

The Antibacterial Activity Screening of the Extracts of Some Moroccan Medicinal Plants

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Abstract: The antibacterial and antifungal activities of some Moroccan medicinal plants extracts were evaluated against six bacterial species and three yeasts.

The agar diffusion method was used to evaluate the antimicrobial activity of the extracts of the following medicinal plants: *Rosmarinus officinalis*, *Salvia officinalis*, *Psidium guajava*, *Crocus sativus*, *Dysphania ambrosioides*, *Trigonella foenum graecum* and *Cymbopogon citratus*. The used standard antibiotic is the Ampicillin 30µg, and the standard antifungal is the Econazole 30µg.

The highest antibacterial activity was observed for the extracts of *Rosmarinus officinalis* (16,6mm<d<26mm) and *Salvia officinalis* (18mm<d<22mm), which are very close to that of some standard antibiotic (ampicilline 30µg) who recorded zones of inhibition from 24 to 29 mm. In the second class there is *Psidium guajava* extracts, with a moderate antibacterial activity (9mm<d<12mm) against *Pseudomonas.sp.*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Escherichia. coli*. The extract of *Crocus sativus*, *Trigonella foenum graecum* and *Cymbopogon citratus* showed a low antibacterial activity ((8mm<d<9mm), against four bacterial strains among five tested. No antimicrobial activity was obtained for *Dysphania ambrosioides* extracts.

This study proves, in part, that excepted *Dysphania ambrosioides* all the studied medicinal plants extracts have a good antibacterial activity, so they can establish a source of alternative antibiotics in the pharmaceutical industry. On the other hand, the species of these studied medicinal plants could be valuable due to their bioactive compounds. The results confirm also the ethnobotanical importance of the screened plants for the treatment of some bacterial infections.

Index Terms- *Rosmarinus officinalis*, *Salvia officinalis*, *Staphylococcus aureus*, antibacterial activity, antifungal activity

I. INTRODUCTION

Since Antiquity, the humanity used diverse resources of the environment to handle the diseases [1]. At present, medicine with plants is widely used[2]. Consequently, more than 120

compounds resulting from plants are used in modern medicine [3]. Also 5 in 15 % of vegetables inventoried in the world were the object of current researches on bioactive molecules, representing an immense reservoir of new potential medicinal compounds [4-6]. The use of antibiotics of synthesis and mainly the resistance of the pathogenic microorganisms became a public health problem in the world [7,8]. It is important to find new molecules having an antibacterial activity. Many aromatic and medicinal plants which have antimicrobial properties are known for several centuries [9]. It's in this context that our study was realized with an objective to evaluate the antimicrobial activity of some medicinal Moroccan's plants. Therefore, the extracts of seven medicinal plants (*Rosmarinus officinalis*, *Salvia officinalis*, *Psidium guajava*, *Crocus sativus*, *Dysphania ambrosioides*, *Trigonella foenum graecum* and *Cymbopogon citratus*), from different families were tested for their potential activity against bacteria.

II. MATERIALS AND METHODS

A. Medicinal plants:

The studied medicinal plants were collected in the region of El Jadida and one plant of the region of Taliouine (*Crocus sativus*), that is known in Morocco and in the world by the crop of a good quality of Saffron. This cultivated plants are free of pesticides. Medicinal plants, place of harvest and the part used in this study were grouped in the table I.

A. Preparation of the extract

After their collection, the fresh plant material was dried in the oven under a temperature of 50 °C to obtain the dry matter, which is grounded in the electric grinder Moulinex-type to obtain the dry powder. The dried and powdered plant materials (100 g) was macerated for 48 hours at room temperature in a mixture of two solvents, a polar solvent (Ethanol) and a non-polar solvent (Dichloromethane) with a proportion of 50%:50%. The mixture was after that filtered using Whatman filter paper then the filtrat was concentrated in vacuo at 40 °C using a Rotary evaporator until the total elimination of the solvent and the dried crude extract is obtained. The crude extracts of every plants were stored in a freezer at 4 °C until further tests.

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Table I: Name; parts and harvesting site of studied medicinal plants

Scientific name	Common name	Family	The used part	Harvesting site
<i>Psidium guajava</i>	Guava	Myrtaceae	Leaves , fruits	El Jadida
<i>Cymbopogon citratus</i>	Lemongrass	Poaceae	Leaves	El Jadida
<i>Dysphania ambrosioides</i>	Epazote	Chenopodiaceae	Leaves	El Jadida
<i>Trigonella foenum-graecum</i>	Fenugreek	Fabaceae	Leaves	El Jadida
<i>Salvia officinalis</i>	Sage	Lamiaceae	Leaves	El Jadida
<i>Crocus sativus</i>	Saffron crocus	Iridaceae	Wastes	Taliouine
<i>Rosmarinus officinalis</i>	Rosmary	Lamiaceae	Leaves	El Jadida

B. Test microorganisms

Five bacteria species obtained from the Institute Pasteur of Paris Collection (CIP) and from an American Type Culture Collection (ATCC) were used as antimicrobial strains: *Citrobacter freundii* ATCC8090, *Escherishia coli* CIP54127, *Pseudomonas* sp, *Enterococcus faecalis* ATCC19433, *Staphylococcus aureus* CIP 209 (ATCC 25923), *Candida tropicalis* R2 CIP1275.81, *Candida albicans* 48.72 and *Cryptococcus neoformans* CIP960. The bacterial strains were maintained on the Mueller-Hinton agar medium.

The choice of these bacteria is explained by their availability in our laboratory and by the high pathogenic power of these bacteria, since they are at the origin of several infections and diseases in humans, fish in aquaculture or in other animals such as chicken such as urinary tract, respiratory tract, meningitis and other infections [10-24].

C. Antibacterial assay

The antibacterial activity of the crude plant extracts was studied using the disc diffusion method [25]:

The inoculum of bacteria was prepared from colonies in phase of exponential growth from the culture from 18 to 24 hours old on the gelose of Mueller-Hinton (MH).

The cellular density was estimated by counting using a cell of Malassez, adjusted by dilution in sterile physiological water to obtain a final concentration of 106 UFC / ml.

The disks of cellulose of 6 mm as diameter, were saturated by 20 µl of extract solution to 25 mg/ml of solvent (25 mg of the extract dissolved in one ml of solvent), 500 µg of extract by disk. After drying at room temperature, the disks were deposited on Petri dishes containing the test culture medium already sowed with a target germ (MHA to 106 UFC / ml).

After two hours in 4°C, the Petri dishes were incubated in 37 °C during 24 at 72 hours.

The evaluation of the antibacterial and antifungal activity of every extracts were validated by the measure of the diameters of the zones of inhibition appearing around the disks, in comparison with the standard antibiotics (ampicilline 30 µg). Every test was realized in triplicate.

III. RESULTS AND DISCUSSION

A. RESULTS

• Antibacterial activity of the medicinal plants extracts against the tested bacteria

The results obtained in the antibacterial assay are shown in Table II. As it can be seen, excepted the leaf extract of *Dysphania ambrosioides* that showed no antimicrobial activity, all the extracts showed varying degrees of antibacterial activity against the Gram-positive and Gram-negative bacteria tested, with zone of inhibition ranging from 9 mm to 26 mm.

The screening of the antibacterial activity of the studied medicinal plants extract are presented in the table II.

Table II: *In vitro* screening of antibacterial activity of the medicinal plants extracts

The crude extracts	Diameters of the zones of inhibition (mm)				
	Gram-negative bacteria			Gram-positive bacteria	
	<i>Escherichia. coli</i>	<i>Pseudomonas sp</i>	<i>Citrobacter freundii</i>	<i>Enterococcus faecalis</i>	<i>Staphylococcus aureus</i>
<i>Salvia officinalis</i>	18±1,73	20±2,65	20±3,00	22±2,00	21±1,73
<i>Rosmarinus officinalis</i>	16,6±1,15	26±1,03	19,6±2,07	21±2,08	19±2,15
<i>Psidium guajava</i> (leaves)	8±1,00	11±2,00	8±1,00	11±1,73	11±2,65
<i>Trigonella foenum graecum</i>	9±1,00	9±1,73	-	8±0,00	8±1,00

<i>Crocus sativus</i>	9±2,00	9±0,00	-	8±2,00	8±0,00
<i>Psidium guajava</i> (fruits)	9±1,73	12±0,58	-	9±2,00	8±0,00
<i>Cymbopogon citratus</i>	8±1,73	9±1,00	-	9±0,00	-
<i>Dysphania ambrosioides</i>	-	-	-	-	-
Ampicilline30 µg	27	25	25	29	24

The extracts of *Salvia officinalis* and *Rosmarinus officinalis* showed an excellent antibacterial activity against all the germs bacterial tested, are presented by their zones of inhibition between 18 mm and 22 mm against *Escherishia coli* and *Enterococcus faecalis* respectively. The *Rosmarinus officinalis* extract showed a zone of inhibition between 16,6 mm and 26 mm, against *Escherishia coli* and *Pseudomonas sp* respectively, the values are very close to the standard antibiotic used (ampicilline30µg) which registered the zones of inhibition between 24 to 29 mm (Fig 1). With stronger antibacterial action of *Salvia officinalis* extract against Gram+ bacteria (d=21,5mm) compared to Gram- bacteria (d=19,33mm) which are therefore a little resistant to this extract, contrary to *Rosmarinus officinalis* extract, where the Gram- (d=20,73mm) bacteria are the most sensitive, the Gram+ bacteria are somewhat resistant (d=19mm).

The extract of *Psidium guajava* (leaves and fruits) was able to inhibit four bacterial strains among five tested, with a moderate antibacterial activity against *Pseudomonas. sp* (d=12mm), *Enterococcus faecalis* and *Staphylococcus aureus* (d=11 mm), a low antibacterial activity against *Escherichia coli* (d=9mm) and a none antibacterial activity against *Citrobacter freundii*.

The extracts of *Crocus sativus* and *Trigonella foenum-graecum* shows a low activity, presented by a zones of inhibition of 9mm, against all of the tested bacteria with the exception of *Citrobacter freundii* who present no antibacterial activity, followed by the extract of *Cymbopogon citratus* with a low antibacterial activity only against *Escherishia coli*, *Pseudomonas sp* and *Enterococcus faecalis*. Finally, The leaf extract of *Dysphania ambrosioides* did not show any antibacterial activity against any tested bacterium.

• Sensitivity of bacteria to the studied medicinal plant extracts according to their Gram

The sensitivity of bacteria to the studied medicinal plant extracts according to their Gram shows that Gram- bacteria are more resistant than Gram+ bacteria which are more sensitive to the most of studied medicinal plant extracts. Indeed for the extract of *Salvia officinalis*, *Psidium guajava*, *Trigonella foenum-graecum* and *Crocus sativus*, Gram-bacteria are more resistant than Gram+ bacteria. However, for the extract of *Rosmarinus officinalis* and *Cymbopogon citratus*, Gram+ bacteria are more resistant than Gram-bacteria (Fig1).

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B. DISCUSSION

Firstly, the antibacterial activity showed that the leaf extracts of *Rosmarinus officinalis* and *Salvia officinalis* have a strong antibacterial activity against all the tested bacteria (Fig.1).

As regarding to *Salvia officinalis* extract, these findings are in accordance with the results recorded in the investigation of many publications have documented the antibacterial activity of *S.officinalis* extracts against different bacterial species[26-34]. The aqueous extract of *S. officinalis* has already shown antibacterial activity against *Bacillus mycoides*, *Bacillus subtilis*, *Enterobacter cloacae*, *Escherichia coli*, and *Proteus sp* [35]. Similarly, The supercritical fluid sage extracts revealed excellent growth inhibition against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus*[36],the hydroalcoholic extract is actif against *S. aureus*, and *Klebsiella oxytoca* [37] and the ethanolic extract has already shown a strong bactericidal and bacteriostatic effects against both Gram-positive and Gram-negative bacteria [38]. This antibacterial activity of *S.officinalis* extracts is positively related to their phenolic composition [39] such as terpens and terpenoids compounds found in this plant[38] or their oleanolic acid, ursolic acids and their Carnosol and carnosic acid [36,40-42]. As reported rosmanol and ferruginol are also responsible for the biological activity of sage along with the phenolic rosmarinic and salvianolic acids [43]. But, the antibacterial activity of *S.officinalis* extracts can also be due to the presence of interaction between different volatile constituents [44].

About *Rosmarinus officinalis* known for these medicinal virtues around the world [45], according to the results obtained in the present work, Genena and al in 2008 was mentioned that *R. officinalis* leaf extracts obtained by SFE extraction a good antibacterial activity against Gram+ bacteria (*Staphylococcus aureus* and *Bacillus cereus*) and Gram- bacteria (*Escherichia coli*) [46], two years later, other studies mentioned that the extract of *R. officinalis* has antibacterial activity against clinical Methicillin-resistant *Staphylococcus aureus* and against *Listeria* species such as *Listeria grayi*, *Listeria innocua*, *Listeria ivanovii* and *Listeria monocytogene* [47-49]. In 2012, Rosemary leaf extracts showed a stronger antimicrobial activity against *Bacillus cereus*, *C. jejuni*, *S.infantis* [50]. Similar finding was proposed recently in 2015 against *S. epidermidis*, *B. bronchiseptica*, *G. stearothermophilis* [51], against *Enterococcus faecalis*, *Streptococcus mutans* and [52], in 2017 against *E. coli* and *S. aureus* bacteria [53] and in 2018 against *S. aureus*, *S. epidermidis*, *P. aeruginosa*, *B. cereus* [54]. This antibacterial activity of rosemary against all the the tested bacteria could be attributed to the presence of flavonoids, phenolic acids (caffeic, chlorogenic, carnosic and rosmarinic) [49,55,56] and other apolar phenolic compounds [57,58]. Moreever, carnosic acid is more efficient against gram-positive bacteria than rosmarinic acid [59], since, carnosic acid was the major bioactive compound of the

rosemary extract but its derivative and other compounds like carnosol, rosmarinic acid, etc. have also important antimicrobial activity[47].

There is also some evidence that minor components have a critical part in antibacterial activity, possibly by producing a synergistic effect between other components [54,60].

Secondary. Regarding the extracts of *Psidium guajava*, it should be noted that this is the first study on the antibacterial activity of this newly introduced species in Morocco, since it is native to South America, So the results of this study are very interesting. The result indicate that in spite of the lower antibacterial activity of *Psidium guajava* extracts (leaves extract and fruits extract), it remains considerable with a zone of inhibition between 8 en 12 mm. These results are very close to those found by Biswas in 2013, when the ethanol extract showed a zone of inhibition of 6.11 and 11 mm against *Staphylococcus aureus* and *Bacillus cereus*, respectively [61]. Indeed, several other studies confirm the antibacterial effect of *Psidium guajava* [62], the methanol and ethanol extracts showed inhibitory activity against the gram-negative bacteria known by the resistance such as *Acinetobacter baumannii*, *Proteus mirabilis*, *Salmonella* sp, *Shigella flexneri*, *E.coli*, *Pseudomonas Sp* [61,63-65], but also against Gram+ bacteria like *Staphylococcus aureus*, *Staphylococcus haemolyticus*, *Staphylococcus epidermidis* [65,66], and caused by the impermeability of the bacteria to any lipopolysaccharide membrane in the presence of active component of *Psidium guajava* that are the tannins that have an effect of limiting the multiplication of *Staphylococcus aureus* by inhibition phosphorylation activity of bacteria forming its cell wall which can cause multiplication of bacteria[66]. This antibacterial activity possibly due to protein degrading activity of the extract[67], can be associated with the existence of some bioactive ingredients such as; Saponins, Flavonoids, Tannins, Alkaloids, Phenols, and Phyto Sterols in both plants effective against several strains of pathogenic bacteria [64,68].

As regards the extract of *Crocus sativus*, *Trigonella foenum graecum citratus*, which showed an average antibacterial activity with a diameter of inhibition zone between 8 and 9mm against all the tested bacteria excepted *Citrobacter freundii*, which is therefore considered a resistant bacteria. Indeed, the results of *Trigonella foenum-graecum* leaves extract are in accordance with the previous studies which stipulate that the leaves methanol extract has an antibacterial activity against *Escherichia coli* (20 mm), *Pseudomonas aeruginosa* (9,2mm) [69] and against *Staphylococcus aureus* [70], and more than the result observed by Sharma *et al* for the aqueous extract [71]. This antibacterial activity is due to the fact that the leaves of *Trigonella foenum-graecum* are rich in a wide variety of secondary metabolites such as glycosides, alkaloids, phytosterols, proteins, saponins and phytosterols which have been found *in vitro* to have antimicrobial properties [69]. The results of *Crocus sativus* are very similar to those found by Muzaffar in 2016, of the extracts of *Crocus sativus* against *Proteus vulgaris*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*, with a zone of inhibition of 8mm, 6mm, 9mm, 7mm, and 6mm, respectively [72], and also by Okmen in 2016, when aqueous extract of *Crocus sativus* showed a maximum

inhibition zone against *Coagulase- Negative Staphylococci* (d=11 mm)[73]

The antibacterial activity of *Cymbopogon citratus* extract is somewhat lower, since it is active only against three bacteria (*Escherichia coli*, *Pseudomonas sp*, *Enterococcus faecalis*) and inactive against *Citrobacter freundii* and *Staphylococcus aureus*. Bearing, These results remain interesting, since the diameters of the inhibition zones obtained are very close to the other studies, Zulfa *et al* in 2016, found that *Cymbopogon citratus* extracts has an antimicrobial activity against the foodborne pathogens; *Bacillus cereus*, *Escherichia coli*, *K.pneumonia*, and *Staphylococcus aureus* [74], Moreover, Ewansiha and all also confirmed that the *Cymbopogon citratus* chloroform leaf extracts showed an intermediate antimicrobial activity against *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli*[75]. This antibacterial activity can be attributed to the richness of the species in active components like alkaloids, tannis, flavanoids, terpenes and phenolic compounds [76,77], caused membrane disruption or affected the genetic materials of bacteria [78].

Finally, the leaf extracts of *Dysphania ambrosioides* did not show any antibacterial activity against targets bacterial studied, as it was already demonstrated for *Dysphania ambrosioides* collected from Meknés city (Morocco) with no antibacterial effect [79] in contrary to Brazilian[80] or Pakisani [81] *Dysphania ambrosioides* which showed good antibacterial activity. These difference can be related to the geographical localization and environmental factors connected, who are one of the major determinants of plant secondary metabolites [82,83] responsible of the plant antimicrobial activity. Despite this, it should be noted that the absence of inhibition zone does not necessarily mean that compounds are inactive. For example, non-polar compounds may not diffuse into the culture medium [55].

In the other hand, the sensitivity of bacteria to the studied medicinal plant extracts according to their Gram, showed that Gram negative bacteria are more resistant compared to Gram positive bacteria. This finding are in agreement with previous data demonstrating greater activity of the plant extracts towards Gram+ bacteria compared to Gram-bacteria, which can be explained by the fact that Gram-bacteria are more resistant than Gram+ bacteria [51,84-94].

One of the most plausible reasons behind such observation, as also mentioned by others, is the different nature of cell wall among Gram+ and Gram- bacteria[92]. Moreover, efflux pump system of Gram-bacteria may mediate for such difference, by the outer membrane surrounding the cell wall in gram-bacteria and the periplasmic space containing enzymes which are capable of breaking down foreign molecules introduced from outside [95-98].

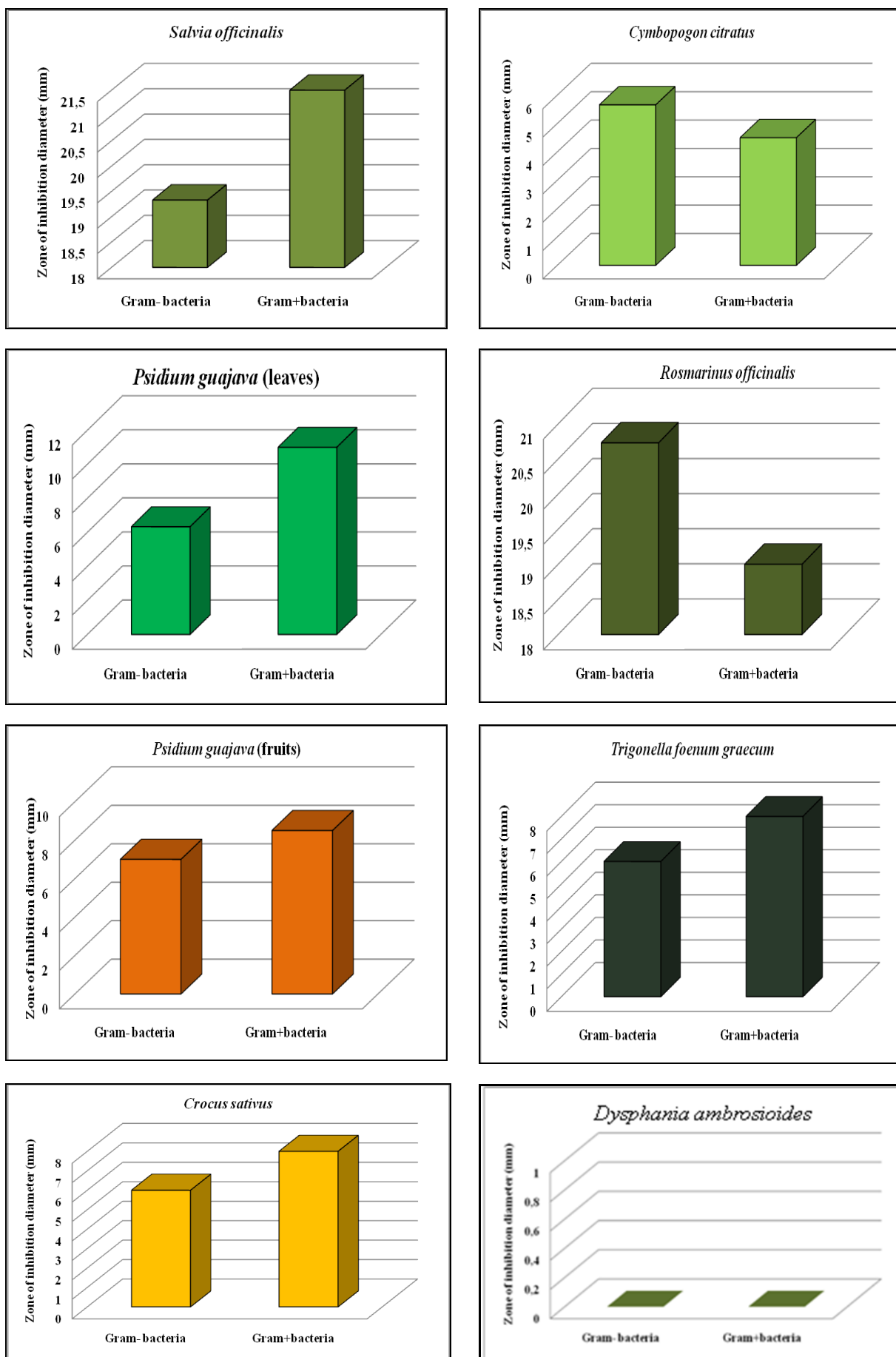


Fig.2: Sensitivity of bacteria to the studied medicinal plant extracts according to their Gram

IV. CONCLUSION

This study proves, in part, that excepted *Dysphania ambrosioides* all the studied medicinal plants extracts have a good antibacterial activity, so they can establish a source of alternative to antibiotics in the pharmaceutical industry, on the other hand, the species of the studied medicinal plants could be valuable due to their bioactive compounds.

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CONFLIT OF INTEREST

The authors declare no conflict of interest.

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