Antioxidant, Anti-inflammatory and Anti-diarrheal Activity of Ethanolic Extract of Some Selected Medicinal Plants Grown in Jordan and Palestine

NOOMAN A. KHALAF, RAJASHRI R. NAIK*, ASHOK K. SHAKYA, NAEEM SHALAN and ATIF AL-OTHMAN

Faculty of Pharmacy and Medical Sciences, Al-Ahliyya Amman University, PO Box 263, Amman 19328, Jordan.
*Corresponding author E-mail: rajashrinaik4011@yahoo.com, rsharry@ammanu.edu.jo

http://dx.doi.org/10.13005/ojc/310408

(Received: September 25, 2015; Accepted: October 31, 2015)

ABSTRACT

Herbs like Rosmarinus officinalis (Lamiaceae), Peganum harmala (Nitrariaceae), Teucrium polium (Lamiaceae), Verbena officinalis (Verbenaceae), Artemisia herba-alba (Asteraceae) and Arum palaestinum (Araceae) have been studied for its various biological activities, the extract of fresh aerial parts of plant have not been reported for their antioxidant and anti-inflammatory activities. Our aim was to study the antioxidant, anti-inflammatory and antidiarrheal activity of ethanolic extract of fresh aerial parts of the plant. Anti-inflammatory activity was evaluated by carrageenan induced paw edema method, DPPH free radical scavenging activity (antioxidant) and effect of extract on gastro-intestinal tract motility was studied. Phenolic content of Verbena officinalis was higher (652.5 mg GAE %) than the other plant extracts. Whereas antioxidant activity is concerned the Rosmarinus officinalis exhibited highest antioxidant activity (IC₅₀= 6.25 µg/ml), and showed excellent anti-inflammatory (65.5%) as compared to ascorbic acid (6.14 ± 0.09 µg/ml) and diclofenac sodium (70.1%) respectively. Rosmarinus officinalis leaves extract showed significant inhibition of the gastrointestinal activity (31.3 %) when compared to all the other ethanolic extracts. The ethanolic extract of Arum palaestinum Bios did not show any significant anti-inflammatory activity and had no significant effect on gastrointestinal tract inhibition.

Key words: Rosmarinus officinalis, Peganum harmala, Teucrium polium, Verbena officinalis, Artemisia herba-alba and Arum palaestinum

INTRODUCTION

Large numbers of conservationist groups have been suggesting that wild medicinal plant be brought to cultivation owing to its therapeutic potential and to meet the growing demand for the herbal medicines. Study conducted among the traditional herbal medicine practitioner of Arab medicine in Jordan, Palestine and the gulf countries suggest that several different plants are being used in herbal therapies. Several medicinal plants and extracted constituents having therapeutic potential are used clinically. Various herbs and spices like Rosmarinus officinalis⁴⁻⁸, Peganum harmala¹⁰⁻¹²,
Teucrium polium\textsuperscript{13-15}, Verbena officinalis\textsuperscript{16-20}
Artemasia herba-alba\textsuperscript{21} and Arum palaestinum\textsuperscript{22-26}
have been reported to exhibit a variety of activities
including anti-oxidant, analgesic, anticancer, antibacterial, anti-inflammatory, CNS stimulant and
depressant activities. The presence of various
phytochemicals like flavones, iso-flavones,
flavanoids, anthocynain, coumarin, lignans,
dihydrochalcones and isocatechins account to the
antioxidant activity in plants. Drug formulations
possessing antioxidant activity have been reported
for the prevention and treatment of certain diseases
like atherosclerosis, Alzheimer’s disease, cancer,
inflammation, and diabetes\textsuperscript{27}. The
chemotherapeutic, cytotoxic, antibacterial and
antifungal potential of \textit{R. officinalis}, \textit{P. harmala}, \textit{T. polium}, \textit{V. officinalis}, \textit{A. herba-alba} and \textit{A. palaestinum}
has been reported. To the best of our
knowledge the antioxidant and anti-inflammatory
activities of fresh aerial plant parts has not been
reported so far. The secondary metabolites vary
according to the weathering conditions with these
criteria in the present study; we report the antioxidant,
anti-inflammatory and anti-diarrheal activity of
ethanolic extract collected from fresh plant.

\section*{Material and Methods}

\textit{1,1-Diphenyl-2-picryl-hydrazyl (DPPH),
loperamide, diclofenac sodium and the other
reagents used throughout the experiment were of
analytical grade and purchased from Sigma Aldrich Co.,
St. Louis, USA.}

\section*{Preparation of crude plant extract}

The plant used for the studies were
obtained locally or purchased from the local market,
north of Jordan Valley and Palestine during Feb-
June 2013. Plant material consisting of mature
leaves of \textit{Rosmarinus officinalis}, \textit{Peganum harmala},
\textit{Teucrium polium}, \textit{Verbena officinalis}, \textit{Artemasia
herba-alba} and \textit{Arum palaestinum}. Washed leaves
different plants were dried using filter paper
sheets and then air dried for 2 hours. Leaves were
cut and grinded using electrical mixer for 5 minutes.
About 200gm of this powdered plant material
soaked in ethanol (1 L, 96%) for 24 hours, stirring
using magnetic stirrer. The final extracts were filtered
using muslin cloth and then through Whatman filter
paper No.1 (Whatman Ltd., England). The solvent
was evaporated using rotary evaporator at 50°C
and the plant extract was stored in refrigerator
between 4 to 8°C. For antioxidant activity, stock
solutions of crude extracts (5 mg/ml) were prepared
by dissolving a known amount of dry extract in
99.5% methanol. The working solutions (1-700 µg/
ml) of the extracts were prepared using suitable
aliquots of stock solution. For other pharmacological
activities the extract was suspended in 10% acacia
gum.

\section*{Animals}

Wistar albino rats (125-160g) and Swiss
albino mice (22-23g) were procured from market.
All animals were acclimatized at room temperature
of 24±2 °C and relative humidity of 55-60%, with
free access to food and water for one week. Animal
ethics committee guidelines were followed for the
experiments on animals. The number of animals
used was the minimum necessary to demonstrate
the consistent effects of the treatment.

\section*{Preliminary test}

\subsection*{Determination of Total Phenolic Content}\textsuperscript{28}

Total phenolic content (TPC) was
estimated following reported method\textsuperscript{27} with slight
modification. Ethanolic solutions of samples (100
µg/mL) were prepared. The sample solutions (1mL)
were treated with Folin-Ciocalteau’s reagent (1:10
dilution, 1mL). Then sodium carbonate (25% w/v;
1mL) was mixed with content, vortexed and diluted
to 10 ml using distilled water. Sample were vortexed
again and the absorbance of resultant solutions
were measured at 725 nm using Thermo-Fisher
spectrophotometer after incubation at 25 °C for 1 h.
Gallic acid was used as a reference standard for
the preparation of calibration curves. Total phenolic
content of the samples is reported as mg of gallic
acid equivalent (GAE/g).

\subsection*{Antioxidant activity (Free radical scavenging
activity)}

Samples were evaluated using DPPH
radical for free radical scavenging activity using the
method reported earlier\textsuperscript{27, 29}. Solutions were
prepared freshly. DPPH (0.002 g %), different
concentration of extract (1 µg/ml – 700 µg/ml) and
ascorbic acid (0.5-25 µg/ml) were prepared in (70% methanol). One ml of DPPH solution was mixed
either with different samples of extract (1ml) or
ascorbic acid (1ml). These solutions were vortexed at 25°C for 30s and kept aside for 30 min. in dark. Using methanol (70%) as blank at 517nm the instrument spectrophotometer was set to zero. The free radical scavenging activity of residual DPPH against the blank was determined using given formula. IC$_{50}$ (concentration of required to reduce by 50% of the initial quantity of DPPH) are expressed as Mean ± SD (n=3) and determined graphically.

\[
\text{Free radical scavenging activity} = \frac{\text{Abs}_\text{control} - \text{Abs}_\text{sample}}{\text{Abs}_\text{control}} \times 100
\]

where Abs$_\text{control}$ is the absorbance at 517 nm DPPH solution and Abs$_\text{sample}$ is the absorbance at 517 nm of DPPH (0.002%) with sample (1mL).

**Anti-inflammatory activity**

Carrageenan-induced paw edema model was used for studying anti-inflammatory activity$^{20}$. The animals (albino rats) were divided into groups of four. Plant extracts were given orally (200-300 mg/kg b.wt.) and the paw volume determined plethysmographically (Ugo-Basel, Italy). The control group received equivalent volume of normal saline. The reference group received diclofenac sodium (10 mg/kg b.wt. p.o.). After half an hour the carrageenan (0.1 mL of 1.0% w/v solution) in sterile saline was injected into the sub-plantar tissue of the rat's right hind paw. The paw volume was determined at hourly intervals for 3 hours (0, 1, 2, 3 h). The percent of inhibition of inflammation was determined using formula:

% inhibition = 100 × (V$_{\text{con}}$ - V$_{\text{sam}}$)/V$_{\text{con}}$ ; where V$_{\text{con}}$ represents edema volume in control and V$_{\text{sam}}$ edema volume in the group treated with sample.

**Effect of extract on gastrointestinal motility$^{29, 31}$**

For the motility studies the mice were fasted overnight with only access to water. The study was performed using the procedure. In which the mice are divided in different groups. Leaves extract samples (100 mg/kg p.o.) containing Tween-80 (0.1 ml, 1%) was administered to different animals. Animals of standard groups received Loperamide (8 mg/kg, p.o.) while the control group received Tween 80 (0.1 ml, 1%). Charcoal meal (0.3 ml, 10% suspension of charcoal in 1% sodium CMC) was given to animals, 15 minutes after the treatment. Twenty minutes later, animals were sacrificed and small intestine was removed. The total length of intestine (T$_{\text{i}}$) and distance travelled by the charcoal plug in the intestine (T$_{\text{c}}$) was measured for each animal. Percentage activity was calculated as:

% inhibition = [(1-(T$_{\text{c}}$/T$_{\text{i}}$)]×100

Where T$_{\text{i}}$ is the percentage of g.i.t. transit in control group and T$_{\text{c}}$ is the percent of g.i.t. transit in treated group.

**Statistical Analysis**

GraphPad Prism 5 (San Diego, CA, USA) software was used for statistical analysis. The statistical significance of differences between the groups was analyzed by one-way ANOVA.

**RESULTS AND DISCUSSION**

The crude extracts obtained, ranged from 6.9 to 8.9 g /100 g (w/w). The preliminary phytochemical studies indicate the presence of phenolic compounds in all extracts. The phenolic content (mg GAE %) were ranged from 22 to 652 mg %. The phenolic content was higher in Verbena officinalis (652 mg GAE%) than the other extract studied. Total phenolic content in Arum palaestinum Bioss (obtained from Palestine and Jordan) were lower than other extracts. Among the 7 extracts and the standard tested for in-vitro antioxidant activity using the DPPH method, the crude ethanolic extracts of Rosmarinus officinalis, Peganum harmala, Teucrium polium, Verbena officinalis, Artemisia herba-alba, Arum palaestinum Bioss (Jordan Velley) and Arum palaestinum Bioss (Palestine) showed free radical scavenging (antioxidant) activity with IC$_{50}$ values of 6.25 ± 0.12, 15.45 ± 1.01, 321.50 ± 2.6, 23.64 ± 1.73, 20.90 ± 0.95, 36.36 ± 1.25, and 69.75 ± 2.16 µg/ml, respectively (Table 1). The IC$_{50}$ value for ascorbic acid was 6.14±0.09 µg/ml. The fresh leave extract of Rosmarinus officinalis, Peganum harmala, Verbena officinalis, Artemisia herba-alba and Arum palaestinum Bioss (Jordan Velley) were having excellent antioxidant activity. The results indicate that the antioxidant activity of the crude extract of Rosmarinus officinalis was comparable to ascorbic acid. This also indicates the presence of better...
antioxidant compounds than ascorbic acid. Further work is required to fractionation of the extract of Rosmarinus officinalis to obtain the principle constituent. The antioxidant activity of Arum palaestinum Bioss obtained from Palestine was higher than the Jordanian variety. This difference in the activity might be due to the weathering and climatic conditions.

As far as anti-inflammatory activity of fresh leaves extract is concerned, the Rosmarinus officinalis showed excellent anti-inflammatory activity against carrageenan induced paw edema (65.5%) at 200mg/kg b.wt. dose. Significant anti-inflammatory activity were observed for Verbena officinalis (45.1%), T. polium (30.5%), Peganum harmala (44.2%) at 200mg/kg b.wt. dose. Significant anti-inflammatory activity were observed for Verbena officinalis (63.6%) and Artemisia herba-alba (44.2%) at 200mg/kg b.wt. dose. The extracts of Arum palaestinum Bioss did not produce significant anti-inflammatory activity.

**Table 1: Antioxidant (in-vitro) and anti-inflammatory activity of ethanolic extract of fresh leaves of plants collected from Palestine and Jordan**

<table>
<thead>
<tr>
<th>Tested Material</th>
<th>Weight of extract (g %, w/w)</th>
<th>TPC (total phenolic content) (mg GAE/100 g)</th>
<th>Antioxidant activity IC₅₀ (µg/ml) (Mean ± SD)</th>
<th>Anti-inflammatory activity (200 mg/kg b.wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rosmarinus officinalis</td>
<td>7.5</td>
<td>232.4 ± 25.2</td>
<td>6.25 ± 0.12</td>
<td>65.5*</td>
</tr>
<tr>
<td>Peganum harmala</td>
<td>7.2</td>
<td>112.1 ± 5.1</td>
<td>15.45 ± 1.01</td>
<td>45.1*</td>
</tr>
<tr>
<td>Teucrium polium</td>
<td>6.9</td>
<td>150.7 ± 10.7</td>
<td>321.50 ± 2.60</td>
<td>50.5*</td>
</tr>
<tr>
<td>Verbena officinalis</td>
<td>8.9</td>
<td>652.5 ± 15.5</td>
<td>23.63 ± 1.73</td>
<td>63.6*</td>
</tr>
<tr>
<td>Artemisia herba-alba</td>
<td>7.2</td>
<td>140.3 ± 5.4</td>
<td>20.90 ± 0.95</td>
<td>44.2*</td>
</tr>
<tr>
<td>Arum palaestinum Bioss (Jordan Valley)</td>
<td>7.8</td>
<td>25.1 ± 3.0</td>
<td>36.36 ± 1.25</td>
<td>35.5*</td>
</tr>
<tr>
<td>Arum palaestinum Bioss (Palestine)</td>
<td>6.9</td>
<td>22.2 ± 2.5</td>
<td>69.75 ± 2.16</td>
<td>30.2*</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>-</td>
<td>-</td>
<td>6.14 ± 0.09</td>
<td>-</td>
</tr>
<tr>
<td>Diclofenac Sod. (10 mg/kg b.wt. p.o.)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>70.1*</td>
</tr>
</tbody>
</table>

*Significant difference (p< 0.05), #No significant difference (p>0.05)
Significant inhibition in gastrointestinal activity was observed with the fresh leaves extract of Rosmarinus officinalis, Teucrium polium and Artemisia herba-alba, which might be due to their direct effect. The maximum percent inhibition (31.3%) was observed with leaves extract of Rosmarinus officinalis. The percent inhibition of g.i.t. transit for loperamide was 54.3%. Other extracts did not affect the gastrointestinal transit of charcoal meal (at 100 mg/kg b.wt. dose).

**CONCLUSION**

In the present study on some of the most popular and traditionally used Jordanian plants (Rosmarinus officinalis, Peganum harmala, Teucrium polium, Verbena officinalis, Artemisia herba-alba and Arum palaestinum), it may be concluded that the extract of Rosmarinus officinalis leaves showed higher antioxidant activity associated with appreciable anti-inflammatory activity. It also inhibits significantly the gastrointestinal motility in comparison to the other extracts. A. plalestinium did not exhibit any anti-inflammatory and had no effect on intestine.

**ACKNOWLEDGEMENTS**

Authors wish to thank Dean, Faculty of Pharmacy and Medical Sciences and Dean, Higher Education & Scientific Research, Al-Ahliyya Amman University, Amman, Jordan for providing necessary facilities.

**REFERENCES**


