION WEST OF ALGERIA

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ABSTRACT

The purpose of this study was to investigate the phytochemical proprieties, antioxidant and antibacterial activities of different extracts of *Pistacia lentiscus* on two pathogenic bacteria. The concentration of total phenols was analyzed using Folin-Ciocalteu's method. Exracts of plant were evaluated for their antimicrobial activities against *Staphylococcus aureus* and *Esherichia coli* using the agar disk diffusion method and the minimal inhibitory concentration. The phytochemical study revealed the presence of major bioactive chemical constituents in different extracts of *P. lentiscus* (flavonoids, alkaloids, saponins, tannins, terpenoids, glycosides and steroids). Results showed that this plant has antioxydant activity and high quantity of total phenols and flavonoids. Antibacterial activity of the aerial parts of *P. lentiscus* against tested bacteria has shown that Gram-negative strains were more resistant compared to the Gram- positive ones. We can conclude that *Pistacia lentiscus* from Dahra region under investigation can be a potential source of useful drugs.

Keywords: *Pistacia lentiscus*; Phytochemicals; total phenol; Flavonoids; Antibacterial activity.

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1. INTRODUCTION

Infusions and decoctions of medicinal plants have long been used in traditional medicine to treat or prevent chronic diseases and to improve the quality of life [1-2]. *Pistacia lentiscus L* is a dioecious shrub belong to the Anacardiaceae family is originally from the Mediterranean region, Morocco and the Iberian Peninsula to the west and the south of France, Turkey, Iraq and Iran to the east, was grown in England since 1664 .It is mainly exported from Scio, the island where it was cultivated for centuries. The resin of Pistacia lentiscus L (mastic) has been used for over 2500 years in traditional Greek medicine to treat several diseases such as gastric pain and peptic ulcers [3]. Several scientific researches have justified the beneficial effect of Mastic to gastric diseases [4].

In Algeria *Pistacia lentiscus* is called darou and people have used mastic as a medicine for gastrointestinal ailments for several thousand years. Previous studies in Algeria have been done on *Pistacia lentiscus* extracts from the aerial part showed antimicrobial, antioxidant, hypotensive and hypoglycemic activities [5-9].

The fixed oil extracted from the fruit of *Pistacia lentiscus* in Constantine region east of Algeria is widely used especially to treat skin problems and respiratory conditions [10].

Infectious diseases caused by resistant microorganisms are associated with prolonged hospital stays, higher costs, and increased risk of morbidity and mortality [11-12].

However, providing new agents with new mechanisms of action is limited and emphasizes the need to develop new drug targets [13-14].

In this context, this work aims to study the phytochemistry and antioxydant activities of wide spread plant in Algeria *Pistacia lentiscus* and to investigate it's antibacterial activity on two bacteria notorious for their resistant to antibiotics.

2. EXPERIMENTAL

2.1. Plant materials

Leaves and stems of Pistacia *lentiscus* were collected randomly in 31 mars 2014 from Dahra region located North West chelef (Algeria).The samples collected were identified. The voucher specimens under code (Pl.D/3/2014) have been deposited at the Herbarium of the

Laboratory of Pharmacognosy and Api-Phytotherapy (LPAP); University of Mostaganem, Algeria, for future reference.

The plant material was dried in dark area for few days and then passed in room temperature (45-50C °) for 24 hours. It was then finely grinded using a coffee grinder mill-type (for leaves and stems), to obtain a fine powder.

2.1.1. Phytochemical screening

Phytochemical analysis of different constituents which have a pharmacological interest was performed on the powdered samples using standard methods as described by [15-20]. Detecting the different phytochemical families that exist in the plant by precipitation reactions or staining using specific reagents to each family of compounds.

2.1.2. Antioxidant activity

The activity of free radicals was carried out by the use of 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) according to Takao et al. (1994) [21].

The test on free radicals have been realised after an ascending Thin Layer Chromatography (TLC). The crude extract (100 µg) of *Pistacia lentiscus* was melted down in methanol with 1 mg/ml concentration and deposited on the plates (silica gel TLC is flat. DC Glasplatten kiselgel.2-25 micro m, layer thickness 0.25 mm, medium pore diameter 60 Å). The chromatograms are developed in butanol-acetic acid- distilled water mixture in the proportions 60-15-25 respectively; the plates were dried and sprayed with a solution of DPPH at 2 mg / ml of methanol. The active compounds appear as white or yellow spots on a purple background, after 30 min of reaction.

2.1.3. Total phenolic content

The powder (30 g) was put in ethanol (80%, v / v; 360 mL) to macerate for 48 h. and then filtrate with vacuum filtration, refrigerated for 24 h then decanted and concentrated under reduced pressure the crude extracts of aerial part of the plant, were used for our analyzes.

Total phenolic concentration was analyzed using Folin-Ciocalteu's method [22]. 0.125mL of the crude extract of the plant or gallic acide was added to 0.625 mL of Folin Ciocalteu. resting for 5 min, 0.5 mL of sodium carbonate (75g/L) was added , the mixture was shaken and leave to rest for 2 h. The absorbance of the samples was measured at 760 nm using

UV–vis spectrophotometer. Concentrations were expressed as mg of gallic acid equivalent/g of lyophilized extract. The same procedure was used for making standard curve. All experiments were carried out in triplicates.

2.1.4. Total flavonoids

The aluminum chloride colorimetric method was used for mesure the total flavonoids concentrations [23] .Total flavonoids content was expressed as mg quercetin equivalents (mg QE)/g extract.

2.1.5. Thin Layer Chromatography

Extracts of plant was melted in methanol with 1 mg/ml concentration. 10 µml of extracts were deposited on the analytical plates (2.5 cm above from the bottom) and dried on air for thirty minutes and kept in saturated developing chambers containing mobile phase and let run 3/4th of the height of the prepared plates [24] .There solvent system contains: Acetonitrile-alcohol isopropylic-water-(1:1:3) (v / v / v). as mobile phase. The TLC was visualized under UV at 366 nm, and the Rf were calculated.

2.2. Tested microorganisms

The antibacterial effect of plant was investigated on clinical bacterial strains and references , gram negative : *Escherichia coli* ATCC 25922 and gram positive : *Staphylococcus aureus* ATCC 29213, which are known to cause infections specially in urinary tract.

2.2.1. Preparation of plant extracts for antibacterial test

2.2.1.1. Percolation method: In this method the coffee press is used to drive steam of the active ingredients, 3 g of drug with 30 ml of water (concentration 10%). After 5 minutes at the temperature of about 100C ° (percolation through the coffee maker), we obtain a solution that was evaporated (in open air) then recovered with DMSO.

2.2.1.2. Extraction with methanol: flavonoids were extracted with methanol. 1 g of plant powder placed in a glass container (vial) with 10 ml of MeOH 70%, and then heated at 70C ° for 5 minutes (This destroyed the plant tissue, preventing oxidation or enzymatic hydrolysis). The samples let macerate overnight (about 24 hours); after a first filtration on filter paper, and then evaporated (in the open air) and recovered with DMSO.

2.2.1.3. Infusion method: Boiling water with plant powder infused for 10 minutes, then

filtered, and then evaporated (in the open air) and recovered with DMSO.

2.2.1.4. Decoction Method: Plants put in aerlenmeyer flask. Cover with cold water (10% concentration) and bring to boil. Evaporated until reduced by about a third, filtred, and then evaporated again (in the open air) and recovered with DMSO.

2.2. 2. Antibacterial assay procedure

2.2.2.1. Inoculum preparation

The inoculum is prepared from conserved colonies. Revived strains was taken and homogenized in 5 ml of Mueller- Hinton broth and incubated for 3 to 5 hours at 37C °, inoculum was set to 10^5 CFU /m L [25].

2.2.2.2. The antimicrobial activities

Agar disc diffusion method was used, the microorganisms were spread on MH agar plates by cotton swab ,sterilized disk (5mm) were soaked with 50µl of plants extracts (10%) . for the negative control the disks were soaked with sterilized water . Céphalexin (Lexin) (100mg/ml) was the positive control and then incubated for 24h at 37 ^{0}C. The evaluation of the antimicrobial activities was by measuring the diameter of the inhibition zone

2.2.2.3. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) determination

The minimum inhibitory concentration (MIC) is the lowest concentration of a substance that prevents visible growth of a bacterium [24]. For this test, various concentration of the stock (100, 50, 25, 12.5, 6.25, 3.12) mg/ml were tested against the tow microorganisms.

Extracts at different concentrations were added to Mueller Hinton broth to make up 1 ml of solution than after 1 ml of McFarland standard of each bacteria suspension (10^6 UFC/ml) was added .Tubes were incubated at 37 ^{o}Cfor 18-24 h. Tow control tubes were prepared, one containing the extract without the organism and the second containing the medium with the inoculum. The minimum bactericidal concentration (MBC) defined as the concentration those results in microbial death. This was done only on some extracts having high antimicrobial activity.

3. RESULTS

Phytochemical study showed that the plant Pistacia *lentiscus L* contained flavonoids, alkaloids,

saponins, tannins, glycosides, terpenoids and steroids, and does not contain anthraquinons and quinons. Quantitative Analysis showed that this plant contained high quantity of flavonoids.

Results revealed that total phenolic content in the extract of *Pistacia lentiscus L* was (114.95± 12 mg GAE/g DM), the concentration of flavonoids was 25.212±2.13 mg EQ/g of extract (Table 2).

The methanolic extract of aerial parts was used to study the antioxidant activity. The use of methanol like solvent permits the extraction of phenolic compounds from the leaf and stem of *P. lentiscus*. The plant presented a better scavenging efficiency toward DPPH radical manifested in the TLC analysis UV light with yellow and white spot in purple background, The TLC analysis UV light of *Pistacia lentiscus L* showed five spot ((Rf) = 0.04; 0.11; 0.30; 0.41; 0.93) .Extract in methanol confirms the presence of various group of phytochemicals (coumarins, xanthons, flavonols, phenolic acid and flavone) .Different retardation factors (R_f)values of phytochemicals also reflect an idea about their polarity (figure 1).

Results of antibacterial activities of *P. lentiscus* against *S. aureus* and *E. coli* showed a remarkable antibacterial effect against gram positive strains *S. aureus* , the higher inhibitory activity was with decoction method with diameter (25.5±0.5) .However no inhibitory effect was noted with gram negative strains *E. coli* (table 3 and 4).

Results of MIC and MBC determination (Table 5) and the ratio of MBC/MIC (table 6) showed that *S aureus* was more sensitive to different extracts of *P. lentiscus*, *E coli* was resistant to different extracts of this plant.

Table 1. Phytochemicals screening of aerial of *Pistacia lentiscus L*

Extract constituents	**leaves**	**Concentraction (m/g of plant)**
		Aerial part
Flavonoids	+++	0.223
Alkaloids	++	nd
Glycosides	++	nd
Tannins	+++	nd
Saponins	+++	nd
Steroids and terpenoids	++	nd
Quinons	-	nd
Anthraquinons	-	nd

(- Absent,+ present,+++ present in high quantity ,nd not determined).

Table 2. Levels of total phenolic (mg of Gallic acid equivalent per 1g lyophilized extract) and flavonoid contents (expressed as mg Quercetin equivalents/g extract contents in aqueous extract of *Pistacia lentiscus L* .

species	Total phenolic (mg GAE/1g)	Total flavonoids (mg QE/ g)
leaf and stem	114.95±6.25	25.212±2.13

Table 3. Effect of differents extracts of aerial parts of *Pistacia lentiscus L* microorganisms *Against Staphylococcus aureus and Esherichia coli*

Microorganisms	**Aerial parts**				***Cephalexin***
	P	M	I	D	
***Escherichia coli* (G^-)**	6±0.7	5±0.2	5±0.1	7±0.9	56.5
***Staphylococcus aureus* (G^+)**	14±1	18±0.7	16±0.5	25.5±0.5	33

Disc diameter :5mm. **P**: percolation. **M**:extraction with methanol. **I**: infusion. **D:** decoction

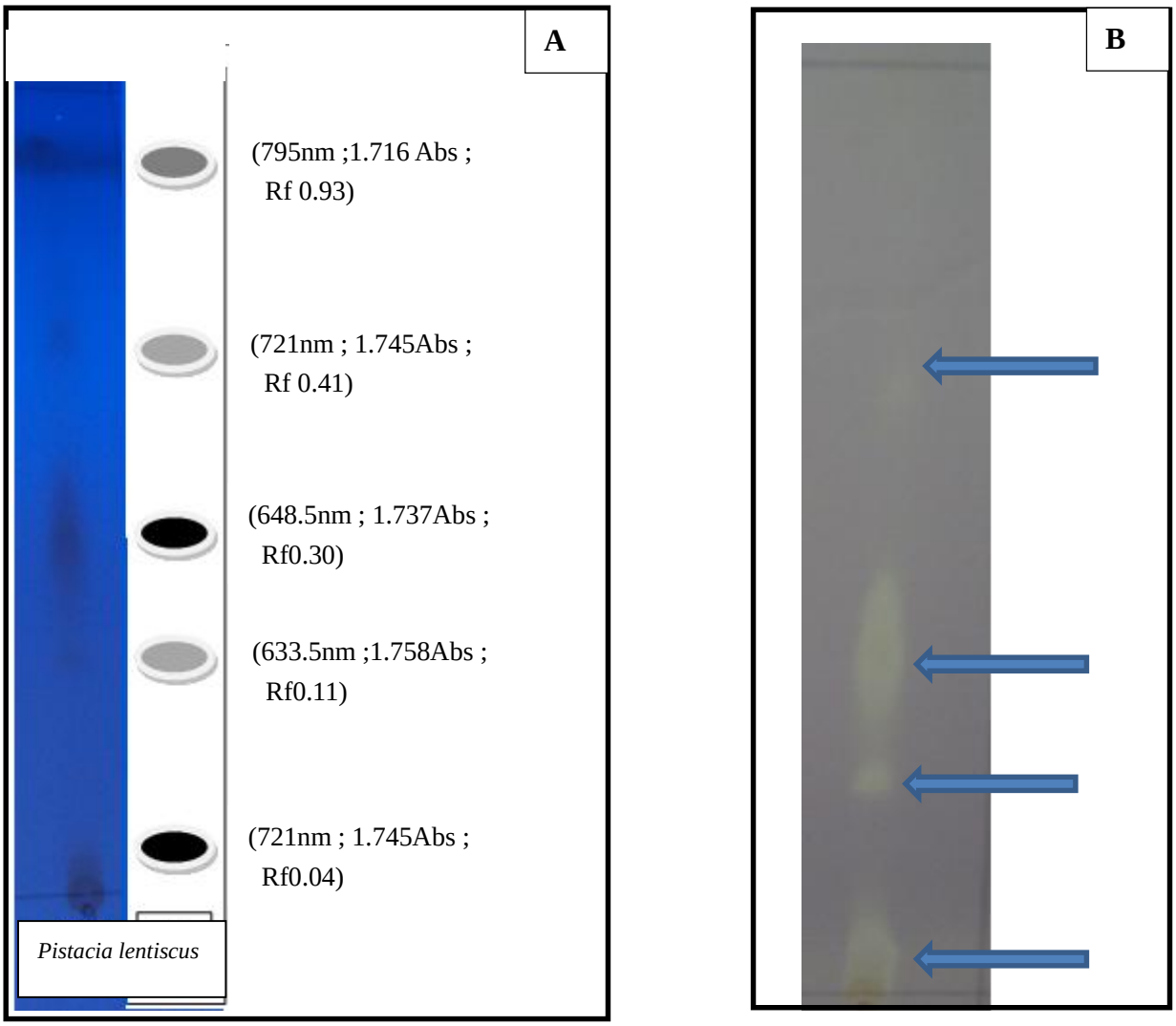


Fig.1. A: TLC profiles of contents of *Pistacia lentiscus* aerial part (UV 336 nm).

B: TLC after spraying with a methanolic solution of DPPH at 2 mg / ml; *Pistacia lentiscus* (Rf) = 0.04; 0.11; 0.30; 0.41)

Table 4 . The inhibitory effect of extracts of plant on 02 pathogenic strains (*Staphylococcus aureus and Escherichia coli).*

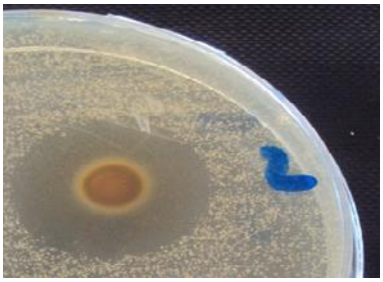
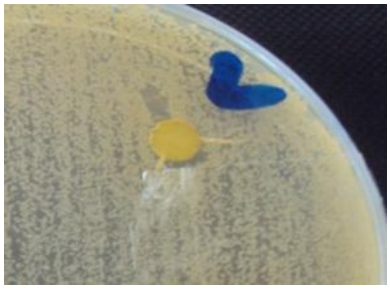
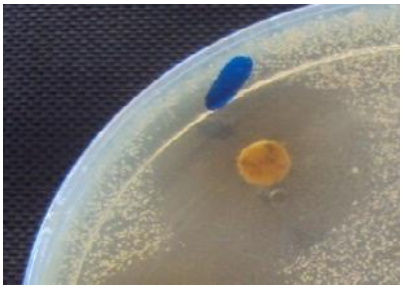
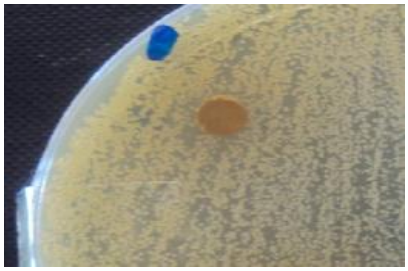
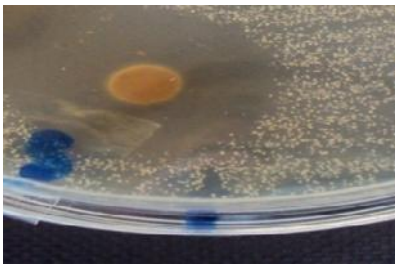
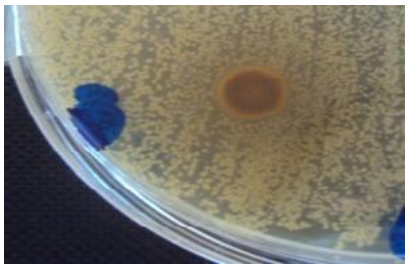
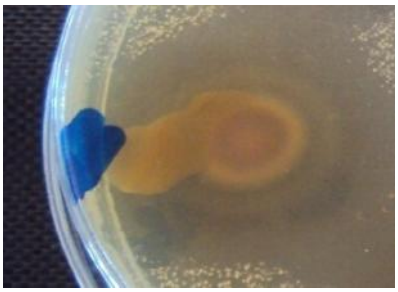
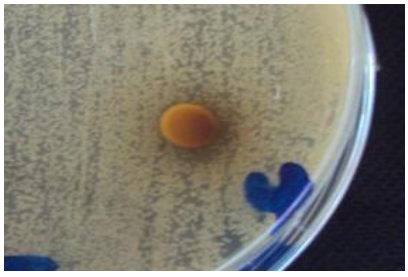
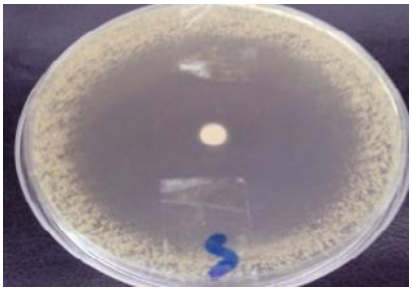
Method of extraction of *Pistacia lentiscus* 100mg/ml	*Staphylococcus aureus*	*Escherichia coli*
Percolation		
Extraction with methanol		
Infusion		
Decoction		
Cephalexin (Lexin) ***(100mg/ml)***		

Table 5. Minimum Inhibitory Concentrations (mg/ml) and Minimum Bactericidal Concentration (mg/ml) of the different extracts

	CMI and CMB (mg/ml)	*Staphylococcus aureus*				*Escherichia coli*			
		M	P	I	D	M	P	I	D
Pistacia	CMI	50	50	50	50	+100	+100	+100	50
lentiscus	CMB	50	50	50	50	+100	+100	+100	100

CM(mg /ml) = 100; C1= 50 ; C2= 25 ; C3= 12.5 ; C4= 6.25 ; C5= 3.125, +100: resistant in 100 mg/ml

Table 6. Ratio of CMB / CMI

Aerial part	**method**	**bactericidal activity**			
		Staphylococcus aureus		*Escherichia coli*	
	Maceration	1	bactericidal	R	resistant
Pistacia	Percolation	1	bactericidal	R	resistant
lentiscus	Infusion	1	bactericidal	R	resistant
	Decoction	1	bactericidal	2	bacteriostatic

MBC /MIC <2 :bactericidal; MBC /MIC >2 : bacteriostatic; MBC /MIC≥32: resistant

4. DISCUSSION

Plants are a source of natural bioactive compounds with high medicinal activities for the treatment of various diseases [27].The use of plants for local remedies is a traditional custom .In Algeria the mastic tree is widespread in the forest belt.This study provides support to traditional and alternative use of *P. lentiscus* against various infections.

Phytochemical studies of *P. lentiscus* showed that the major components were flavonoids, alkaloids, saponins, tannins, glycosides, terpenoids and steroids, however the absence of anthraquinons and quinons. Our results are in agreement with many researches on Algerian *P. lentiscus* **[**8-28-29-30].

The TLC analysis UV light, allowed the identification of different metabolites of yellow and green (coumarins), orange (xanthons), [31]. Flavonols (yellow colours), phenolic acids (blue

fluorescent), flavones (purple chestnut), [30] .The plant also exhibited a better scavenging efficiency toward DPPH radical.Our results are in agreement with those of [8-28-29] who found that *P.lentiscus* exhibited a great reducing power.

In a study realized by Rodriguez-Perez et al. (2013) [33], 46 phytochemical compounds were identified, 20 of which were identified for the first time in *P. Lentiscus* leaves. Flavonoids, phenolic acids and their derivatives were the most abundant compounds, those with the highest concentrations being myricetin glycoside, catechin, β-glucogallin, and quercitrin gallate.

The total phenolic concentration was (114.95±6.25 mg GAE/g of plant extract) in the aerial parts of *P. Lentiscus* from Dahra region. Our results showed low concentrations of phenolic contents compared with others authors Zitouni et al (2016) [8]**,** found that the Total phenolic concentration of *P. Lentiscus* from Nedroma region ,west of Algeria were in leaves (216.289 ± 20.62 mg GAE/g DM) was significantly higher than those revealed in stems (121.399 ± 3.354 m g/g).

Comparing to others studies, the flavonoid contents in aerial parts of *P. Lentiscus* in this work were higher 25.212±2.13 (mg QE/ g) than their of Zitouni et al. (2016) [8], which found 19.162 ± 0.436 and 16.788 ± 0.733 mg QE/ g in leaves and stems ,respectively, and 12.93 ± 1.69 mg/g found by Atmani et al (2011) [29] and 8.21 ± 0.09 mg/g by Krimat et al (2014) [32].

Antimicrobial activities of the aerial parts (leaves and stems) of *P. lentiscus* against tested bacteria have shown that Gram-negative strains were more resistant compared to the Gram positive ones. Similar findings have been reported by other authors (Arab k et al. (2014)[35]**;** Benhammou N et al .(2007) [36]; Taoufik H et al. (2015)[37] who found that Gram positive strains were more sensitive to this plant than Gram negative strains.The higher inhibitory effect was with decoction method, Decoction involves first mashing and then boiling in water to extract oils, volatile organic compounds and other chemical substances as tannins that are stable to heat ,it is the traditional method that have been used since time in Algeria to treat many illness. Our results are in agreement with the results of bouabdelli et al. (2012) [9] who found that the decoction method had the highest antibacterial effect with 43. 3 %,

followed by percolation with 28. 3 %,

Literature reports that for antimicrobial *P. lentiscus* has been observed that it has strong antifungal but low antibacterial activities. *Pistacia lentiscus* L. has found to be effective against *Sarcinalutea*, *Staphylococcus aureus* and *E. coli* and it also has antimycotic activity [38]. Results of MIC and MBC confirme the antibacterial results and showed that *Staphylococcus aureus* was more sensitive to this plant compared to *E. coli* which was more resistant to *P. lentiscus* extracted with different mode of extraction.

4. CONCLUSION

This work aims to investigate the phytochemical and antibacterial activities of *Pistacia lentiscus L* from Dahra region (Algeria). This study showed that *Pistacia lentiscus* is very rich on flavonoids, alkaloids, saponins, tannins, glycosides, terpenoids and steroids. Also this plant contained phenolic and flavonoids contents. The plant presented a better scavenging effectiveness toward DPPH radical .The antibacterial activities against *S aureus* and *E. coli* prepared with four mode of extraction (percolation, maceration in methanol, decoction and infusion) showed that Gram positive bacteria was more sensitive to the different extracts .The higher effect was noted with decoction method. Other research are required to isolate and identify the different phytochemical compounds from the crude plant extracts for proper drug development.

5. ACKNOWLEDGEMENT

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