Effects of the chronic ingestion of an infusion of *Ruta chalepensis* on the vasomotor responses of rat aortic rings

[Efectos de la ingestión crónica de una infusión de *Ruta chalepensis* sobre las respuestas vasomotoras de anillos de aorta de rata]

Lourdes CARTAS HEREDIA\(^a\), Dieter MASCHER\(^a\), Marco Antonio JUÁREZ OROPEZA\(^b\), José FARIÁS\(^a\) & Cristina PAREDES CARBAJAL\(^a\)

\(^a\) Department of Physiology, School of Medicine, UNAM, P.O. Box 70 -250, Mexico D.F. 04510, Mexico
\(^b\) Department of Biochemistry, School of Medicine, UNAM, P.O. Box 70 -159, Mexico D.F. 04510, Mexico

**Contactos | Contacts:** Cristina PAREDES CARBAJAL E-mail address: cparedes@unam.mx

**Abstract**

*Ruta chalepensis*, is used, in traditional medicine, as emmenagogue, abortive, and analgesic. We analyzed, in male Wistar rats, the effects of the chronic intake of an infusion of *Ruta chalepensis* (20 g/L) on the vasomotor responses of, either intact or endothelium-denuded aortic rings, to phenylephrine or carbachol. Only in rings with endothelium significant effects were observed. The infusion induced a leftward shift of the concentration-response curve to phenylephrine and an increase in maximal tension development. These effects were abolished by indomethacin. In these rings, inhibiting the synthesis of nitric oxide, in the presence of indomethacin, induced a leftward shift of the concentration response curve to phenylephrine, as well as an increase in maximal tension. These results suggest that the chronic ingestion of a *Ruta chalepensis* infusion induces an endothelium dependent increase in the synthesis/release of cyclooxygenase-dependent vasoconstrictor prostanoids, and an increase in the basal synthesis/release of nitric oxide.

**Keywords:** Nitric Oxide, Prostanoids, Endothelium, Ruta chalepensis.

**Resumen**

*Ruta chalepensis*, se utiliza en la medicina tradicional como emenagogo, abortivo y analgésico. Se analizaron, en ratas Wistar macho, los efectos de la ingesta crónica de una infusión de *Ruta chalepensis* (20 g/L), sobre las respuestas vasomotoras de anillos de aorta con y sin endotelio, a la fenilefrina o al carbacol. Se observaron efectos significativos sólo en anillos con endotelio. La infusión indujo un desplazamiento a la izquierda de la curva de concentración-respuesta a fenilefrina y un incremento en la tensión máxima desarrollada. Estos efectos fueron abolidos por la indometacina. La inhibición de la síntesis de óxido nítrico, en presencia de indometacina, produjo un desplazamiento a la izquierda de la curva de concentración-respuesta a la fenilefrina, así como un incremento en la tensión máxima. Estos resultados sugieren que la ingesta crónica de una infusión de *Ruta chalepensis* induce un incremento en la síntesis/liberación de prostanoides vasoconstrictores dependientes de la ciclooxigenasa y un aumento en la síntesis /liberación basal de óxido nítrico.

**Palabras Clave:** Óxido nítrico, prostanoides, endotelio, *Ruta chalepensis*.
INTRODUCTION

*Ruta chalepensis* belongs to the *Rutaceae* family; common names of *Ruta* are Garden Rue, Herb of Grace, Herbygrass Citronelle Marron, Rue and Ruda. It is a hardy, evergreen shrub of up to one meter tall, with a characteristic grayish color and a sharp unpleasant odor. The leaves are small, oblong, deeply divided, pinnate and granular dotted. The flowers are small yellow and in clusters. The fruits are round, small and lobulated. The taste is rather bitter, even more when dried. Rue is a herb with an ancient history. The genus name "Ruta" comes from the Greek word "reuo", to set free, showing its reputation as a freer from disease. It is native to Mediterranean Europe, and Western Asia. It is a very popular and attractive garden shrub which is grown not only for ornamental but also as a flavoring agent in food and beverages and mainly, for medicinal reasons. (Abu-Hamdah, 2001).

The aerial part of *R. chalepensis* is used in traditional herbal medicine in many countries for the treatment of a wide variety of diseases. It has been recommended in herbal treatment of insomnia, headaches, nervousness, abdominal cramps, and renal troubles. The plant may be part of ophthalmic, sedative and hypnic herbal preparations, rue oil is commonly used as antitussive, rubefacient, for certain dermatoses such as eczemas and psoriasis (Atta and Al-Kofahi, 1998; Alzoreky and Nakahara, 2003; Raghav et al., 2006; El Sayed et al., 2000; Al-Okbi et al., 2002), and also, as an antiviral agent (Vigneau, 1985). Rue oil is a powerful local irritant (Martindale, 1982). The most frequent intentional use of the plant has been the induction of abortion (Al-Qarawi, 2005). This plant has, also, been used as an inductor of uterine contractions (Morton, 1981; Hoet, 1980; Fleurentin and Pelt, 1982; Browner, 1985); as well as in some diseases of the respiratory system (Dafni et al., 1984; Nagaraju and Rao, 1990).

Pharmacological studies carried out with different extracts of various *Ruta* species have reported many effects which include anti-epileptic, sedative, and hypnotic actions (Font-Quer, 1962; Di Stasi et al., 2002, Gonzalez-Trujano et al., 2006), analgesic, antipyretic, anti-inflammatory effects (Al-Said et al., 1990; Atta and Alkofahi, 1998; Giganda and Laborde, 2003; Lauk et al., 2004), antispasmodic, emmenagogue, antihelminthic and antibacterial actions (Font-Quer, 1962; Di Stasi et al., 1994): In the cardiovascular system *Ruta* has been shown to have positive chronotropic and inotropic effects on isolated right atria and to prolong the AV-node refractoriness in isolated rat hearts, suggesting cardiotonic and anti-arrhythmic activities (Chiu and Fung 1997; Khor et al., 2008).

Phytochemical investigations of *R. chalepensis* have reported that the leaves and young stems of the plant are a rich source of several alkaloids, coumarins (Ulubelen et al., 1988), flavonoids, phenols, amino acids, furocoumarins and saponins (Zeichen et al., 2000). Other of the active principles of clinical importance are the psoralens, responsible for hepatotoxicity and methyl-nonyl-ketone, which accounts for effects on the uterus (Günaydin and Savci, 2005). Psoralens or furocoumarins are and photoactive chemicals that applied to the skin and exposed to sunlight produce redness, hyperpigmentation and blistering (Heskel et al., 1983). The sedative-hypnotic potentiation, anxiolytic, anticonvulsant (Mansour et al., 1990) and antinociceptive effects suggest that Ruta chalepensis induces a depressant activity on the CNS (Gonzalez-Trujano et al., 2006).

Several studies have reported the effects on the cardiovascular system of some active compounds isolated from Ruta (Chiu and Fung, 1997; Ushida et al., 2008; Xia et al., 2005; Son et al., 2001; Raghav et al., 2006), however, in alternative medicine an infusion of the whole aerial parts of the plant is commonly used. Therefore, the aim of the present study was to analyze, in adult male rats, the effects of the chronic ingestion of a *Ruta chalepensis* infusion on the vasomotor responses of either intact or endothelium-denuded aortic rings to the alpha-adrenergic agonist phenylephrine and to the M2 muscarinic agonist carbachol.

MATERIALS AND METHODS

**Animals**

Two groups of five adult male Wistar rats (starting weight 150 - 180 g) were used. The animals were randomly allocated to either a control group or an experimental group. Animals of either group were kept during 116 days individually in acrylic cages with free access to food (Purina Chow), and exposed to 12 h light- dark cycle in a temperature controlled room (20 - 23°C). All animals were cared in compliance with the guidelines of Animal Care (NOM-062-ZOO, MEXICO). Rats from the control group had free access to tap water whereas those from the experimental group, instead of water, had access only to a Ruta infusion (20 mg/ml).
In vitro measurements of vascular responses

At the end of the 116 day period, rats, now weighing 250 - 300 g, were killed by cervical dislocation and decapitation. Immediately thereafter a midsternal thoracotomy was performed and the thoracic aorta was excised and placed in a dissection chamber filled with aerated Tyrode’s solution. Under a stereoscopic microscope the aorta was cleaned of connective and adipose tissue. From the central portion of the aorta 2 mm-long rings were cut carefully to avoid damage to the endothelium. In every other ring, the endothelium was thereafter removed by gently rubbing the intimal surface.

For each experiment a pair of rings from the same aorta (one with intact endothelium, the other without a functional endothelium) was used. Each of these rings was suspended horizontally in the same miniature organ chamber (volume 0.5 ml) between two stainless steel hooks. One of the hooks was fixed to the chamber wall while the other was attached to an isometric force transducer (Grass, FT 03, Grass Instruments, Quincy, Mass., U.S.A.). The vessels were continuously superfused with prewarmed (37ºC) aerated (95% O₂ and 5% CO₂) modified Tyrode’s solution (composition in mM: NaCl, 137; KCl, 2.7; MgCl₂, 0.69; NaHCO₃, 11.9; NaH₂PO₄, 0.4; CaCl₂, 1.8 and glucose, 10; pH was adjusted to 7.4). The rings were initially stretched until resting tension reached 2 g and allowed to equilibrate for one hour; during this period the resting tension was continuously monitored (Grass, Model 79 Polygraph, Grass Instruments, Quincy, Mass., U.S.A) and, if needed, readjusted to 2 g by further stretching.

Before starting an actual experiment, the functional integrity of the endothelium was confirmed using phenylephrine and carbachol responsiveness as described elsewhere (Paredes-Carbajal et al., 1995).

Experimental protocol

The contractile response of pairs of aortic rings to increasing concentrations of phenylephrine (10⁻⁹ - 10⁻⁵ M) (concentration-response curve to phenylephrine) was initially recorded and, once tension development in response to highest phenylephrine concentration reached its peak value, superfusion was switched to solutions having in addition to phenylephrine, successively increasing concentrations of carbachol (10⁻⁹ - 10⁻⁵ M) concentration-response curve to carbachol). After a 30 min washout period in Tyrode’s solution, the concentration-response curves were repeated in the presence of the cyclooxygenase inhibitor, indomethacin (10⁻⁶ M). Finally, after another washout period in Tyrode’s solution the curves were obtained in the presence of both indomethacin (10⁻⁶ M) and the competitive inhibitor of nitric oxide synthase no-nitro-L-arginine methyl ester (L-NAME, 300 µM).

The comparison of the means ± SD of the concentration-response curves obtained in rings from the experimental group with those from rings of the control group was used to evaluate the effects of the chronic ingestion the Ruta chalepensis infusion on the vasomotor reactivity.

Data Analysis

The contractile responses induced by phenylephrine are expressed as tension increment, in grams, above the basal tension (imposed on the vessel throughout the experiment). Carbachol-induced relaxations are expressed as the percent of the maximal tension induced by phenylephrine (10⁻⁵ M), PD₂ (-Log of the mean molar concentration of agonist producing 50% of the maximal response) was determined with the software package Graph Pad Prism (San Diego, CA., USA). Data are expressed as means ± S.D. for tension development and as means ± S.E. for PD₂ values.

Comparisons of means were made by One Way Analysis of Variance (ANOVA) and differences between groups were evaluated using Student-Newman-Keuls method (Graph Pad Prism software; St. Louis, MO.). A P value of 0.05 or less was considered significant.

Reagents

Ruta chalepensis was harvested in January 2005 at a farming located at 2325 m above sea level in the town of San Nicolás Tlamínca, Texcoco, Mex. MEXICO. Botanically it was identified in IMSS Herbarium (Mexico D.F), where a copy of reference was deposited, with the number 11, I32 (voucher).

The aerial part of the plant was dried at room temperature, and stored away of sunlight. The infusion was prepared, using raw dry material (stems and leaves) (20g/L), with boiling distilled water. Afterwards, the infusion was kept overnight at room temperature and filtered before its use instead of drinking water in the experimental group.

Glucose was from E. Merck (Darmstadt, Germany); all other chemicals were from Sigma (St. Louis, MO., USA). Indomethacin was dissolved in 4% sodium carbonate. L-phenylephrine hydrochloride, carbachol (carbamoylcholine chloride)
and NO-nitro-L-arginine methyl ester hydrochloride (L-NAME) were dissolved in deionized water.

RESULTS
Rats of the experimental group apparently were healthy and no adverse effects of Ruta infusion on either behavior or the growth curve were observed.

Concentration-response curves to phenylephrine
Rings with a functional endothelium
Rings excised from rats of the experimental group, developed more tension in response to phenylephrine (10⁻⁹ - 10⁻⁵ M) than the rings excised from the control rats group (compare filled and open circles in Figure 1). Tension developed in response to the highest phenylephrine concentration tested (10⁻⁵ M) (maximal tension) amounted to 3.53 ± 0.33 for the experimental group and 2.45 ± 0.21 g, the control group. The concentration-response curve to phenylephrine of rings from the experimental group was shifted to the right of that of rings from the control group, PD₂ values were 6.88 ± 0.03 and 7.12 ± 0.03, respectively.

Table 1 summarizes the maximal tension and the PD₂ values for this series of experiments.

Addition of indomethacin (10⁻⁶ M) to the perfusion solution containing phenylephrine induced a marked reduction in the maximal tension developed by aortic rings from both groups (Table 1). Actually, in the presence of indomethacin maximal tensions was no longer significantly different between the two groups, i.e. inhibition of the cyclooxygenase abolished the increased response to phenylephrine of the rings from the experimental group. These results strongly suggest that the above described increase of tension development in the experimental group is caused, at least in part, by an increased synthesis/release of an endothelium- and cyclooxygenase-dependent vasoconstrictor prostanoid. In the presence of indomethacin the concentration response curve to phenylephrine of rings from the experimental group was shifted to the left of that rings from the control group (6.50 ± 0.01 and 7.00 ± 0.05 respectively, P < 0.05, Table 1).

Table 1
Effects of chronic administration of Ruta chalepensis infusion on the concentration-responses curves to phenylephrine (10⁻⁹ - 10⁻⁵ M) in rat aortic rings with endothelium

<table>
<thead>
<tr>
<th>Groups</th>
<th>PD₂</th>
<th>Max. tension (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.12 ± 0.03</td>
<td>2.45 ± 0.21</td>
</tr>
<tr>
<td>Control + indom.</td>
<td>6.50 ± 0.01₁</td>
<td>0.93 ± 0.2₁</td>
</tr>
<tr>
<td>Control + indom. + L-NAME</td>
<td>6.39 ± 0.02²</td>
<td>2.04 ± 0.25²,³</td>
</tr>
<tr>
<td>Ruta ch.</td>
<td>6.88 ± 0.03ᵃ</td>
<td>3.53 ± 0.33ᵃ</td>
</tr>
<tr>
<td>Ruta ch. + indom.</td>
<td>7.00 ± 0.05₁ᵃ</td>
<td>1.33 ± 0.27₁ᵃ</td>
</tr>
<tr>
<td>Ruta ch. + indom. + L-NAME</td>
<td>6.58 ± 0.07²,³ᵃ</td>
<td>3.31 ± 0.14²,a</td>
</tr>
</tbody>
</table>

PD₂ –log of mean molar concentration causing 50% of maximal response to phenylephrine (10⁻⁵ M). Max. Tension (g) developed in response to phenylephrine (10⁻⁵ M). (+) indom. with indomethacin 10⁻⁶ M. (+) L-NAME with L-NAME (300 µM). Data of max. tension are presented as mean ± SD. Data of PD₂ are presented as mean ± SE. ¹,²,³ Denotes significant differences within the same group. ⁾ Denotes significant differences between groups. n=5 for all groups.
The addition of L-NAME (300 µM) in the presence of indomethacin (10⁻⁶ M) increased maximal tension development in aortic rings from both the experimental and the control group, however the increase in tension development was almost twice as large in the experimental group (from 1.33 ± 0.27 to 3.31 ± 0.14 and from 0.93 ± 0.20 to 2.04 ± 0.25 g, respectively). These results strongly suggest that the basal synthesis/release of NO is increased in the experimental group. In the presence of both indomethacin and L-NAME the concentration-response curve to phenylephrine of rings from the experimental group was shifted to the right of that of the control group (PD₂ 6.58 ± 0.07 and 6.39 ± 0.02 respectively, P < 0.05).

**Figure 1.** Concentration response curves to phenylephrine (10⁻⁹ - 10⁻⁵ M) of aortic rings with endothelium. The curves represent the contractile response to phenylephrine of aortic rings from the control group (●), the experimental group (○), the experimental group in presence of indomethacin (10⁻⁶ M) (▼), and the experimental group in presence of both indomethacin and L-NAME (300 µg/ml) ( ◊ ). The data are expressed as the mean ± S. D. of rings from five rats of each group.

Table 2
Effects of chronic administration of *Ruta chalapensis* infusion on concentration-responses curves to phenylephrine (10⁻⁹ - 10⁻⁵ M) in rat aortic rings without endothelium

<table>
<thead>
<tr>
<th>Groups</th>
<th>PD₂</th>
<th>Max. tension (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.66 ± 0.05</td>
<td>3.24 ± 0.38</td>
</tr>
<tr>
<td>Control + indom.</td>
<td>7.00± 0.08¹</td>
<td>2.56 ± 0.54</td>
</tr>
<tr>
<td>Control + indom. + L-NAME</td>
<td>6.39 ± 0.15²,³</td>
<td>2.46 ± 0.66</td>
</tr>
<tr>
<td><em>Ruta</em> ch.</td>
<td>7.41 ± 0.01ᵃ</td>
<td>3.86 ± 0.61</td>
</tr>
<tr>
<td><em>Ruta</em> ch. + indom</td>
<td>7.16 ± 0.05¹</td>
<td>3.46 ± 0.69</td>
</tr>
<tr>
<td><em>Ruta</em> ch. + indom. + L-NAME</td>
<td>6.64 ± 0.06²,³ᵃ</td>
<td>3.53 ± 0.80</td>
</tr>
</tbody>
</table>

PD₂ –log of mean molar concentration causing 50% of maximal response to phenylephrine (10⁻⁵ M). Max. Tension (g), tension developed in response to phenylephrine (10⁻⁵ M). (+) indom. with indomethacin 10⁻⁶ M. (+) L-NAME with L-NAME (300 µM). Data of max. tension are presented as mean ± SD. Data of PD₂ are presented as mean ± SE. ¹²,³ Denotes significant differences between groups. n=5 for all groups.
Rings without endothelium
Maximal tension developed by endothelium-denuded aortic rings from both groups, in response to either phenylephrine alone or to the combination of phenylephrine and the enzyme inhibitors, was quite similar (Figure 2, Table 2). However, under each of the experimental conditions the PD₂ values calculated from the concentration-response curves differed significantly between the groups (Table 2).

![Figure 2](image-url)

**Figure 2.** Concentration-response curve to phenylephrine ($10^{-9} - 10^{-5}$ M) of aortic rings without endothelium. The curves represent the contractile response of the aortic rings to phenylephrine from the control rats (●), the experimental group (○), the experimental group in the presence of indomethacin ($10^{-5}$ M) (△), and the experimental group in the presence of both indomethacin and L-NAME (300 µg/ml) (◊). The data are expressed as the mean of the rings from five rats of each group. For clarity SD bars were omitted.

Concentration response curve to carbachol
The concentration response curve to carbachol ($10^{-9} - 10^{-5}$ M) of phenylephrine-precontracted rings from the experimental group showed a significant (P<0.05) shift to the right of the corresponding curve of rings from the control group. PD₂ amounted to 5.95 ± 0.04 and 6.50 ± 0.02, respectively. This shift could be induced by the increased synthesis of vasoconstrictor prostanoids mentioned above. The maximal relaxation induced by carbachol was, however, similar in both groups, 65.88 ± 15.32 % in the control group and 56.29 ± 7.72 % in the experimental group (Figure 3, Table 3).

**Table 3**

<table>
<thead>
<tr>
<th>Groups</th>
<th>PD₂</th>
<th>Max. Tension (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.50 ± 0.02</td>
<td>65.88 ± 15.32</td>
</tr>
<tr>
<td><em>Ruta</em> ch.</td>
<td>5.95 ± 0.04*</td>
<td>56.29 ± 7.72</td>
</tr>
</tbody>
</table>

PD₂ –log of mean molar concentration causing 50% of maximal relaxation induced by carbachol ($10^{-5}$ M). Max. tension (%), mean percent decrease in tension compared with maximal tension developed in response to phenylephrine ($10^{-5}$ M). Data of maximal percent relaxation are presented as mean ± SD. Data of PD₂ are presented as mean ± SE. * Denotes difference is significant (p < 0.05). n = 5 for all groups.
DISCUSSION

This study analyzed the effects of the chronic intake of a *Ruta chalepensis* infusion on the responses of aortic rings (with or without endothelium), to the alpha-adrenergic agonist phenylephrine, and to carbachol a predominantly muscarinic 2 agonist. The experiments showed that, in rings with endothelium, the ingestion of *Ruta chalepensis* induces a marked increase in the maximal tension induced by phenylephrine as well as a shift to the left of the concentration-response curve to this agonist. Both effects were reverted by the inhibition of the cyclooxygenase by indomethacin.

These results suggest that the chronic intake of *Ruta chalepensis* induces an increase in the synthesis and/or the release of cyclooxygenase-dependent vasoconstrictor prostanoids. Since this increase in the maximal tension, was not observed in rings without endothelium, it may be assumed that these vasoconstrictors are synthesized by the endothelium. In regard to this interpretation it may be mentioned that Al Mofleh *et al.* (2010), in a very different experimental model, also concluded that an infusion of *Ruta chalepensis* induces a cyclooxygenase-dependent increase in the synthesis of prostanoids.

Since, in the presence of indomethacin in rings with endothelium, the increase in phenylephrine-induced tension caused by the inhibition of NO synthesis, was almost twice as large in the experimental group, it, may be assumed that the chronic ingestion of *Ruta chalepensis* induces, also, an increase in the NO synthesis. It is plausible that the synthesis/release of NO increases as a compensatory mechanism to the enhanced release of vasoconstrictor prostanoids. The increased synthesis/release of NO could, however, also be caused by the action of one or several of the bioactive compounds that are known to be present in *Ruta* (see below). Since the relaxation induced by carbachol in rings with endothelium was similar in both groups, it may be assumed that the chronic ingestion of *Ruta* increases the basal tension-dependent synthesis/release of NO but not its receptor dependent release. On the other hand, the rightward shift of the concentration-response curve to carbachol in the experimental group is, probably, due to the increased release of vasoconstrictor prostanoids discussed above.

Several bioactive compounds have been isolated from *Ruta chalepensis*, including rutin, quercetin, other flavonoids, and terpenoids. It is well established that flavonoids from a great variety of plants produce endothelium-dependent NO/cGMP-mediated vasodilation in vessels of different vascular territories and/or mammals. For example, a previous report from our laboratory suggested, that the relaxing effect of an extract of the mistletoe *Psittacanthus caliculatus* is mediated by the flavonoid quercetin shown to be present in the extract (Rodríguez *et al.*, 2000).
2003). More recently Fuentes and Alarcón (2010) reported, that, Bauhinia candida which is rich in flavonoids reverts the functional impairment of endothelial NO synthesis, in aortic rings from rats with alloxan induced diabetes.

Regarding the effects of rutin and quercetin on the cardiovascular system, several studies report that these compounds have vasodilator effects when administered, either in vivo or in vitro (Chiu and Fung, 1997; Ushida et al., 2008).

Xia et al., (2005), also, reported that rutin and quercetin induce endothelium dependent vasorelaxation in rat thoracic aorta. These authors proposed that the vasodilator effect of quercetin might be mediated by guanylyl cyclase- and cyclooxygenase-dependent pathways, while the vasodilation by rutin might be via a nitric oxide-guanylyl cyclase pathway.

In the present study we explored the vasomotor effects of the chronic ingestion of a *Ruta chalepensis* infusion, simulating, in this manner, the use of this plant in traditional medicine. Under these conditions, the most relevant finding of the study was a marked increase of the vasoconstrictor effects of phenylephrine in aortic rings with endothelium. Therefore, if future investigations confirm such an effect in other vascular beds, the use of this infusion in traditional medicine could be too risky.

**CONCLUSIONS**
In rats, the chronic ingestion of a *Ruta chalepensis* infusion induces an endothelium-dependent increase in both the synthesis/release of cyclooxygenase-dependent vasoconstrictor prostanoids and the basal synthesis/release of nitric oxide.

**ACKNOWLEDGEMENTS**
We want to thank to Dr. Carlos Hernández-Díaz for his careful revision of the manuscript, and Mrs. Ana Lilia Ocampo-Belmont for her skillful technical assistance.

**REFERENCES**


Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas/422