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Evaluation of the Antioxidant and Anti-Inflammatory Effect of *Moringa Stenopetala* from the Algerian Sahara

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KEYWORDS

Moringa stenopetala;

Extracts;

Antioxidant effect;

Anti-inflammatory effect;

Polyphenols

ABSTRACT:

Introduction: *Moringa stenopetela*, an indigenous plant to the arid regions of Africa, exhibits nutritional and medicinal properties.

Objectives: This study aimed to evaluate the antioxidant and anti-inflammatory potential of *M. stenopetala* sourced from the Algerian Sahara.

Methods: The methanolic and aqueous extracts were obtained by maceration, and then subjected to phytochemical investigations to quantify the total polyphenols content. The in vitro antioxidant effect of extracts was evaluated by 2, 2-Diphenyl-1-picrylhydrazyl (DPPH). The *in vivo* anti-inflammatory effect was investigated by evaluating the paw edema development induced by carrageenan injection.

Results: The results of the quantitative analysis showed the richness of the extracts with total phenols and flavonoids. The latter were respectively very abundant in the methanolic extract of the leaves with contents of the order of 101.081 mg GAE/g DM and 73.826 mg QE/g DM. Moreover, all extracts showed interesting antioxidant potential, whose methanolic root bark and Methanolic leaf extract were the most effective with an inhibitory concentration of 50% of the order of 0.159± 0.014 and 0.169 ± 0.004 mg/ml respectively. Additionally, these extracts exhibited marked anti-inflammatory effects, with varying inhibition levels across extract types. After 5h, we recorded the percentages of edema inhibition of 44.27%, 47%, 69.38% and 55.64% for stems, roots, seeds and leaves aqueous extract, respectively.

Conclusions: This study suggests that *M. stenopetala* can be a source of natural antioxidant and anti-inflammatory agents, which can replace synthetic with harmful side effects.

1. Introduction

Medicinal plants have long been used in traditional medicine for their therapeutic properties, particularly in treating diseases associated with oxidative stress and inflammation which negatively impact human health [1, 2]).

Moringa stenopetela is a member of the Moringa genus (Moringaceae family), which includes 13 species indigenous to Africa and Asia [3].

M. stenopetala is rich in essential nutrients, including calcium, vitamins, proteins, and iron, making it a valuable food source. Additionally, it contains a variety of bioactive molecules such as polyphenols, flavonoids, alkaloids, and essential oils, which contribute to its therapeutic properties [4].

All the parts of this species are used for various purposes. For example, the leaves and fruits are consumed as vegetables because they are rich in proteins of high nutritional value [5]). The pods are often used as animal fodder [6]).The seeds are used to purify turbid water [7]).Various parts of the plant have been used traditionally to treat diseases such as diabetes, malaria, hypertension and digestive disorders [8].

Previous studies have highlighted several biological activities of *M. stenopetala* extracts obtained from different organs. Aqueous leaf extracts have a hypotensive effect tested on experimental animals [9].The ethanolic root extract has shown antitrypanosomal activity [10]. Extracts obtained from various plant parts have represented antimicrobial properties [11]. The seed powder was found to have an effective power to eliminate toxic heavy metals [12].

2. Objectives

Secondary metabolites are biomolecules derived from plants which play a key role in therapy, especially in the discovery of future drugs without any harmful side effects. So, in the present study, we aimed to quantify total phenols and flavonoids and screened for the antioxidant and anti-inflammatory effects of *Moringa stenopetela* species harvested from Algerian Sahara using two solvents (water and methanol) and various organs of the plant.

3. Methods

Plant material and extract preparation

The whole plant (roots, stems, leaves and seeds) of the species *Moringa stenopetala* (Figure 1) was harvested in April 2019 from El Kantara region, Wilaya of Biskra (semi-arid climate), Algeria (Latitude: 35° 13′ 00″N; longitude: 5° 42′ 37″E) (Figure 2). Before analysis, the different plant organs were dried at room temperature (25°C) for two weeks, then, they were powdered and stored in glass flasks.

Aqueous and Methanolic extracts are prepared by maceration of fifteen grams (15g) of each plant part powder in 150 mL of distilled water and methanol, respectively. After 24h, the extracts were filtered using Whatman No. 1 filter paper, then, subjected to solvent evaporation under reduced pressure at 50°C using a rotary evaporator [13,14]). The obtained extracts was stored at 4°C prior to further analysis.

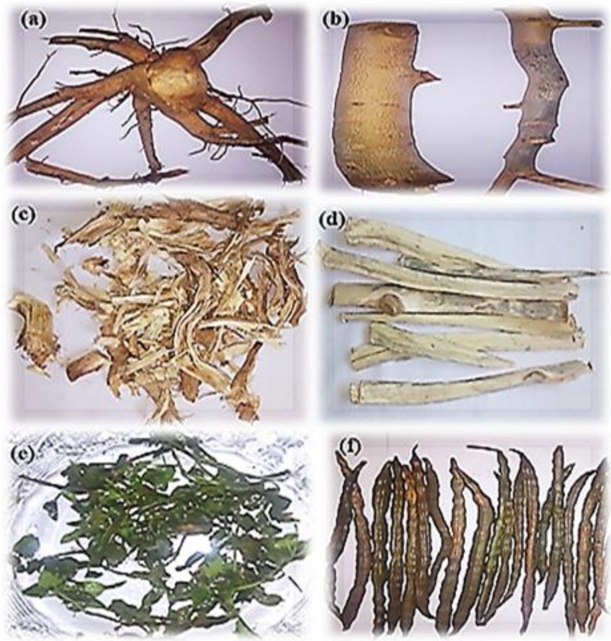

Figure 1. Different parts of *Moringa stenopetala*. (a): root; (b): stem; (c): root bark; (d): stem bark; (e): leaves; (f): seeds Capsule.

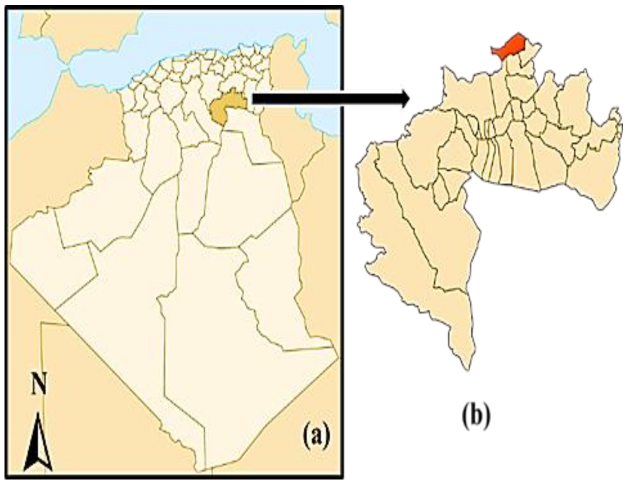

Figure 2. Sampling site. (a): geographical location of Wilaya of Biskra; (b): El Kantara region.

Animals

Thirty male Wistar rats, weighing between 250 and 280 grams obtained from Pasteur institute-Algeria were used in an anti-inflammatory study. Animals were housed in Sidi Bel Abbes pet shop under controlled conditions of temperature (25°C) and light/dark circle (12h/12h). The animals had access to water and a standard diet, ad libitum. The experimental protocol

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was approved by the ethics committee of Sidi Bel Abbes.

Quantitative polyphenols evaluation

Total phenol determination

The total phenol content in the extracts was determined using the Folin-Ciocalteu method as described by Muller et al. (2010) [15]. Using a spectrophotometer, the absorbance was read at 765nm against a blank. The total phenol content was extrapolated from the equation of the gallic acid calibration curve (y=0.0037x+0.065; R^2=0.9851) and expressed in terms of milligrams of gallic acid equivalent per 100 grams of dry matter (mg GAE/gDM).

Total flavonoids content

The flavonoids content was determined by the aluminum trichloride method as described by Topçu et al. (2007) [16].The absorbance was read using a spectrophotometer at 510 nm against a blank. The total flavonoid content was calculated using the calibration curve equation (y=0.0048x+0.0103; R^2=0.9983) and expressed as mg of quercitin equivalent (QE) per 100 grams of dry matter (mg QE/gDM).

***In vitro* antioxidant effect**

The *in vitro* antioxidant effect of methanolic and aqueous extracts was assessed using the stable free radical diphenylpicrylhydrazyl (DPPH) assay according to the method of Benhammou et al. (2009) [17]. Briefly, Fifty microliter of various concentrations of the aqueous and methanolic extracts were added to 2 mL of 0.004% methanol solution of DPPH. After 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. The percentage inhibition of the DPPH free radical was calculated using the following equation:

$$\boldsymbol{I}\% = \left[\frac{\boldsymbol{A0 - At}}{\boldsymbol{A0}}\right] \boldsymbol{X\ 100}$$

Where A0 is the absorbance of the control (containing all reagents except the test compound), and At is the absorbance of the test compound. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting the inhibition percentage against extract concentrations. The BHA, BHT and α-Tocopherol are used as positive controls.

***In vivo* anti-inflammatory effect**

The anti-inflammatory effect of aqueous extracts was investigated in carrageenan-induced inflammatory paw edema according to Winter et al. (1962) [18] protocol. Healthy male Wistar rats were divided into five groups of six animals each. Before the experiment, they were fasted for 16 h with free access to water. Fifteen minutes after the peritoneal injection of tested solutions (400 mg/kg of each aqueous organ extract), edema was induced by injecting 100 µL of carrageenan solution at 1% (w/v) at the sub-plantar region of the left hind paw. Diclofenac (20mg/kg) was used as a standard anti-inflammatory drug. The volume of each rat’s paw was measured before and after a carrageenan injection, and then after 1h, 2h, 3h, 4h and 5h using a digital calliper. The inhibition percentage of edema was calculated as follows:

$$\%\ \boldsymbol{Inhibition} = \frac{\boldsymbol{(Vt-V0)control-(Vt-V0)tested}}{\boldsymbol{(Vt-V0)control}}\ \boldsymbol{X100}$$

Where Vt is the paw volume after carrageenan injection and V0 is the paw volume before carrageenan injection (mm).

Statistical Analysis

The collected data are expressed as mean ± standard deviation (Mean ± SD). Statistical analysis was performed using IBM SPSS v28. Graphs were created using Microsoft Office Excel 365.The comparison between solvents was done using two independent samples: the Student test, the Welch test, or the Mann-Whitney test. The comparison of edema evolution was carried out using one sample Student test or Wilcoxon test. The comparison between organs was established using one-way ANOVA, Brown Forsyth, Welch, or Kruskal-Wallis tests, sustained if significant with a Tukey post hoc test or Mann-Whitney test. The significance level is 0.05.

4. Results

Total phenols and flavonoids content

The results of the quantification of total phenols and flavonoids in *M. stenopetala* methanolic and aqueous extract are represented in the Figure 3 and Figure 4, respectively. The results obtained showed that the leaf extracts were the richest in total phenols, with contents of around 101.08±11.58 and 87.43±6.92 mg GAE/g

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DM respectively for the Methanolic and aqueous extracts. In addition, high levels of total phenols were recorded in the aqueous and methanolic extracts of the root bark, with values of the order of 74.5±3.95 and 73.06±7.33 mg GAE/g DM. The most reliable levels were found in grain extracts, where we recorded levels in the order of 9.82±1.48 and 18.82±0.62 mg GAE/g DM in the aqueous and methanolic extract, respectively. Significant differences ($p<0.05$) were observed between aqueous and methanolic extracts in capsules, roots, and stems; however, no significant difference was noted in the other groups.

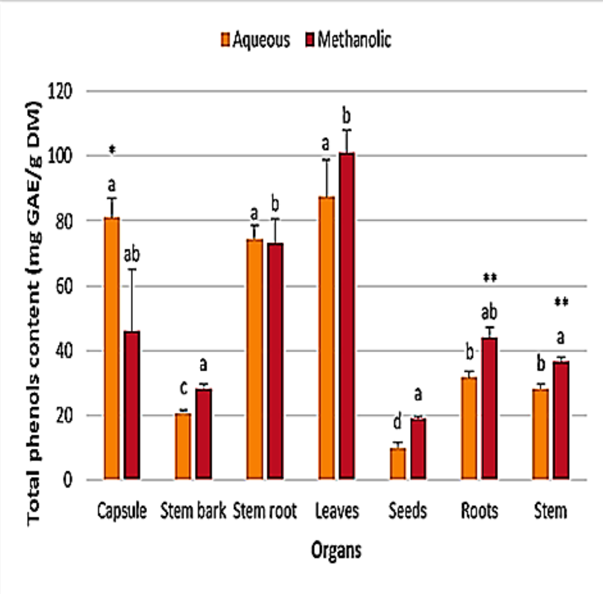

Figure 3. Total phenol content in aqueous and Methanolic extracts of different plant organs. Means with different letter are statically significant at $p<0.05$.

Flavonoid content results showed a high level of these compounds in the methanolic leaves extract (73.82±3.75 mg QE/g DM), methanolic capsule extract (34.93±1.81 mg QE/g DM) and aqueous root bark extract (32.50± 16.95 mg QE/g DM). Intermediate levels of flavonoid were observed in the capsule and leaf aqueous extract (27.22±3.8 and 25.84±15.23 mg QE/g DM). However, the lowest content was obtained from the aqueous seed extract with a value of 9.24± 0.12 mg QE/g DM. In comparing solvents, no significant difference ($p\geq0.05$) was observed between aqueous and methanolic extracts except for capsules and leaves. In addition, using aqueous extraction, no significant difference was found between all organs, while a significant difference ($p<0.05$) was recorded between organs using methanolic extraction.

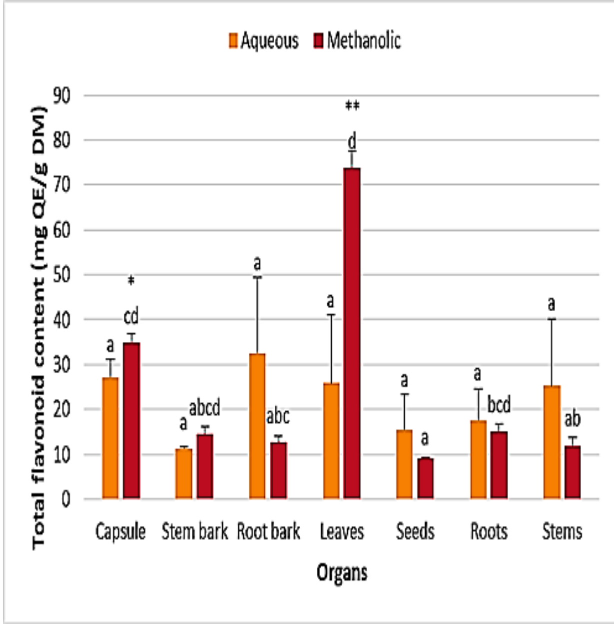

Figure 4. Total flavonoid content in aqueous and Methanolic extracts of different plant organs. Means with different letter are statically significant at $p<0.05$

Antioxidant effect

The antioxidant effect of different plant organs prepared with two solvents (water and methanol) was assessed by calculating the inhibitory concentration 50 (Table 1). A lower value of the latter means a significant antioxidant effectiveness. Higher antioxidant potential was given by the root bark methanolic extract followed respectively by the leaves methanolic extract and capsule aqueous extract. In general, the methanolic and aqueous extracts of all the organs showed a very significant antioxidant effect, but this effect was less than that obtained by the standards used.

Table 1. Antioxidant activity expressed in term of IC_{50} obtained from different solvents and organs

Extracts	**IC_{50} (mg/ml)**	
Leaves	AQ	0.693 ± 0.016^{a}
	MeOH	0.169 ± 0.004^{a}
Seeds	AQ	2.569 ± 0.571^{c}

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	MeOH	2.849 ± 0.905[b]
Capsule	AQ	0.198 ±0.009[a]
	MeOH	0.443 ±0.009[b]
Stem	AQ	2.761 ±0.144[c]
	MeOH	2.032 ± 0.079[d]
Stem bark	AQ	5.644 ± 0.483[d]
	MeOH	2.372 ±0.125[d]
Root	AQ	1.689 ± 0.134[b]
	MeOH	1.279 ± 0.044[c]
Root bark	AQ	0.746 ± 0.097[a]
	MeOH	0.159 ± 0.014[a]
BHA[A]	6.14±0.41	
BHT[A]	12.99±0.41	
α-Tocopherol[A]	13.02±5.17	

AQ: Aqueous extract; MeOH: Methanolic extract; [A]: Reference compounds (µg/ml); Values expressed are means ± SD of three parallel measurements. Means with the same letter are not significantly different at *p<0.05*.

Anti-inflammatory effect

The obtained results of the anti-inflammatory effect of *M. stenopetala* extracts are shown in the Figure 5 and Figure 6.

After 1 h of injection of carrageenan, a significant increase in paw volume was observed in rats receiving physiological water (control) compared with the other groups which received a dose of 20 mg/kg of Diclofenac (standard drug) and a dose of 400 mg/kg of aqueous extract of each plant organ. The paw edema decreased progressively after 3 and 4 hours, with the aqueous leaf extract being the most effective compared with the other plant organs tested (Figure 5). The calculation of the inhibition percentage showed better protection by the aqueous extract of leaves and roots after 3h with percentages of the order of 64.44% and 53.97% respectively. In addition, the aqueous seed extract exhibited a high inhibition of paw edema after 5h with a percentage of 69.38%. In all periods, Diclofenac at the dose of 20 mg/kg showed significant inhibition with percentages between 58.15% and 88.14% (Figure 6).

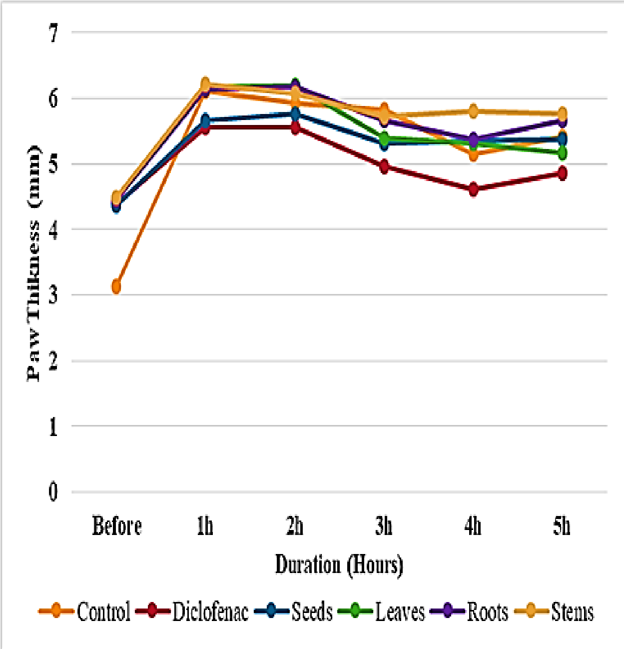

Figure 5. Effect of the aqueous extract of the different organs of the plant on the development of rat paw edema.

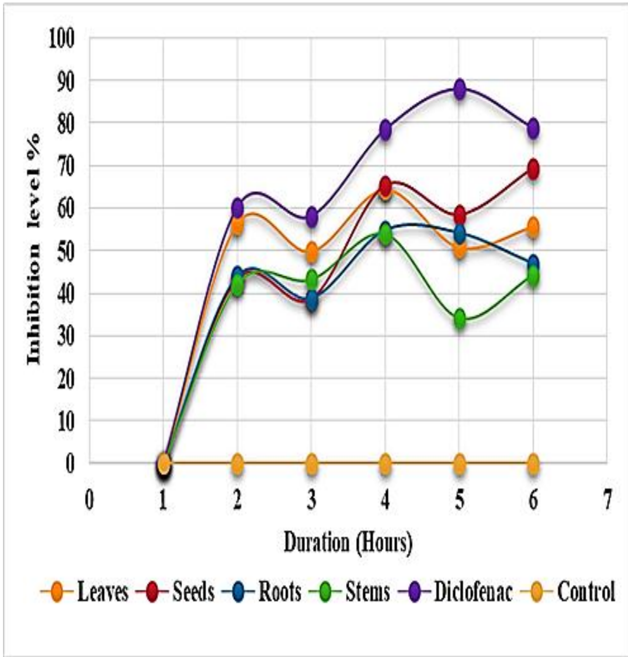

Figure 6. Inhibition percentage of aqueous extract obtained from different organs.

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At the end of the experimental follow-up, the rats were sacrificed under anesthesia and the blood was collected by cardiac puncture to be used for the measurement of proteins, albumin, platelets and white blood cells. Even though the protein levels in all groups were lower than those in the control, no significant difference was observed (*p = 0.336*). Additionally, albumin levels did not differ significantly (*p = 0.617*), with a higher level noted in the root extract-treated group. Moreover, the change in blood cell count was consistent across all groups (*p = 0.794*) and for all types of white cells. Finally, platelet counts were virtually identical in all groups (*p = 0.179*), with a notably low mean observed in the Diclofenac-treated group.

Table 3. Protein levels and blood cell count variation

	C	D	Leaves	Seeds	Roots	Stem	*p*
Alb	2.48± 0.20	2.52± 0.21	2.73 ± 0.29	2.53± 0.46	3.17± 1.44	2.71 ± 0.18	0.617
Prot	9.95± 4.28	7.63± 0.07	6.92 ± 0.85	7.55± 1.63	7.35± 0.53	7.38 ± 0.13	0.336
WBC	4.56± 1.64	3.56± 2.54	5.16 ± 2.47	3.19± 2.19	4.35± 2.28	3.88 ± 1.75	0.794
Neu	1.66± 1.29	1.75± 2.26	1.65 ± 0.97	1.57± 1.27	2.37± 2.40	1.44 ± 0.96	0.965
Lym	2.13± 0.82	1.12± 0.42	2.76 ± 2.31	1.43± 0.93	1.29± 0.41	2.25 ± 0.71	0.129
Mon	0.74± 0.87	0.57± 0.50	0.70 ± 0.84	0.14± 0.15	0.24± 0.21	0.10 ± 0.07	0.255
Eos	0.03± 0.03	0.12± 0.20	0.03 ± 0.02	0.04± 0.04	0.03± 0.04	0.07 ± 0.07	0.879
Bas	0.02± 0.02	0.02± 0.01	0.03 ± 0.01	0.02± 0.01	0.03± 0.02	0.05 ± 0.05	0.433
Plt	844 ± 229	257 ± 75	802 ± 90	776 ± 144	648 ± 337	740 ± 136	0.179

C: Control group (received physiological water 10ml/kg); D: Diclofenac 20mg/kg; Alb: Albumin (g/l); Prot: Proteins; WBC: White blood cells ($\times 10^9$ /l); Neu: Neutrophils; Lym: Lymphocytes; Mon: Monocytes; Eos: Eosinophils; Bas: Basophils; Plt: Platelets. $p \geq 0.05$ means a non-significant difference compared to the control group.

5. Discussion

Ours findings showed the richness of the methanolic and aqueous extracts obtained from different parts of *Moringa Stenopetala* by total phenols and flavonoids. Our results are in good agreement with Mekonnen et al. (2003) [19] and Habtemariam et al. (2015) [20], who revealed a high content of total phenol in leaf extracts of *M. stenopetela*. These same authors reported the following levels: 24 to 48 mg GAE/g DM (leaf aqueous extract) and 11.8 mg GAE/g DM (leaf ethanolic extract with 70% ethanol). These levels are lower than those obtained in our study. On the other hand, Habtemariam (2015) [20] reported the highest amount of phenolic compounds in the leaf hydroacetonic extract mainly represented by chlorogenic acids and quercetin glycosides.

Moreover, various studies have analysed the total flavonoid content in different parts of the *Moringa stenopetala* species, including the study of Abo El-Fadl et al. (2020) [21] who obtained values of 10.477 mg QE/g and 22.16 mg QE/g from leaf ethanolic and methanolic extract of *M. stenopetela* collected in Ethiopia, respectively. Comparing with our results, the flavonoid content in our leaf extracts was higher than that found by the latter. In addition, the flavonoid content in our seed extracts was lower than that obtained by Nibret et al. (2010) [22], who reported a value of the order of 99.72 mg QE/g.

The difference observed in the concentrations of total phenols and flavonoids is attributed to various factors such as geographical location, environmental conditions, harvested time and plant tissues [23].

Moringa stenopetala has been found to have significant antioxidant potential [19, 24]. Many works have reported the antioxidant potential of the studied plant. In this context, several studies have focused on the antioxidant potential of leaf extracts, the antioxidant activity of *Moringa stenopetala* leaves observed in the present study is lower than the findings of Habtemariam (2015) [20] and Ntshambiwa et al. (2023) [25], who reported an IC_{50} values of 90.3±4.6 and 53.21±0.005 respectively using DPPH free radical assay. This effect is due to the presence of hydrogen atom donor molecules such as phenolic compounds. In our study, the high antioxidant potential of the methanolic leaf and root bark extract is due to the high content of total

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phenol and flavonoids content estimated by quantitative analysis. These results are in good line with those of Tebeka et al. (2014) [26], who reported a positive correlation between the antioxidant capacity and the total phenolic content in *Moringa stenopetala* leaf extracts. Thus, flavonoids like quercetin-3-O-rhamnoglucoside and quercetin 3-O-glucoside have been extracted and represent the main constituents of *M. stenopetala* demonstrated high antioxidant effects [27].

In our study, the aqueous extract obtained from different organs of *M. stenopetala* exhibited an essential anti-inflammatory effect. Our obtained results are in agreement with those of Tamrat et al. (2017) [28], who reported the highest reduction of inflammation obtained by aqueous leaf extract at the dose of 400mg/kg in which the percentage inhibition of inflammation ranged from 47 and 67%. The acute inflammation after carrageenan injection is represented by two phases; the first phase (1h-3h) is characterized by the release of mediators such as histamine, serotonin and bradykinin, while the second phase (3h-5h) is contributed by the prostaglandins which play major role in development of inflammation [29]. In the present study, the aqueous extracts of all organs of the plant showed a significant inhibition of an increase of paw edema starting from the second hour after carrageenan injection. Generally, the anti-inflammatory effect of *Moringa stenopetala* aqueous extracts could possibly be due to the action of flavonoids and the presence of other compounds such as saponins, alkaloids and tannins [30,31]). Also, it is possible that these bioactive compounds could have an inhibitory effect on mediators such as prostaglandins, bradykinin and leukotriens [32]. Finally, the difference in inflammation inhibition observed between the different parts of the plant might be, at least in part, due to variability in composition and concentration of bioactive components responsible for this effect [28].

6. Conclusion

This work focused on the *Moringa stenopetela* species, known for its medicinal, nutritional and agricultural properties. Overall, the study highlighted the potential health benefits of *Moringa stenopetala* from the Algerian Sahara, particularly its antioxidant and anti-inflammatory properties. Our findings showed the richness of this plant in phenolic compounds, with variable levels of total phenols and flavonoids depending on the type of organ and the solvent used. On the other hand, all tested extracts exhibited a significant antioxidant and anti-inflammatory effect. The latter is due to the presence of the bioactive molecules responsible for these effects. Further research will be needed to determine the chemical composition of the plant and establish its mechanism of action. It is also crucial to investigate the potential synergistic effects of combining *Moringa stenopetala* extracts with other natural compounds or standard drugs to enhance antioxidant and anti-inflammatory activity. This could be a valuable area for future studies.

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