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Screening of antioxidant activity and the total polyphenolic contents of six medicinal Moroccan's plants extracts

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- ✓ *Salvia officinalis.*

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Abstract
The Moroccan medicinal plants *Crocus sativus, Psidium guajava, Rosmarinus officinalis, Salvia officinalis, Trigonella foenum-graecum, Cymbopogon citratus, and Dysphania ambrosioides* were selected to evaluate their chemical's activity. The total polyphenolic contents and the antioxidant activity were assessed using the Folin-Ciocalteu method and 2, 2-di- phenyl-1-picrylhydrazyl (DPPH). The results showed that the highest antioxidant activity was found in the plant *Crocus sativus, Psidium guajava, Rosmarinus officinalis, Salvia officinalis, and Cymbopogon citratus.* However, *Dysphania ambroisioides* and the *Trigonella foenum-graecum* extracts did not showed a strong free radical scavenging activity. *Rosmarinus officinalis*, wastes of *Crocus sativus, Dysphania ambrosioides, Psidium guajava*'s extracts are rich in polyphenols that may have other activities than antioxidant activity. However, due to the diversity and complexity of the natural mixtures of phenolic compounds in these plant extracts, it is not easy to characterize every compound and assess its antioxidant activities. Each herb contains generally different phenolic and non-phenolic antioxidants. The results suggest that these plants may constitute an alternative of certain synthetic additives in food-processing, pharmaceutical, cosmetic industry and agri-food industry on large scale.

1. Introduction
The medicinal plants are a major interest for medicine and for the scientific experiment, which took a tremendous increase. Several natural substances with therapeutic virtues were isolated from plants.
The necessary substances for the creation of new medicines were always obtained from the medicinal plants by the research teams [1]. However, concerning the number of superior plant species on the planet (angiosperms and gymnosperms) estimated at 250000[2], the potential of plants as sources for the production of a new-medicine remains widely unexploited [3]. Indeed, only 6 % of the total number of species were tested for their biological activity, and 15 % were estimated on the phytochemical plan [4]. Furthermore, we are more confronted with the resurgence of the new diseases, as those which caused by oxidative stress. However, the antioxidants of synthesis can cause toxicologic problems, that's why the research for new natural antioxidants from vegetable sources was developed [5].
Among the compounds characterized by the best antioxidant activity, there are the polyphenolic compounds. Indeed, their role of natural antioxidants arouses more and more interest in cancer prevention and treatment, inflammatory and cardiovascular diseases [6].
The antioxidant activity corresponds to the ability of the compound or product to scavenge free radicals [7]. The most known antioxidants are ß-carotene (provitamin A), the ascorbic acid (vitamin C), the tocopherol (vitamin E) and the polyphenolic compounds [8].
The choice of our investigated plants is based on two criteria: first, in this domain there is no study in Morocco that deals with these plants, and the second criterion is that these plants have several biological and chemical

activities (Table1) due to their active components, that allow them to be used in traditional medicine in the treatment of some diseases.

Table 1: Some chemical and biological activities of the studied Moroccan medicinal plants

Plant name	**Some chemical and biological activities**
Psidium guajava	Antimicrobial, anti-cancer, astringent, anti-nociceptive, antioxidant, spasmolytic, anti-diarrheal[9-14].
Cymbopogon citratus	Antispasmodic, antifungal, antimicrobial and insecticidal, anti-nociceptive, acaricidal effects, anti-cancer, antioxidant, anti-inflammatory, anti-fungal, anti-bacterial, hypocholesterolemic, gastro-protective, anti-bacterial ulcer, co-digestion of waste, phytotoxic, neurobiological, biosorption activity, insecticide [15-21]
Dysphania ambrosioides	Anti-ulcers, vermifuge, galactogen, antitumoral, antifungal, insecticide, anthelmintic vermifuge, insecticides, molluscicide, allelochemics, anti-parasite [22-27].
Trigonella foenum-graecum	Antidiabetic, hypoglycemic, antifungal, antioxidant, cytoprotective, antiulcer, immune-modulator, antidiabetic, hypertriglyceridemi, carminative, toning, antidiabetic, antiulcer, hypoglycemic, chemoprophagus, anti-inflammatory, antipyretic, low cholesterol, anti-parasitic, galactogen, anti-fatigue, stimulates uterine contractions [28-37].
Salvia officinalis	Antioxidant, anti-hyperglycemic, anti-hyperlipid, antimicrobial, anti-cancer, antiviral, anti-genotoxic, anti-inflammatory, acaricide, gastroprotective, melanogenic, adsorptive-floculative [38-49].
Crocus sativus	Antioxidant, antimicrobial, antinociceptive, anti-inflammatory, neurostimulant, antihypertensive, antiepileptic, antitussive, antigenotoxic, cytotoxic, aphrodisiac, anxiolytic, relaxant, antidepressant, anticarcinogene, antinociceptive, anti-atherosclerosis, anti-hyperlipidemia [50-73].
Rosmarinus officinalis	Antibacterial, antifungal, anti-inflammatory, antidepressant, anti-ulcer, antioxidant, anti-cancer activity, immunoregulator, diuretic, antispasmodic, emmenagogue, neurostimulant [43,74-107].

The purpose of this study was to evaluate the total phenolic content and the antioxidant activity of seven Moroccan medicinal plants and to find out new potential sources of natural antioxidants.

2. Material and Methods

2.1. Plant material

The plants used in this study were collected in January 2019 in El Jadida city , in the region of Casablanca-Settat located in the northern part of Morocco, except *Crocus sativus* which was imported from Taliouine, a town in Taroudant Province, Souss-Massa region, located in the southern part of Morocco (Figure 1). This city is known for saffron production and is the main exporters of this spice worldwide.

The botanical characteristics, used part, and the harvesting site of the studied Moroccan medicinal plants, are presented in the Table 2.

2.2. Preparation of the extracts

Each plant was fragmented, placed in a drying oven at 47°C, and macerated in two solvent ethanol/dichloromethane (50/50), with a soft agitation during 24 h. The dichloro-ethanoïque extract of every plant, was recovered after a filtration of the mixture by a filter paper. the ethanol/dichloromethane was evaporated at reduced pressure. The crude extract is obtained as a solid product.

2.3. Determination of polyphenols contents

The principle of this dosage is adapted by Singleton and Rossi (in 1965) with the reactive of Folin-Ciocalteu [110]. Briefly, 2.5 mL of Folin-Ciocalteu reagent (diluted10 times) was added to 0.5 mL of aqueous extract (diluted 200times). Sodium carbonate Na2CO3 (75g/L) was added (what favours an alkaline environment to activate the redox reaction). the obtained mixture was incubated in a water bath at a temperature of 50°C during 5 min.Then, the absorbance was measured at 760 nm by a spectrophotometer UV-3100 PC VWR

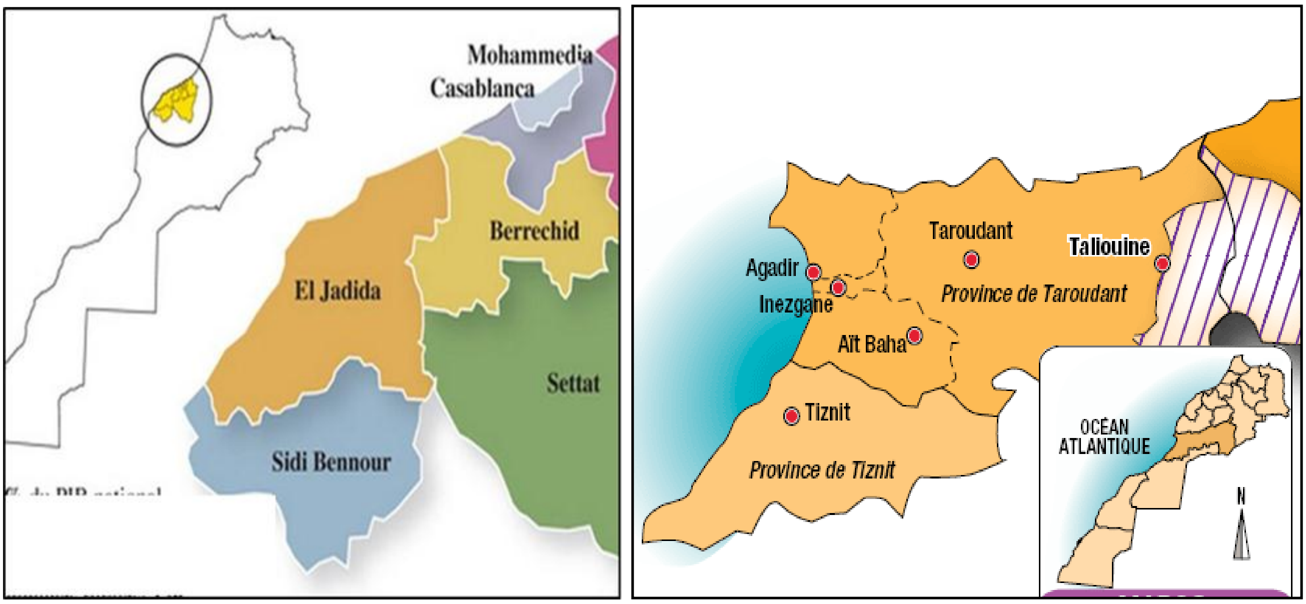

Figure 1 : El Jadida and Taliouine cities locations [108,109].

Table 2: Botanical characteristics of the studied Moroccan medicinal plants.

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

The total polyphenols content was calculated from the calibration curve established with a solution of gallic acid (calibration range 0- 80µg/ml)(Figure 1). The negative control of the reaction was a polyphenol content free [111]. To assure the performance of experiments, every dosage was done in triplicates.
The results were expressed by milligram of gallic acid equivalent (GAE) per gram of dry weight (mg GAE/gdw).

2.4. Determination of the antioxidant activity

The antioxidant activity of the plant extracts was determined by a DPPH (diphenyl-1-picrylhydrazyl) assay. The free radical scavenging capacities of samples were evaluated by IC_{50} values (concentration of samples required to scavenge 50% of DPPH$^{\cdot}$ radicals). The percentage of DPPH inhibition was calculated using the following formula:

$$I\% = [(Ac – As)/Ac] \times 100$$

I%: percentage of DPPH inhibition
Ac: the negative control's absorbance;
As: the sample's absorbance tested [112].
The standard of the reaction is the Butylatedhydroxytoluene (BHT).
All the tests were made in triplicates and the results were expressed as a mean of the three assays.
Briefly, the ethanolic solution of extract was prepared at different concentrations from 0 to 5000 µg/ml. DPPH$^{\bullet}$ (0.04g/l) was added to 0.5 ml of each solution. Also, the negative control was prepared by adding 0.5 ml of methanol to 1.5 ml of the DPPH methanolic solution. Discolorations were measured by the spectrophotometer at

517 nm after incubation of the mixture for 30 min at room temperature in the dark. The absorbance of the positive control (BHT) was measured in the same conditions as well as the extracts. The percentage of DPPH inhibition (I%) was calculated and the IC50 values for all the samples were determined by «Origin®Pro8» software.

2.4. Correlation between antioxidant activities and polyphenolic contents.

The correlation between the antioxidant activities and phenolic contents in different medicinal plant extracts was assessed as reported previously [113].

2.4. Statistical analysis

The results were expressed as mean ± standard error (mean ± SD) of three measurements. The correlation between polyphenols contents and antioxidant activity was established by regression analysis.

3. Results and discussion

3.1. The polyphenols content

The polyphenols content was calculated according to the reference Gallic acid after plotting calibration curves (Figure 1). The polyphenols content of different plants extracts, presented on mg GAE/g dry weight or mg GAE/gdw, are given in (Figure 2).

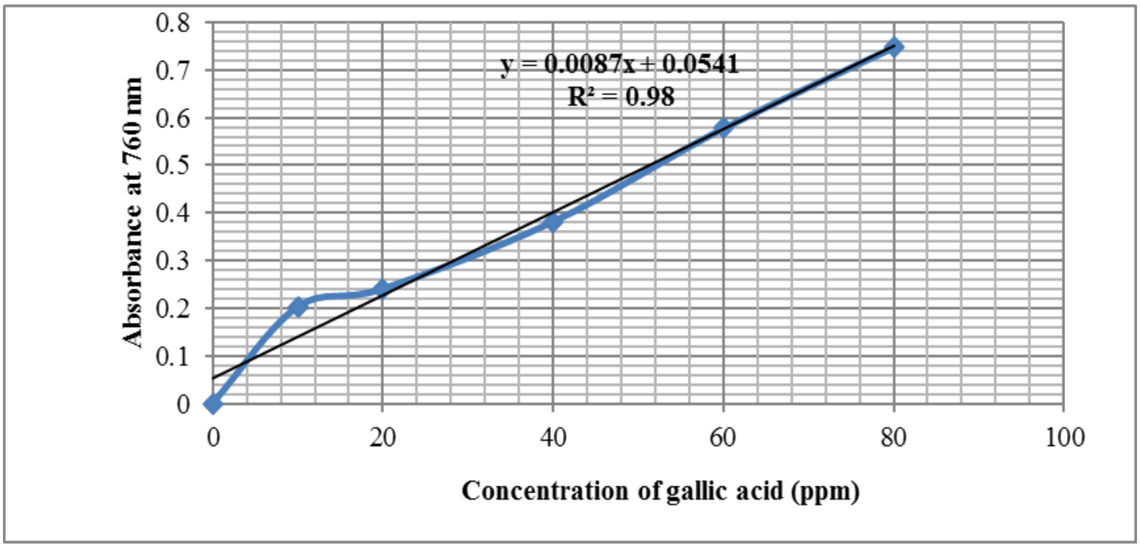

Figure 2: The calibration curves of gallic acid

The Folin–Ciocalteu procedure has been proposed as a means to rapidly estimate the level of total phenolics in foods and supplements [114]. As can be seen in the figure 2, the contents of polyphenols of the studied plants were in range of 0.82 ± 0.12 mg GAE/gdw (*Cymbopogon citratus)* to 47.73 ± 2.21 mg GAE/gdw (*Rosmarinus officinalis*). It is well-known that a wide variation was observed on total phenolic content in different aromatic and medicinal plants were 6.80 to 32.10 mg GAE/gdw [115].

Three of these plants show the highest levels of polyphenolic compounds (Figure 3), which are the extracts of *Rosmarinus officinalis*, wastes of *Crocus sativus* and *Dysphania ambrosioides* with a polyphenolic content of 47.73 ± 2.21 mg GAE/gdw, 35.58 ± 2.76 mg GAE/gdw and 29.47 ± 1.94 mg GAE/gdw respectively. Then, the extract of *Psidium guajava*'s fruits that show a medium polyphenol content in the range of 18.09 ± 3.41 mg GAE/gdw. Finally, the remaining extracts which show the weak polyphenolic contents and which are *Salvia officinalis*, *Psidium guajava's* leaves, *Trigonella foenum-graecum* and *Cymbopogon citratus,* with polyphenol contents of 6.07 ± 1.55 mg GAE/gdw, 2.52 ± 1.12 mg GAE/gdw, 2.16 ± 0.67 mg GAE/gdw and 0.82 ± 0.12 mg GAE/gdw, respectively.

The mean value of total phenolic compounds in dichloromethane/ethanol extract of *Rosmarinus officinalis* was 47.73 ± 2.21 mg GAE/gdw, these results seem in line with previous data [116,117]. However, Nadia *et al.* found a lower value of 10.42 mg GAE/g for ethanol/water rosemary extract [118]. Furthermore, Erkan *et al.* obtained a higher value of 162 mg GAE/g for the methanol extract of rosmary [119]. This difference can be attributed to differences in the conditions of extraction, the season of the harvest and the bioclimatic area [117]. The total phenolic compounds in the extracts of *Crocus sativus's* wastes were found to be 35.58 ± 2.76 mg

GAE/gdw. According to the literature reports, Sengul *et al. reported* a very close value (42.29 mg GAE/g dw) for the methanolic extract of aerial parts of *crocus sativus* [120]. Recently, similar results were reported for the petals ethanol:water extract with values of 48 mg GAE/gdw [121], respectively. Alltoghether, this data highlighted the richness of this species with phenolic compound [122].

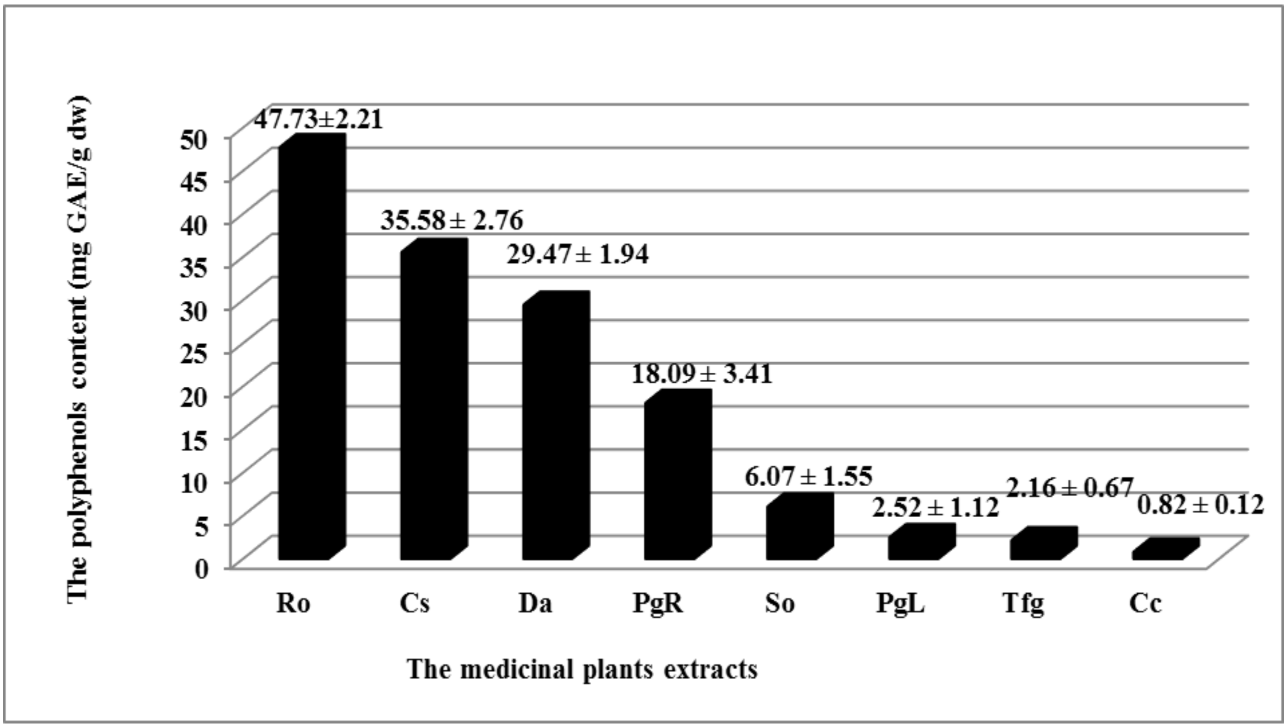

Figure 3: The polyphenols content of the medicinal plants extracts (mg GAE/gdw)
(**PgL:** *Psidium guajava'* sleaves, **Ro:** *Rosmarinu sofficinalis* **So:** *Salvia officinalis*, **Cc:***Cymbopogon citratus*, **PgR:***Psidium guajava's* fruits, **Cs:** *Crocus sativus,* **Da**: *Dysphania ambrosioides,* **Tfg:***Trigonella foenum-graecum)*

Regarding the extract of *Dysphania ambrosioides*, the total phenol content was found to be 29.47 ± 1.94 mg GAE/gdw. Our results are in agreement with the previous research that reported the highest content of polyphenols of the plants from *Chenopodiaceae*, especially of flavonoids [123]. Previous result has shown a content of polyphenols of 59.45 mg GAE/gdw for the aqueuse extract of *Dysphania ambrosioides* [124]. In other hand, Zohra *et al.* has reported that the methanolic extract contains 87.69 ± 1.41 mg GAE/gdw of polyphenolics content in dry plant material [125].

For, the extract of *Psidium guajava*'s fruits which has a polyphenols content of 18.09±3.41 mg GAE/gdw), this value remains significant compared to the values obtained by other studies, which recorded lower values of 0.6 mg GAE /gdw and 10.81±0.16 mg GAE/gdw [126,127], respectively.

Concerning the extracts that have shown a low polyphenolic content. The*Salvia officinalis*extractthat contains6.07 ± 1.55 mg GAE/gdw is very close to that obtained by Roby and colleagues of 5.95 mg GAE/g dw [128].

The *Psidium guajava* leaves extracts showed a low polyphenolic content not exceeding 2.52 ± 1.12 mgGAE/gdw, as reported previously [129].

Trigonella foenum-graecum extract showed a polyphenolic content of 2.16 ± 0.67 mg GAE/gdw which is corroborating with previous result with a value of 3.42 ± 0.123 mg GAE/gdw for the aqueuse extract [130].

Cymbopogon citratus extract revealed a polyphenolic content of 0.82 ± 0.12 mg GAE/gdw, similar to the result obtained by Yoo *et al.* who concluded that the lemon grass belonged to the low polyphenolic group [131].

3.2. DPPH· scavenging activity

The DPPH (1,1-Diphenyl-2-picrylhydrazyl radical) and ABTS [2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid diammonium salt)] assays have been widely used to determine the free radical-scavenging activity of various plants. The DPPH IC50 values (IC50 value is the concentration of the sample required to inhibit 50% of radical) are calculated from the curves that show the variation in inhibition (%) as a function of the concentration of the extract (Figure 4).

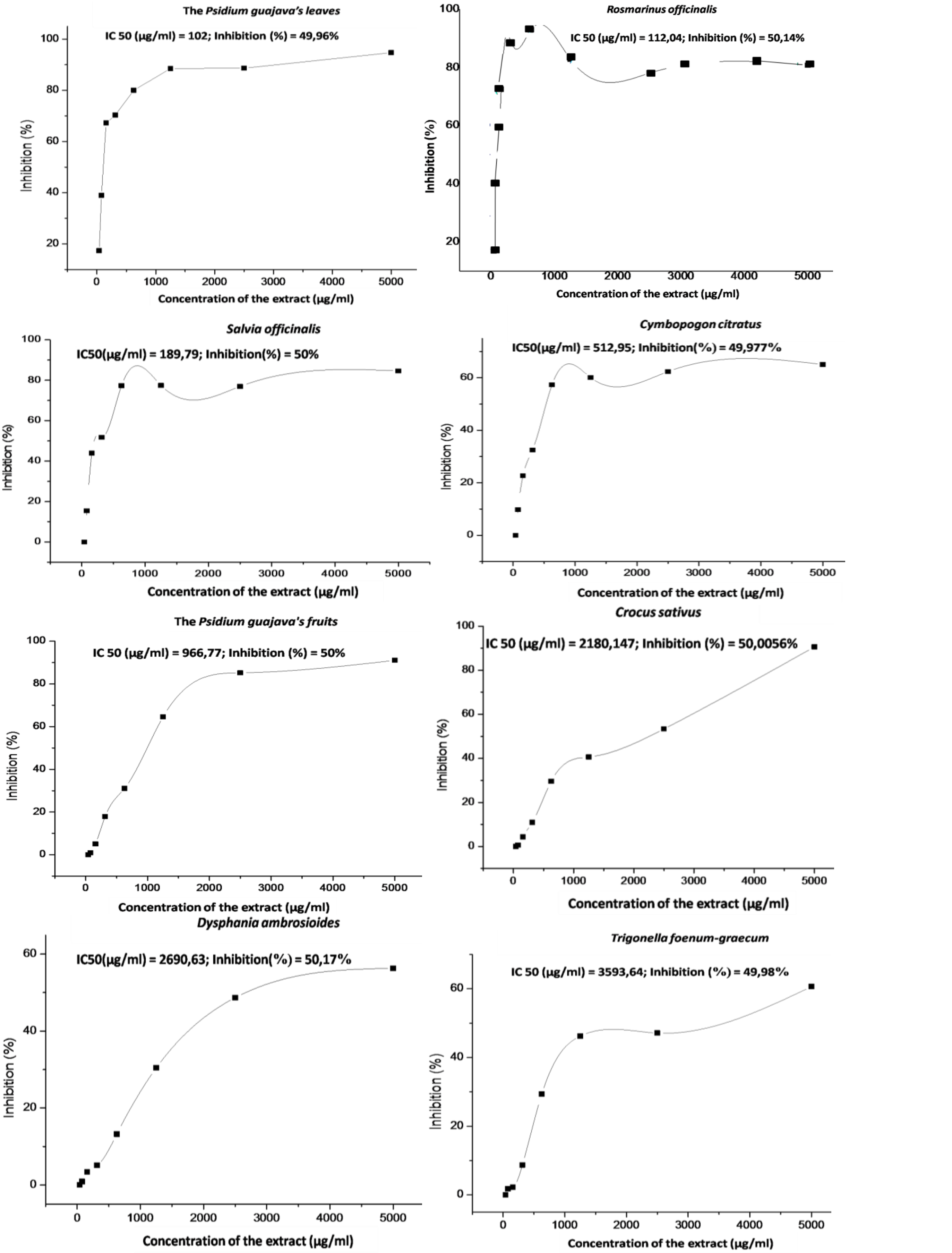

Figure 4. Effect of different concentration of the studied medicinal plants extracts in free radical scavenging tests (DPPH)

The IC_{50} values of each extract are presented in Figure 5. The following hierarchy of antioxidant activitieswas found: *Psidium guajava* leaves ≫ *Rosmarinus officinalis* ≫ *Salvia officinali s*≫ *Cymbopogon citratus* ≫ *Psidium guajava* fruits ≫ *Crocus sativus* ≫ *Dysphania ambrosioides* ≫ *Trigonella foenum-graecum.*

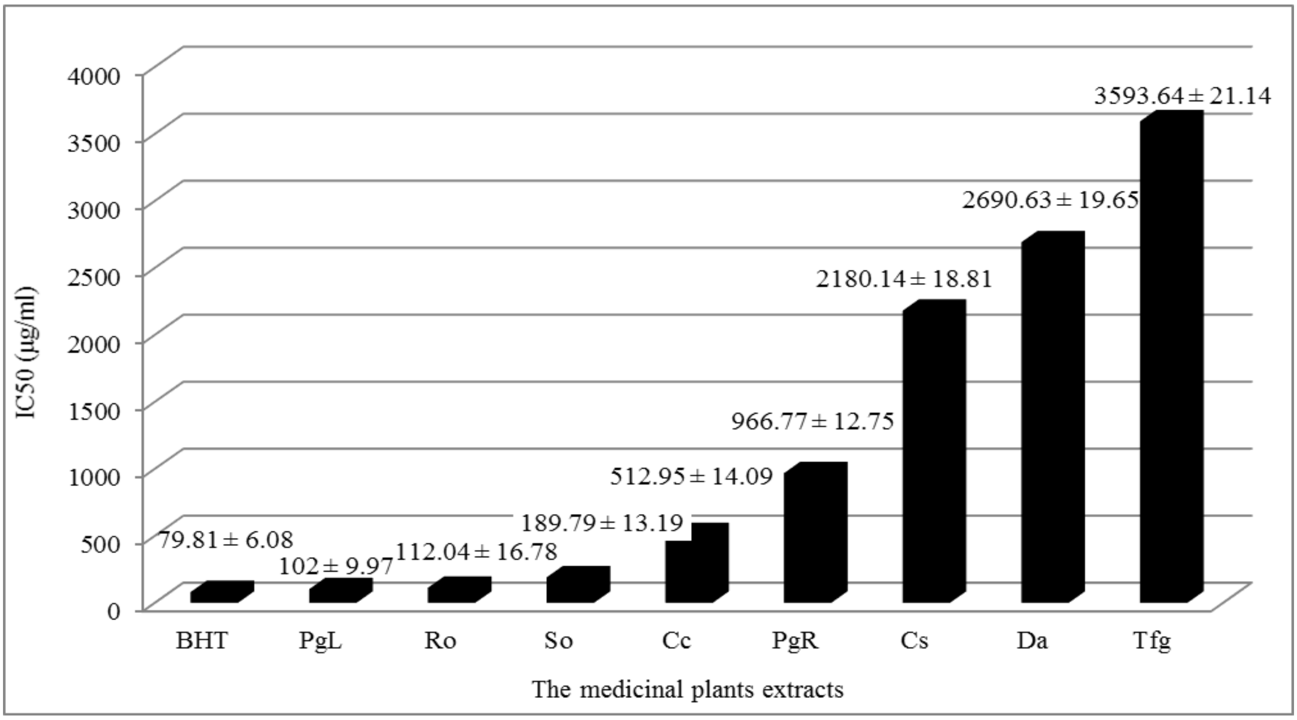

Figure 5: The IC50 values of the medicinal plant extracts (µg/ml)
(**PgL:** *Psidium guajava's* leaves, **Ro:** *Rosmarinus officinalis* **So:** *Salvia officinalis,* **Cc:***Cymbopogon citratus,* **PgR:***Psidium guajava's* fruits, **Cs:** *Crocus sativus,* **Da**: *Dysphania ambrosioides,* **Tfg:***Trigonella foenum-graecum)*

Concerning the *Psidium guajava*, it is important to note that this is the first time that the antioxidant activity of this species, recently introduced in Morocco, has been assessed, since it is native to South America. Indeed, the leaves extract of *Psidium guajava* showed very high antioxidant activity compared to the other studied plants, C_{50}value of 102 ±9.97 µg/ml which is very similar to that found by Cedric *et al*.(IC_{50}= 102.831 µg/ml) [1]. This high antioxidant activity may be due to the blocking of chain reaction by the linoleic acid[132] or by a free radical scavenging activity of quercetin, quercetin-3-o-glucopyranoside, morin [14] and ferulic acid [133], as well as other phenolic compounds such as flavonoids, phenolic acids and carotenoids. However, the effectiveness of flavonoids as effective antioxidants is dependent upon the environment [133]. A number of factors may influence the behaviour of flavonoids and may result in alterations to their efficacy as antioxidants [133,134]. In contrast, the fruit extract of *Psidium guajava* recorded C_{50}value of 966.77 µg/ml showing an average antioxidant activity, close to the results of Ademiluyi*et al*.(C_{50}= 920 µg/ml) which is interpreted as a high antioxidant activity due to the richness of this fruit in phenolic compounds[135]. The similar result is obtained for other species such as *Coccinia grandis* (C_{50}=910 µg/ml) also considered to be a good source for antioxidant compounds [126].

The *Rosmarinu sofficinalis* leaf extract, had a very low IC_{50} value of 112.04 ± 16.78 µg/ml similar to IC_{50} value found by Bianchin*et al*. (127.33 ± 0.04 µg/ml) [116] and Fadili*et al.* with IC_{50}value of 109.98 µg/mL [136]. These results showed that *Rosmarinus officinalis* leaf extracts contains compounds which are responsible for scavenging free radicals, such as carnosic acid, carnolsol and rosmarinic acid [137,138] with high antioxidant activity other than phenolic compounds like, cyclic diterpenediphenols, epirosmanol, rosmanol, isorosmanol, and caffeic acid derivatives [74,139].

For the extract of the leaves of *Salvia officinalis*, the IC_{50}value was also very low (IC_{50}=189.79 µg/ml), and close to that found by Jeshvaghani *et al* for the methanolic extract (IC_{50}=233 µg/mL) [11] and much lower than the Serbian *S. officinalis* (IC_{50}=16.93 mg/ml) [140].

Moroccan *S. officinalis* extract showing a very high DPPH radical scavenging activity may related to the presence of the phenol carbonic acid, rosmarinic, carnosic, salvianolic, caffeic, chlorogenic and ferulic acid and its derivates or antioxidant enzymes[141-143]. Indeed, rosemary and sage extracts can be used as antioxidants for food preservation [144]. For *Cymbopogon citratus* leaves extract, the IC_{50} value is slightly higher (IC_{50} =512.95 µg/ml)with other previous results with a considerable antioxidant properties due to polyphenols compound as carnosic acid, *p*-coumaric acid, cinnamiac acid, quercetin, rutin, and chlorogenic acid [145] and other compounds such as alkaloids, saponins, tannins, anthraquinones and steroids [146]. These phytochemical constituents contribute directly to antioxidant activity [147].

For the *Crocus sativus* petals extract, even that with high IC_{50} value compared with the previous extracts in this work (IC_{50}=2180.14 µg/ml), but the DPPH radical scavenging activity obtained from this value was higher than the value reported by Tamegart *et al.* which found an IC_{50}=5300µg/ml and concluding that the extract has very high antioxidant activity [148]. This antioxidant capacity of the *Crocus sativus* petals extract can be explained by the presence of strong pigment compounds such as cyanidins, known as one of the important compounds that could react as antioxidant [149] and the intensity of color in the flowers could play an important role in their antioxidant activity [150]. The petals are also rich in flavonoids and especially kaempferol aglycone, glycosides and esters, compounds known for their antioxidant capacity [151].

The extract of *Dysphania ambroisioides* showed a high IC_{50} value of 2690.63 µg/ml, with very low antioxidant activity, contrary to several previous works which have concluded that this plant is endowed with a considerable antioxidant power [125,152,153].

Trigonella foenum-graecum extract showed an IC_{50} value of 3593.64 µg/mL that indicates a very low antioxidant activity, consistent with results obtained by Singh *et al.* [154] and by Kenny *et al.* who concluded that the very low antioxidant activity of this species can be explained by the fact that complex carotenoids and tannins are not abundant in fenugreek [130].

3.3. Correlation between antioxidant activity and polyphenols content

The correlation between antioxidant activities and total polyphenolic contents of the extracts of the medicinal Moroccan plants had a low correlation coefficient (R^2=0.003, (Y=0.013X+3,267)) (Figure 6). This result suggests that only 0.3% of the antioxidant capacity of the extracts of the medicinal Moroccan plants due to phenolic compounds. Therefore, the results showed that antioxidant properties of plant extracts couldn't always be attributed to their phenolic content.

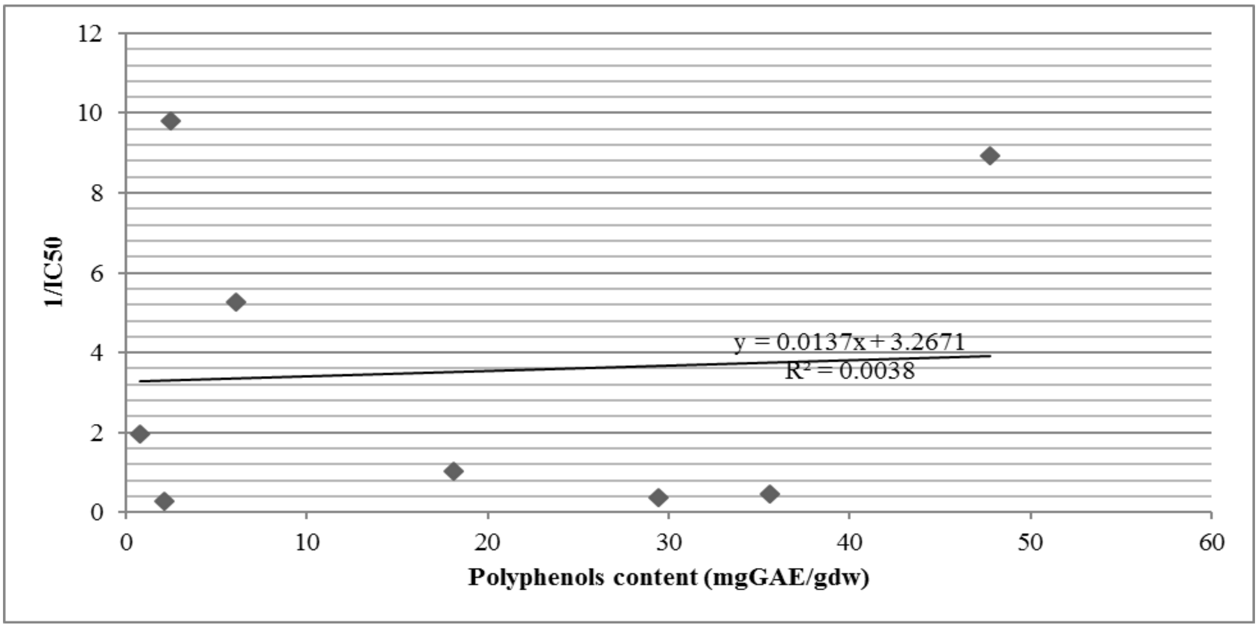

Figure 6: Correlation graphs of DPPH (1 /IC50 values) and total polyphenolic contents in the different medicinal plant extracts

Several previous pieces of research has shown that there is no correlation between the antioxidant activity and total polyphenolic [155-159]. Indeed, several reasons to explain the ambiguous relationship between the antioxidant activity and total phenolics. Firstly, total phenolics content did not include all the antioxidants, such as ascorbic acid, carotenoid and tocopherol [160]. In addition, the synergism between the antioxidants in the mixture makes the antioxidant activity not only dependent on the concentration, but also on the structure and the interaction between the antioxidants [161]. Finally, different methods to measure antioxidant activity with various mechanisms may lead to different observations. However, the polarity of the compound and the physical state of the lipid system also affected the behaviour of the antioxidants [160]. Moreover, the no correlations confirm that phenolic compounds are not the only contributor to the antioxidant activities of the medicinal plant extracts, these plants may contain several other non-phenolic antioxidants [162] such as nitrogen compounds, carotenoids, ascorbic acid, vitamin E and β-carotene might also be accountable in enhancing the antioxidant activity of the plant extracts [163,164]. Sengul *et al.* explained this lack of correlation by the presence of some other phytochemicals such as tocopherol and pigments as well as the synergistic effects among them [120]. On the other hand, total phenolic content determined according to the Folin- Ciocalteu method is not an absolute measurement of the number of phenolic materials. Different types of phenolic compounds have different antioxidant activities, which are dependent on their structure. The extracts possibly contain different type of phenolic compounds, which have different antioxidant capacities [120]. Moreover, the flavonoids, luteolin and apigenin glycosides, possessed a weak to moderate antioxydant activities [165].

Conclusion

In general, our results indicate that all studied medicinal plants in these work have an antioxidant activity and could be helpful in protecting the DNA against free radicals [166], with the exception of *Dysphania ambroisioides* and *Trigonella foenum-graecum* preventing various human oxidative stress and related diseases[167] such as hepatic oxidative stress, fibrosis, cirrhosis and hepatocellular carcinoma [168], and are a reliable and a safe alternative to synthetic antioxidants causing side effects and complications to human health. Moreover, their antioxydant activity seems to be attributed not only to phenolic compounds but also to other plant metabolites. Morever, *Rosmarinus officinalis*, wastes of *Crocus sativus*, *Dysphania ambrosioides*, *Psidium guajava*'s fruits extracts are rich in polyphenols that may have others activities than antioxidant activity. However, due to the diversity and complexity of the natural mixtures of phenolic compounds in these plant extracts, it is not easy to characterize every compound and assess its antioxidant activities. Each herb contains generally different phenolic and non-phenolic antioxidants. Upon this study, we can state that *in vitro and in vivo* studies are warranted to further confirm the advantageous quality of these extracts.

These results also point out the benefit of the use in the traditional medicine and could constitutes an alternative of certain synthetic additives in food-processing, pharmaceutical and cosmetic industry.

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Conflict of interest : The authors declare no conflict of interest.

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