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ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF THE ESSENTIAL OILS AND PHENOLIC EXTRACTS OF MYRTUS COMMUNIS AND ZYGOPHYLUM ALBUM FROM ALGERIA

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ABSTRACT

The present study deals with the evaluation of the in vitro antioxidant and antibacterial activity of phenolic extracts and essential oils of two medicinal and aromatic plants *Zygophyllum album* and *Myrtus communis* by using the 2,2- diphenyl-2-picrylhydrazyl radical ,total antioxidant power and agar diffusion methods and minimum inhibitory concentration (MIC) determination. Moreover, the extracts were investigated for their polyphenolic, flavonoids, tannins and anthocyans content by using the Folin-Ciocalteu assay, the aluminium trichlorid method, reaction with vanillin and colometer method based on differentiation of absorbance, respectively. The results showed that the highest antioxidant capacity was exhibited by the aqueous extract of *Myrtus communis* with IC_{50}= 29,080mg/ml. All extracts possessed more or less antibacterial activity against the tested Gram- positive and Gram – negative bacteria *Bacillus subtilis* and *E.coli* were the more susceptible microorganisms to all extracts and essential oils. Pronounced antibacterial activity was observed by the methanolic extract of *Zygophylum album* (MIC value=25 µg/ml). Morever, the results showed that the phenolic compounds and flavonoids were abundant in Myrtle aqueous extracts.

Key Words: antimicrobial; antioxidant; plant; essential oil; phenolic extract.

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1. INTRODUCTION

There is a popularity and scientific interest to screen essential oils and extracts of plants used medicinally in all over the world [1]. The main volatile constituents of the essential oils have been used historically in the pharmaceutical, food and perfume industries because of their antibacterial properties, culinary and fragrance, respectively. Antioxidants have been widely used as additives to avoid the degradation of foods. Also, are compounds that react with free radicals, neualizing them and thereby preventing or reducing their damaging fects in the human body [2]. Lipid oxidation is so responsible for deterioration of fats and oils resulting in change color, flavor and nutritive value, while oxidative stress is involved in the pathogenesis of numerous diseases.

The problems regarding application of conventional antibiotics, including antimicrobial resistance, environmental problems, cancerogenity, side effects and high costs, have reinforced a tendency to replace synthetic antimicrobials with natural alternative agents [3]. Plant based products are among the alternative agents examined in order to replace conventional antibiotics. Accordingly, extensive research has been carried out in order to evaluate the antimicrobial effect of the essential oils and extracts which showed the ability to inhibit the growth of various pathogenic microorganisms [4].

With that in mind, the halophyte *Zygophyllum album L.* (Zygophyllaceae) might be of interest. This species has long been used in traditional medicine as a remedy for rheumatism, gout, asthma and as diuretic. Nowadays, it is still used against aches and thirst, for wound care or treatment of dental caries, and to wash clothes and hair. Some phytochemical data on *Z. album* have been reported [5], although the chemical composition and biological activities of *Z. album* have not been fully elucidated.

Myrtus Communis L. is an evergreen shrub belonging to the Myrtaceae family that grows wild around the Mediterranean region. It is commonly known as myrtle. Its leaves are pleasantly scented making it valuable for the perfume industry. It has white star-like delicate flowers and the fruit is a round, dark-blue berry containing the seeds. Many medicinal and nutraceutical properties have been attributed to myrtle, which has been used since ancient times in folk medicine. It is traditionally used as an antiseptic and wound healing, disinfectant, hypoglycaemic agent, with anti-hemorrhagic, antimicrobial and antioxidant properties [6].

Here, we report the composition and antibacterial and antioxidant activity of the essential oil and various extracts of *Myrtus communis* and *Zygophylum album* from Algeria as well as total phenolics, total flavonoids, condensed tannins, and total anthocyans of these plants.

2. EXPERIMENTAL

2.1. Plant material

Myrtle (*M. communis* var. italica L.) aerial parts were collected at the flowering stage in July 2014 from Honaine region (North East of Tlemcen-west of Algeria. In the case of *Z.album*; fresh aerial parts were collected in August 2014 from Sidi Khouiled region (sahara of Ouargla).

The sampling was done by a randomized collection of 15–20 shrubs and sub-shrubs in an area of about 200 m^2 each. Myrtle leaves, and areal parts of *Z.album* were isolated manually in our laboratory to obtain a weight of 500–700 g of each part. Botanical identification of this species was carried out according to Africain flowering plants database and by local experts.

2.2. Bacterial strains

The bacteria used in the antibacterial tests were: Gram-positive (*Bacillus subtilis, Staphylococcus aureus, clostridium sp*.) and Gram-negative (*E.coli, salmonella typhi and Shigella sp*.)

All strains isolates from different environnement, all bacterial strains have been provided by Laboratory of Microbiology, Faculty of Biology, and University of Mascara, Algeria.

2.3. Essential oil isolation

The plant samples were separately water distilled in a Clevenger type apparatus for 3 h (time fixed after a kinetic survey during 30, 60, 90,120, 150, 180 and 210 min). According to the method recommended by the French standard method AFNOR (2000), all experiments were done in triplicates and results were expressed on the basis of dry matter weight. The essential oil was stored at 4 C in the dark.

2.4. Polyphenols extraction

The plant materials was dried at ambient temperature and stored in a dry place prior to use. The plant was washed well with water, dried at room temperature in the dark, and then ground in an electric grinder to give a coarse powder. In this study, samples were extracted by decoction (10%), maceration with ethanol (8%) and by extraction with solvents of increasing polarity (Dichloromethan and methanol/soxhlet) methods.[7,8].

2.5. Phytochemical screnning by colometer method

All plant extract were tested for the presence of different families of compounds according to methods reviously described [9], [10].

Table 1. Phytochemical screnning of phenolic extracts of *Zygophylum album* and *Myrtus communis*

	Myrtus communis				***Zygophylum album***			
	Aqu.E	**Eth.E**	**DCM.E**	**Met.E**	**Aqu.E**	**Eth.E**	**DCM.E**	**Met.E**
Alcaloids	+++	-	-	-	-	-	-	++
Free anthraceniq derived	+	-	-	-	-	-	+	-
anthraquinons	-	-	-	-	-	-	-	-
C-hétérosides	+	+	+	+	+	+	-	++
Anthocyanes	+++	+	+	+	-	-	+	+
Saponins	-	+++	-	-	+++	+	+	++
Tannins	+++	-	-	-	+	+	+	+
Flavonoids	+++	-	-	-	+	-	+	++

According to the screnning results and based on the richness on compounds, two phenolic extracts were selected for study; aqueous extract of *Myrtus communis* and methanolic extract of *Zygophylum album*

2.6. Polyphenols analysis

The total phenolic in extracts content was determined by spectrometry using ‘‘Folin-Ciocalteu’’ reagent assay [11]. Gallic acid was used as a standard for the calibration curve. The total phenolic content was expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW).

2.7. Condensed tannin content

Condensed tannins were transformed by the reaction with vanillin to anthocyanidols. Condensed tannin contents of each organ (three replicates per treatment) were expressed as mg catechin equivalents per gram (mg CE/g) through the calibration curve with catechin.

2.8. Total flavonoid content

Total flavonoid content was measured according to [12]. Total flavonoid contents were expressed as mg catechin equivalents per gram (mg CE/g).

2.9. Total anthocyans content

Total anthocyans content was evaluated by colorimetry using a UV-visible spectrophotometer

The concentration of anthocyanin pigment in the extract is expressed in mg equivalent cyanidin-3 glucose per liter of solution or mg of cyanidin-3 equivalent glucose / g dry matter (Cg / g dM) [13].

2.10. Antioxidant activity assays

DPPH scavenging assay

The hydrogen atom donation ability of chemical compounds in leaves and stems was measured on the basis to scavenge the 2,2-diphenyl-1-picrylhydrazil free radical [21]. Fifty microliter of various concentrations of the extracts in methanol were added to 1950 µl of a 0.025 g/l methanol solution DPPH. After a 30-min incubation period at room temperature, the absorbance was read against a blank at 515 nm. DPPH free radical scavenging activity in percentage (%) was calculated using the following formula:

$$\text{DPPH scavenging activity (\%)} = (A_{blanc} - A_{sample}/A_{blanc}). 100$$

Where: A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), A_{sample} is the absorbance of the test compound.

Extract concentration providing 50% inhibition (EC_{50}) was calculated from the graph plotted of inhibition percentage against extract concentrations. The ascorbic acid methanol solution was used as positive control.

Total antioxidant power

During this test, hydrogen and electron is transferred from the reducing compound (antioxidant extract) to the oxidant complex (PPM). This transfer depends on the redox potential of pH of the medium and the structure of the antioxidant compound.

The method comprises introducing 300 .µl of the extract of the leaves mixed with 2.7 ml of a reagent consisting of H_2SO_4 (0.6 M), NaH_2PO_4 (28 mM) and ammonium molybdate (4 mM).

The tube is then incubated at 95 ° C for 90 minutes. After being cooled, the absorbance is measured at 695 nm. The control consisted of 300 .µl of methanol mixed with 2.7 ml of the reagent mentioned above; the calibrators, controls and samples are incubated under the same conditions. The results are expressed in mg of gallic acid equivalents per gram of dry material (mg E AG/g Ms).

2.11. Antibacterial activity assays

- **Bacterial strains and Antibiotic susceptibility test**

The antibiotic discs Amoxicillin (AX 30µg) several bacterial strains were isolated from different environments and subjected to disk diffusion method using 06 different antibiotics according to [14]. The antibiotic discs Amoxicillin (AX, 30µg), Oxacillin (OX, 5µg),

Cefazolin (CZ, 30µg), Gentamycin (CN, 10µg), penicillin (P, 30 µg), and Spiramycin (SP, 10 µg) were used and the susceptibility was determined by the inhibition zone in mm [14]. In the end, seven strains were selected for their antibiotic resistance According to the standardization of susceptibility in human medicine at the national level, and the recommendations of the Committee on Antimicrobial the French Society for Microbiology (2008): three of them from Meslam Taib Hospital, Mascara (*E. coli*, *Staphylococcus aureus*, *Enterococcus faecalis*), three from wastewater (*Salmonella typhi*, *Shigella sp*, *Bacillus subtilis*) and the last one (*Clostridium sp.*) from the great Sabkha Oran.

Table 2. Antibiotic resistance profile of tested bacterial strains

	P	**AX**	**OX**	**CZ**	**SP**	**CN**
Escherichia coli	R	R	R	R	R	R
Salmonella typhi	R	R	R	R	R	R
Shigella sp.	R	R	R	R	R	R
Bacillus subtilis	R	R	R	R	R	R
Clostridium sp.	R	R	R	R	R	I
Staphylococcus aureus	R	R	R	R	R	I
Enterococcus faecalis	R	R	R	R	R	R

R: resistant;I:intermediate

- **Disc diffusion test**

Antimicrobial activity was determined by the agar disc diffusion assay [15]. Inoculm for the assays were prepared by diluting scraped cell mass in 0.85% NaCl sterile solution, adjusted to McFarland scale 0.5 and confirmed by spectrophotometric reading at 580 nm. Cell suspensions were finally diluted to 10^6 CFU /ml. The extracts were dissolved in dimethyl sulfoxide (DMSO) or distilled water. Petri plates were prepared with 20 ml of sterile Mueller Hinton agar (Sigma, Paris, France) surface inoculate by suspension of cell (200 µl). The test cultures were swabbed on the top of the solidified media and allowed to dry for 10min. The tests were conducted at a concentration of the sterile phenolic extract (100mg/ml) and essential oils (250 µl/ml) of *M.communis* and *Z.album* in Sterile filter paper discs (6 mm). The loaded discs were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. The plates were incubated at 37C for 24 h. Gentamicin (10µg) and oxacillin (30µg) was used as positive controls. Negative controls were performed using paper discs loaded with 20 µl of the aqueous DMSO.

The antimicrobial activity was evaluated by measuring the zone of growth inhibition surrounding the discs. After that, the inhibition zones were measured in millimetres by Vernier callipers. All tests were repeated two times to minimize test error. An inhibition zone of 14 mm or greater (Including diameter of the disc) was considered as high antibacterial activity [16].

- **Determination of MIC by Microdilution Method**

MIC of the compounds under study was determined by the microdilution method as described by [17]. All wells were filled with 50 µL of Muller Hinton broth (MHB). Extracts were dissolved in DMSO and added to the first well (50 µL). Serial two-fold dilutions were made then. An over-night culture of bacteria suspended in MHB was adjusted to turbidity equal to 0.5 McFarland standards so 10^4 CFU^{-1} of bacterial inoculums size. Each test included two growth controls consisting of the medium with the solvent (DMSO) and medium with bacterial suspension. Each plant extract was run in duplicate. The test plates were incubated at 37°C for 18 h. Then the turbidity was measured every two hours using micro-plate reader (TECAN brand) at 620 nm wavelength. The MIC was taken as the minimum concentration of the dilutions that inhibited the growth of the test microorganism.

2.12. Statistical analyses

All extractions and determinations were conducted in triplicates and results were expressed on the basis of dry matter weight. Data are expressed as mean ± SD. The means were compared by using the one-way and multivariate analysis of variance (ANOVA). The differences between individual means were deemed to be significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Extraction yields

The yield of decoction for aqueous extract of *M.communis* was 24.65% (w/v) and was about 0.52±0,03% (w/v) for the essential oil wich is very higher than the yiels of methanolic extract of *Z.album* 25,03±0,1% and 0,05±0,8% for The essential oil ,it had a pale white/yellow color with a strong musk and floral odour. It was difficult to recover it because of its doughy and grassy aspect.

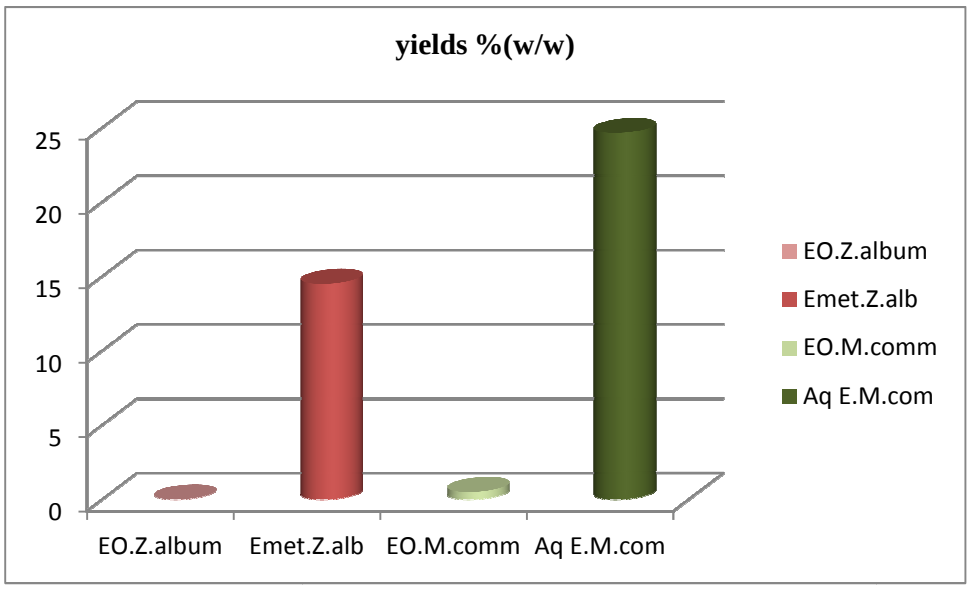

Fig.1. the yield of different extracts of plants

3.2. Phenolic compound content

Table 3. Total phenolic, flavonoids, tannins and anthocyans content

		Total Phénols (mg GAE/g DW)[a]	Total flavonoids (mg EC/g DW)[b]	Condensing tannins(mg EC/g DW)[c]	Anthocyans (mg Cg/ g dM)[d]
Z.album	**Met.E**	6,766±0,628	1,610±0,020	4,349±0,569	1,80±0,01
M.communis	**Aqu.E**	121,23±0,77	7,256±0,57	18,78±0,86	11,79±0,30

[a:] mg acid galic equivalent/g dry weight. [b, c] :mg catechin equivalent/g dry weight.[d]: mg E cyanidine-3 glucose/ g dry weight

Table 03 summarises the results from the quantitative determination of the tannins, flavonoids and proanthocyanidins of the different phenolic extracts of *Z.album* and *M.communis* and their respective total phenol contents. Total phenol and total tannin contents were determined as gallic acid equivalents in milligrams per gram (mg GAE/g) while total flavonoid and condensed tannin contents were calculated as catechin equivalents in milligrams per gram (mg CE/g). The total phenol contents varied between the two plants: Myrtle aqueous extract had higher total phenol content (121.67 mg GAE/g) than methanolic extract of *Z.album* (6.766 mg GAE/g).

A study by [18]shows that the content of the higher total phenols was noticed in the leaves of *myrtus var. italica* (33.67 mg GAE / g),These levels are lower than our results against by the

content of total phenols leaves Myrtle Greece is much higher compared to our plant, it is of the order of 373 mg GAE / g

Concerning total flavonoid and condensed tannin contents, the highest values were observed in M.communis (7.25and 18.78 mg CE/g, respectively). Significant differences were also found in anthocyanins contents among different plants, representing 11.79 mg Cg/ g dM) in *M.communis*, 1,80 mg Cg/ g dM) in *Z.album*.

3.3. Antioxidant activity

Table 4. antioxidant activities of the essential oils and phenolic extracts of plants

		DPPH (IC_{50}, µg/ml)	Total antioxidant power (mg /g DW)
Phenolic extract	***Z.album***	1107,031±1,55	10,99±11,43
	M.communis	29±0,80	68,05±7,55
Essential oil	***Z.album***	6018±0,20	2,44±9,14
	M.communis	615±1,13	36±1,22
Synthetic antioxidant	**Ascorbic acid**	39,53±0,05	-
	BHT	25±0,20	-

IC_{50} value: the effective concentration at which the antioxidant activity was 50%; ; 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals were scavenged by 50%; ; values are given as mean ± SD (n = 3); means with different capital letter within a row are significantly different ($P < 0.05$).

Free radical scavenging properties of phenolic extracts and essential oils from the two plants are presented in Table 3. Lower IC_{50} value indicated higher antioxidant activity. The aqueous extract of myrtle leaves (IC_{50} = 29±0, 80) showed higher scavenging ability on DPPH radicals when compared to those reported for essential oils of leaf (IC_{50} = 600 lg/ml). In addition, DPPH scavenging ability of the aqueous extracts of myrtle leaf was approximately same with that of synthetic antioxidant BHT (IC_{50} = 25 µg/ml). Unlike to *Z.album*, its represent a very lower antioxidant activity(IC_{50}=6016, and 1017,031) for the essential oil and methanolic extract respectively. The antioxidant activity of phenolic extracts and essential oils of the two plants was also evaluated by the total antioxidant power method (Table 4). As for antiradical scavenging activity, all phenolic extracts of plants (IC_{50} = 29±0,80 µg/ml for *M.communis*,

IC_{50} = 1107,031 µg/ml for *Z.album*) showed higher of the total antioxidant power than that of essential oil.

The stronger activity of *M.communis* should be related to its higher phenolic content (121.23mg/l) as measured by gallic acid test (Table 3). Antioxidant activities of the essential oils and the phenolic extracts from myrtle and zygophylum plants were tested by the DPPH radical scavenging, and total antioxidant power assays. Our results showed that all phenolic extracts of plants showed higher scavenging ability on DPPH radicals when compared to those reported for essential oils. In addition, DPPH scavenging ability of *M.communis* was higher than that of *Z. album* plant suggesting the presence of specific bioactive components in these two organs that may be responsible for the antioxidant activity. Thus, it has been reported that free radical-scavenging activity is greatly influenced by the phenolic composition of the sample [19].

3.4. Antimicrobial activity

Antimicrobial activity was assayed against seven pathogen bacteria (*S. aureus, clostridium sp., E. fecalis, S. typhi, E. coli, shigella sp.,* and *B. subtilis*). These microorganisms have different growth properties. The results of disc diffusion test and minimum inhibitory concentration of essential oils are listed in Tables 3-4 and figure 2. *Z.album* showed some activity on Gram positive and Gram negative bacteria while *M.communis* was found to have a low activity. The higher efficacy of *Z.album* was confirmed by the agar dilution method.

Table 5. Disc diffusion test

		Inhibition diameter (Ø mm)					
		S. aureus	E. coli	Shigella	Salm.typhi	clostri	B.subt
M. communis	**E.O**	07±0,2	07±0,01	07±0,2	09±0,02	09±0,01	08±0,3
	AQ.E	10±0,07	09,5±0,02	10±0,1	08,5±0,03	10±0,01	11±0,01
Z. album	**E.O**	11±0,1	08±0,3	09±0,05	10±0,06	09±0,05	10±0,03
	Met.E	09±0,06	15±0,05	10±0,03	09±0,03	11±0,2	14±0,01
Oxacillin 5µg		-	13±0,2	-	-	-	-
Gentamicin 10µg		20±0,1	30±0,02	9	-	10	10

- :No inhibition zone.

All phenolic extracts and essential oils showed a varying degree of antimicrobial activity against bacteria.For *Z.album* ; the methanolic extract was the most effective on the different strains than the essential oil and exhibited a stronger antibacterial activity against *E.coli* and

B.subtilis (11-14mm repectively) this can be proved by the study of [20] witch found that the methanolic extract of *Zygophyllum quatarense* exhibited the highest antibacterial effect followed by the chloroform extracts, In the case of *M.communis*, this plant had a variable and significant antibacterial activity. Selon [21,22], predominant materials of the essential oil are also known for their antimicrobial activity. Alcohols, aldehydes and esters can contribute to the overall antimicrobial effect of essential oils [23]. Monoterpenes such as α-pinene and limonene are among the major components that contribute to high antimicrobial activity of *myrtus communis* [24].

These activities are related to their contents in active compounds, which had a wide range of biological and pharmacological activity. The plant contains also, a class of secondary metabolites which are produced by plants primarily as a defence against herbivores or against infection by microorganisms [25,26].

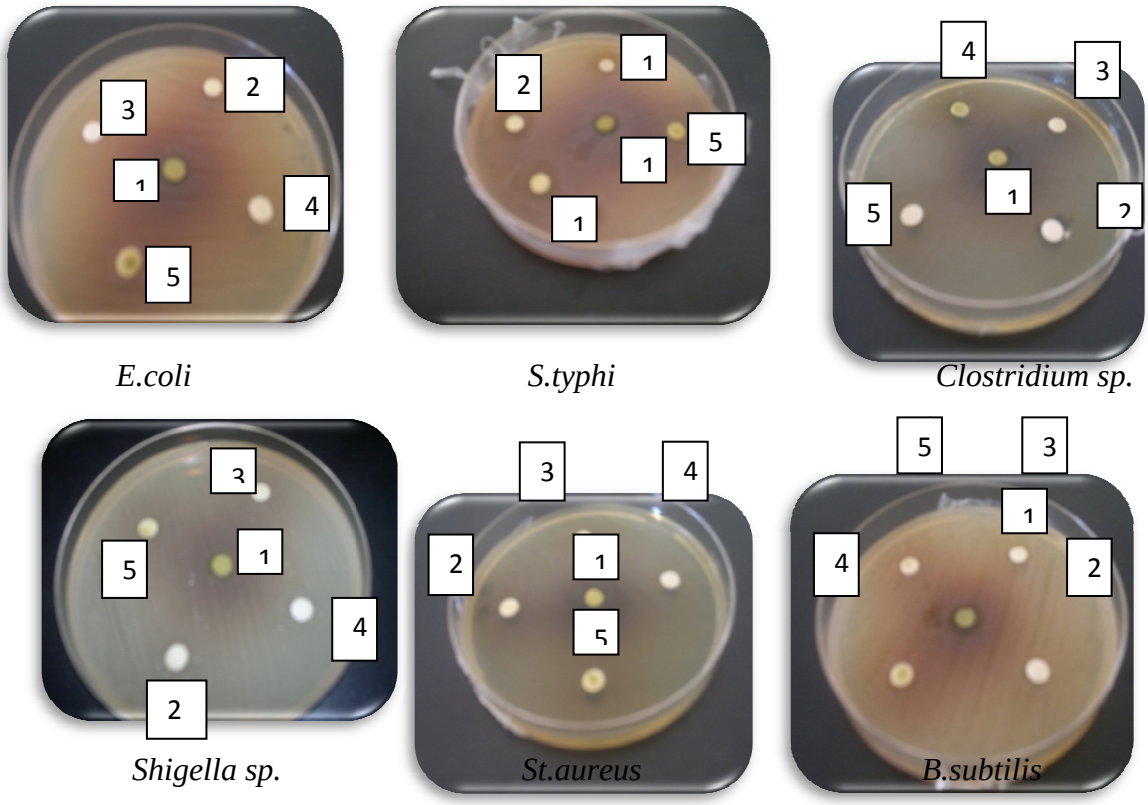

Fig.2. Antibacterial activity of Z .album and M.communis

1 : Methanolic extract of *Z.album*, 2 : Eseential oil of *Z.album*, 3 : Aqueous extract of *M.communis*, 4 : Essential oil of *M.communis*, 5 : ATB (OX5µg /CN 10µg).

Table 6. Minimum inhibitory concentration (MIC %,v/v)

	Polyphenols mg/ml		Essential Oils µl/ml	
	M.com	**Z.alb**	**M.com**	**Z.alb**
E. coli	100	25	250	250
S. typhi	100	100	250	125
Shigella sp.	50	50	250	250
B. subtilis	100	50	250	250
Clostridium sp.	50	50	250	250
St. aureus	50	50	250	125

The MIC was (25-100) mg/ml for methanolic extract of *Z.album*, a significant reduction in growth was revealed after 18 h of incubation. Strains were also susceptible to the aqueous extract concentration of the *M.communis* (50-100mg/ml). However *Z.album* was more active than *M.communis* against all strains especially against *E.coli* (25 mg/ml) for methanolic extract.and phenolic extracts were more actifs than essential oils with higher MIC.

The results presented in this study (Tables 2 and 3) are in line with the small number of published papers on the effects on bacterial growth of M. *Communis* and Z.album, With regard to the *M. communis*, have reported the antimicrobial activity of essential oil of leaves against S. *Aureus, S. epidermidis, E. coli, B. Subtilis* and *Serratia Marcescens* [27,28] have demonstrated the activity of *Z.album* extract against E. *Coli S Aureus and Candida albicans*. However, it is difficult to compare the data with the literature because several variables influence the results, such as the different chemical composition due to the environmental factors (such as geography, temperature, day length, nutrients, etc) of the plants.

4. CONCLUSION

According to the results, it may be concluded that the extracts of the phenolic compounds and essential oils of *M.communis* and *Z.album* revealed considerable antimicrobial activity. Furthermore, the examined extracts have a high reducing activity and scavenging activity for DPPH radical in vitro.

These activities were found are probably in relationship with the structure of the phenolic compounds and the composition of the essential oils.

5. ACKNOWLEDGMENTS

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