Antioxidant and Anti-inflammatory Activities of Methanolic Extract of Marrubium deserti de Noé Leaves

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Abstract:
The objective of the present study was to determine the pharmacological properties of the methanolic (MeOH) extract of Marrubium deserti leaves. For this purpose, antioxidant activity was carried out by DPPH and Ferric reducing power (FRAP) assays respectively while in vivo anti-inflammatory activity was tested by carrageenan-induced paw edema model. The Phytochemical investigation revealed the presence of several biocompounds, and total phenolic and flavonoid contents were also determined to support our results and revealed a high proportions of polyphenols (184 ± 0.78 mg GAE/g extract) and flavonoids (28.48 ± 0.40 mg QE/g extract). The MeOH extract demonstrated great pharmacological properties with a dose-effect relationship. Thus, a great antioxidant effect was recorded in both DPPH and FRAP assays with a respective IC50 of (15.1 μg/ml) and (80.01 ± 1 µg EAA/g of extract) and were considered significant (P<0.05) when compared to respective standards. On the other hand, anti-inflammatory results suggested that the plant extract could effectively oppose the inflammation caused by carrageenan at the dose of 200 mg/kg with significant decrease (84.1 %) of inflammation. These encouraging results suggest that our plant could be a good candidate to treat more effectively pathologies related to oxidative stress and inflammation.
INTRODUCTION

The pharmaceutical industry is today facing new challenges and it obviously became very difficult to prevent or cure diseases, in particular chronic pathologies (Tannoury and Attieh, 2017), and when we visualize the history of pandemic situations, it is very urgent to bring new effective ideas to face more effectively these pathologies. That's why testing, developing new drugs is mandatory, and plants may be the key for that (Eddouks et al., 2014).

There is a balance between the production of Reactive oxygen species (ROS) and their elimination via a process called detoxification (Di Giovanni et al., 2001). But when this balance is disturbed, the oxidative stress damage generated by this species can cause harmful damages at the cellular and molecular levels (Jakubczyk et al., 2020) which can affect dramatically the integrity of the body. For example, a study made by Phaniendra et al. (2015) showed that radicals of H₂O could damage DNA or RNA, which results in mutations or even cell death.

The fact that opioids and non-steroidal anti-inflammatory drugs (NSAIDs) are often the best alternatives to treat immediately inflammation and reduce pain sensation (Holdgate and Pollock, 2004); the side effects of these medications could be hazardous when used for a long-term period or not correctly used (Butler et al., 1992). This includes; cardiovascular, gastrointestinal, renal, or respiratory complications (Wu et al., 2013; Farshchi et al., 2009).

The human body is well equipped to face these stressful situations via its natural barriers and especially the innate immune system which plays a crucial role in the prevention and limitation of infections, allergens, and chemical irritants (Platts-Mills and Woodfolk, 2011). The bioactive compounds present in plants possess several pharmacological properties such as antioxidant, antibacterial, anticarcinogenic, antithrombotic, and antidiabetic properties (Chaves et al., 2020; Flefel et al., 2019; Othman et al., 2011).

M. deserti is a native plant which is widely used in Mediterranean countries as diuretic medication but also to treat many chronic illnesses like asthma and diabetes. This species seems to be involved at the molecular level as an antigenotoxic agent (Edziri et al., 2012).

Our previous work made on M. vulgare (Ghedadba et al., 2014) showed the remarkable biological activities of this species which attracted our curiosity to continue the investigation on another species of the same family named M. deserti.

This study aimed to evaluate for the first time the possible antioxidant and anti-inflammatory activities of Methanolic extract of M. deserti de Noé leaves as well as determine the phytochemical profile of this species.

MATERIALS AND METHODS

Plant Material

M. deserti was collected around the region of “Daya Mouguel”, Bachar. The leaves of the plant were then cleaned and grinded after drying period to obtain fine powder, and then put away from moisture and light to prevent a photo-oxidation process.

Experimental animals

Wistar albino rats (150-180 g) of either sex were used for this study. These animals were provided by the Institute of Agricultural Sciences and Veterinary. Animals were given fresh water ad libitum and placed at an ambient temperature (25 °C).

Institutional ethical permissions

All tests performed in this study are in compliance with the Institutional Ethical Committee of our faculty (approval no. 14/DBO/FSNV/UB2/2017).

Preparation of plant extract

The methanol extract of M. deserti was prepared using 500 g of the plant leaves powder...
and macerated with 3L of (methanol: water, 80:20 v/v), for 72h at room temperature, the obtained extract was stored in darkness at 4 °C.

**Phytochemical Screening**

The preliminary screening of MeOH extract was performed as described by Fransworth (1966) to reveal the presence of the main secondary metabolites classes based on precipitation and coloration reactions.

**Total polyphenols content (TPC)**

This assay was performed as previously described by Singleton and Rossi (1965), with a slight modification. Briefly, 0.25 mL of the diluted sample was added to 1.25 mL of Folin-Ciocalteu reagent (FCR) and 1 mL of (NaHCO₃). The Absorbance was then taken at 765 nm after 2h of incubation. The TPC was expressed as gallic acid equivalent (GAE) mg/g extract.

**Total flavonoids content (TFC)**

This test was done according to Ramful et al. (2011) approach. A volume of 1 ml of each sample was added to 1 ml of (AlCl₃) solution, the absorbance was measured after 10 minutes at (λₘₐₓ= 430 nm) and TFC results were expressed as quercetin equivalent (QE) mg/g extract.

**Antioxidant activity**

**DPPH radical scavenging assay**

The DPPH assay was performed according to the method of Braca et al. (2002), Different concentrations of diluted methanolic extract ranging from (5-1000 μg/mL) were added to equal volume (1.95 ml) of DPPH solution (0.0024%; w/v).

The mixture was then vortexed and kept for 30 min in the dark, the absorbance was recorded at λ=517 nm. Noting that Sophorin, Quercetin, and Gallic acid were used as standards. The inhibition percentage of DPPH radical was calculated using the following formula:

\[
\% \text{Inhibition of DPPH} = \frac{Ab \ Control - Ab \ Sample}{Ab \ Control} \times 100
\]

**FRAP assay**

For the evaluation of this test, we used the protocol of Chu et al. (2000), with minor modifications. A mixture composed of 2.5 ml of Potassium phosphate buffer, 2.5 ml of \((C₆FeK₃N₆, 1\% \text{ w/v)}) and 1 ml of plant extract at different concentrations (50 - 500 μg/ml) was prepared. After 20 min of incubation at 50 °C; a volume of 2.5 ml of \((C₂HCl₃O₂, 10\% \text{ w/v})\) was added, followed by the same volume of water and 0.5 ml of (FeCl₃, 0.1% w/v). After 30 min of incubation at 28 °C; the absorbance was then recorded at 600 nm noting that α-tocopherol and ascorbic acid were used as standards.

**Anti-inflammatory activity**

To carry out this test, we used Yimer et al. (2020) protocol, animals first fasted for 16 hours with free access to water, then, 100 μl of 1% carrageenan suspension was administered in the right paw of the rat via sub-plantar injection. Noting that all tested samples were given orally 1h before carrageenan injection. The rats were subdivided into 4 groups as follows:

- **Group I** received 0.9% NaCl (10 ml/kg BW) and was served as control,
- **Group II** received (200 mg/kg BW) of methanolic extract,
- **Group III** received standard diclofenac (100 mg/kg) and served as the second first standard.
- **Group IV** received standard Aspirin (100 mg/kg).

The diameter of the inflamed paws was recorded during 5h following the injection of carrageenan, and the percent inhibition of inflammation was calculated according to the following formula:
Statistical analysis

Data obtained from this study were analyzed by one-way ANOVA followed by Dunnett’s multiple comparisons test and each value was expressed as mean ± SD. Results were considered highly significant at p < 0.001.

Table 1. Qualitative screening of *M. deserti*

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Test name</th>
<th>Analysis</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Mayer’s Test</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>Ferric chloride test</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>Liebermann Burchard test</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Shinoda’s test</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Triterpenes</td>
<td>Liebermann Burchard test</td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

Determination of antioxidant capacity

In order to better understand the oxidative stress process related to the pathophysiology of illnesses, plants are good candidates for that via the study of the pharmacological potential of their bioactive compounds, which lead to further therapeutic application (Kasote et al., 2015).

The evaluation of antioxidant activities of *M. deserti* revealed a highly significant free-radical inhibitory ability of methanolic extract in both DPPH and FRAP assays with a dose-dependent manner, and this could be due to the antioxidant compounds included in *M. deserti* extract in which, they may interacted with the radical DPPH and gained one or more electron or hydrogen atom and converted it into α-α-diphenyl-β-picryl hydrazine have (Liang and Kitts, 2014). While in FRAP test, these bioactive compounds may capture and converted ferric ions (Fe³⁺) into ferrous ions (Fe²⁺) (Shahidi and Zhong, 2015).

When we look at the results presented in table 2; it seems that the MeOH extract of *M. deserti* contain a high proportion of polyphenols (184 ± 0.78 mg GAE/g) and flavonoïds (28.48 ± 0.40 mg QE/g extract) and this could explain the remarkable antioxidant activities of our plant since we know that there is a strong relationship between the phenolic content in a plant and the possible antiradical activity expressed (Ning et al., 2012). Those secondary metabolites act as chain breakers to prevent free radicals accumulation in an organism (Kainama et al., 2020).

Another study (Nimse and Pal, 2015) suggested that antioxidant agents present in plants may also oppose the lipid peroxidation process in cell membranes and even prevent low-density lipoprotein (LDL) oxidation and we know the important role played by LDL in the transport of cholesterol (Mahley et al., 1984).

For each test, the IC₅₀ was also calculated (Figure 1), thus; in DPPH assay, the value was remarkable for the MeOH extract (15.1±0.03 μg/ml) and was considered very close to those values reported in Quercetin and Gallic acid (5.27±0.19 μg/ml and 8.64±0.51 μg/ml) respectively, but on the other hand, it seems that our plant possessed better activity than Sophorin (22.15±0.07 μg/ml).

In FRAP assay, the antioxidant activity of *M. deserti* was considered the best (80.01±1 μg/ml) among the two tested standards namely; ascorbic acid and α-tocopherol (99.87±0.55 μg/ml).
Another study (Maisetta et al., 2019) correlated the strong antioxidant activity of their plant with its high tannin content and explained this by the fact that tannin enhanced the electron transfer process which is consistent with our findings. A compound called glycosidic phenylpropanoid ester which was recently identified in *M. deserti* seems to have strong antioxidant properties (Zaabat et al., 2010).

A recent study made by (Kim et al., 2020) showed that terpenes compounds such as oxygenated monoterpenes are able to provide relevant protection against oxidative stress by acting like reductants in both DPPH and FRAP assays. Noting that in our previous study (Ghedadba et al., 2014) made on *M. vulgare*; we found almost the same result using β-carotene bleaching test as a reference test.

### Table 2. Total polyphenols and flavonoids contents and antioxidant properties of *M. deserti*.

<table>
<thead>
<tr>
<th>Extract and standards</th>
<th>IC50 (μg/ml)</th>
<th>Total Polyphenols mg GAE/g extract</th>
<th>Flavonoids content mg QE/g extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FRAP assay</td>
<td>DPPH test</td>
<td></td>
</tr>
<tr>
<td>MeOH extract</td>
<td>80.01 ± 1b</td>
<td>15.1 ± 0.03c,e 184 ± 0.78</td>
<td>28.48 ± 0.40</td>
</tr>
<tr>
<td>Quercetin</td>
<td>No tested</td>
<td>5.27 ± 0.19</td>
<td>/</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>No tested</td>
<td>8.64 ± 0.51</td>
<td>/</td>
</tr>
<tr>
<td>Sophorin</td>
<td>No tested</td>
<td>22.15 ± 0.07</td>
<td>/</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>99.87 ± 0.55</td>
<td>No tested</td>
<td>/</td>
</tr>
<tr>
<td>α-tocopherol</td>
<td>300.08 ± 0.34</td>
<td>No tested</td>
<td>/</td>
</tr>
</tbody>
</table>

Each value is expressed as mean ± SD (n=3). One way ANOVA followed by Dunnett’s multiple comparison test. Level of significance p< 0.001; MeOH extract vs different standards; "p< 0.001 is statistically significant with a comparison to Ascorbic acid, b"p< 0.001 to α-tocopherol, ’p< 0.001 to Quercetin, ’p< 0.001 to Gallic acid, ’p< 0.001 to Sophorin.
Anti-inflammatory activity

As shown in table 3, the methanolic extract of *M. deserti* exhibited a significant decrease of inflammation at the tested concentration of 200 mg/kg b.w, starting from the 3h and was (4.79 ± 0.2 mm) which was very close to Diclofenac (4.73 ± 0.53 mm) value but not to Salicylic acid (4.16 ± 0.47 mm) value the same hour.

The paw circumference of the methanolic group continued to decrease gradually to reach (4.41 ± 0.2 mm at the end of the experiment) and data were considered highly significant (*P* <0.001) when compared to the control group. In terms of inhibition of edema; our plant showed the highest inhibition value of (84.1 %) but unfortunately was considered lower than aspirin and diclofenac (90 and 85.52 %) respectively.

Our results indicated that the actual bioactive compounds present in MeOH extract of *M. deserti* may inhibit the release of the principal pro-inflammatory mediators of the first and second phases of the inflammations response like histamine, serotonin prostaglandin E2 (PGE2) Cyclooxygenase-1 (COX-1) Cyclooxygenase-2 (COX-2) (Yoon and Baek, 2005).

A recent study made by Saad et al. (2016) demonstrated the anti-inflammatory potential of
the aqueous extract of *M. deserti* with all tested doses and suggested that the actual flavone identified in the plant called apigenin is responsible for this activity which is consistent with our results.

Another study (Yahfouf et al., 2018) demonstrated that flavonoids can significantly decrease the expression of pro-inflammatory genes especially those of cytokines and nitric oxide via downregulation signaling pathways.

It is also interesting to see that another species of the same family called *M. vulgare* demonstrated a potent anti-inflammatory activity by significantly decreasing the release of key cytokine release like tumor necrosis factor-alpha (TNF-α) and interleukins (IL-1β, IL-8) of all tested extracts (Namoune et al., 2018). The researchers linked this remarkable anti-inflammatory effect with the richness of this plant in phenolic and flavonoid content.

Another hypothesis could be the action of alkaloids and terpenes by reducing the permeability of blood endothelial cells which significantly reduces exchanges between the bloodstream and the surrounding tissues (Li et al., 2020). Thus, highly contribute to an important increase of analgesic and anti-inflammatory actions (Shalaby and Hammouda, 2014) and this was reported in many Chinese herbal medicines. Noting that the presence of other bioactive compounds present in our plant may act via synergistic way to exhibit a strong anti-inflammatory effect.

### Table 3. Anti-inflammatory activity of *M. deserti* and standards.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Paw diameter (mm)</th>
<th>Average % of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1h</td>
<td>2h</td>
</tr>
<tr>
<td>Control</td>
<td>6.40 ± 0.5</td>
<td>6.87 ± 0.7</td>
</tr>
<tr>
<td>MeOH extract</td>
<td>6.40 ± 1.0&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>5.94 ± 0.4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>6.37 ± 0.9&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>5.95 ± 0.5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>6.41 ± 0.00&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>5.23 ± 0.71&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Each value is expressed as mean ± SD (n=3). One way ANOVA followed by Dunnett’s multiple comparison test. Level of significance p< 0.001; MeOH extract and standards vs control group; *p< 0.05, **p< 0.01, ***p< 0.001, **ns** is no significant.

### CONCLUSION

The results of this study suggest that *M. deserti* have remarkable pharmacological potential and the phytochemical analysis of the methanolic extract revealed its richness in several secondary metabolites classes, but further investigation is crucial to understand the full potential of this species.

### ACKNOWLEDGMENT

The authors wish to express thanks to the Algerian ministry of higher education and scientific research (MESRS, DGRSDT).

### CONFLICT OF INTEREST

There is no conflict of interest.

### REFERENCES


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