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Full Length Research Paper

Antispasmodic and relaxant activity from the freshwater cyanobacteria *Blennothrix ganeshii* and *Microcoleous lacustris* extracts

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The discovery of novel compounds with pharmaceutical application in cyanobacterias suggests that *Blennothrix ganeshii* and *Microcoleous lacustris* Mexican species can be candidates for the bioprocess of target compounds. The antispasmodic effect of hexane, chloroform, methanol and water extracts of *B. ganeshii* and *M. lacustris* were studied *in vitro* in guinea pig ileum against three spasmogens; acetylcholine, histamine and barium chloride. In all cases, the hexane extract produces a significant antispasmodic effect on the contractions of the rat ileum induced by acetylcholine, histamine and barium chloride. The inhibitory concentration for each was determined. These results show that hexane extract of *B. ganeshii*, and *M. lacustris* possesses both anticholinergic and antihistaminic properties.

Key words: Antispasmodic activity, *Blennothrix ganeshii*, cyanobacteria, *Microcoleous lacustris.*

INTRODUCTION

Cyanobacteria are among the world's most ancient inhabitants. They are single-celled organisms that live in fresh, brackish, and marine water, and use sunlight to make their own food. In warm, nutrient-rich environments, microscopic cyanobacteria can grow quickly, creating blooms that spread across the water's surface and may become visible. Because of the color, texture, and location of these blooms, the common name for cyanobacteria is blue-green algae. Certain cyanobacteria as *Microcystis aeruginosa* (Kützing) Kützing produce cyanotoxins, neurotoxins, endotoxins and hepatotoxins making them dangerous to animals and humans. Several cases of human poisoning have been documented, but a lack of knowledge prevents an accurate assessment of the risks (Carmichael et al., 2001). A large number of cyanobacteria are proving to be rich sources for a variety of bioactive natural products with scientific and *Arthrospira platensis* commercial interest (Skulger, 2000). Some cyanobacteria are sold as food, notably *Aphanizomenon flos-aquae* (Nordstedt). It has been suggested that they could be a much more substantial part of human food supplies (Fogg et al., 1973).

The filamentous mat-forming cyanobacteria, *Blennothrix ganeshii* Watanabe and Komárek and *Microcoleous lacustris* (Rabenhorts) Farlow and Gomont (Oscillatoriaceae) form massive growths in calcareous tropical streams in central Mexico (Valadez et al., 1996; Carmona et al., 2005). There have been no published chemical or pharmacological studies. The present study reports the antispasmodic activity of hexane, chloroform and methanol extracts of cyanobacteria *B. ganeshii*, and *M. lacustris* on guinea pig ileum.

MATERIALS AND METHODS

Source sample collection

B. ganeshii and *M. lacustris* field samples were collected from one spring in Balsas basin, Los Manatiales, Morelos State during February and July 2006 and were taxonomically authenticated at the Phycology Laboratory, Faculty of Sciences, National Autonomous University of Mexico and a voucher specimen of the cyano-

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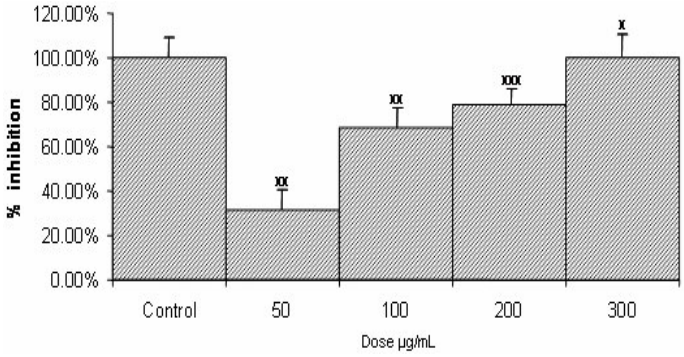

Figure 1. The inhibitory effects of the hexane extract of *B. ganeshii* (50, 100, 200 and 300 µg/ml) on spontaneous contraction ofisolated guinea-pig ileum. Control correspond to responses to papaverine (100 µM), was set as 100% of maximum relaxation. Results are expressed as mean ± S.E.M. *p<0.05, **p<0.01, ***p<0.001 (n=6).

bacteria is stored for reference. After the collection, the samples were kept in an ice chest. Upon return to the laboratory, the samples were washed with distilled water.

Preparation of extract

Blennothrix ganeshii and M. lacustris samples were dried in the shade at room temperature and ground into a fine powder (Pulvex); 100 g of powder each was heated to reflux temperature (Soxhlet) for 3 h with 1.5 L of hexane, chloroform, methanol and water. The solvents were removed under reduced pressure using a rotatory evaporator to constant weight. The percent yields obtained for the hexane, chloroform, methanol and water extracts were of 2.3, 1.16, 3.3 and 2.14% for *B. ganeshii* and 6.46, 1.89, 3.4 and 1.72% for *M. lacustris* respectively. Both extracts were sonicated before addition to the organ bath, acetylcholine, histamine and adrenaline were prepared by adding the substance directly to Tyrode solution.

Biological experimental procedures

Animal's male guinea pigs (250 - 400 g) were used in all experiments. The animals were housed in a cage under conditions of standard light (light on from 7.0 a.m. - 7.0 p.m.), temperature (22 ± 1°C) and room humidity (60 ± 10%) conditions for one week before the experimental sessions. The animals were given a commercial feed prepared by Purina and allowed tap water *ad libitum*.

The procedures involving animals and their care conformed to the international guidelines Principles of Laboratory Animals Care.

Tissue preparation

Male guinea pigs (250 - 400 g) were sacrificed by a blow to the base of the skull and cervical dislocation and 2 cm pieces of the ileum were dissected from the ileum segment 10 - 20 cm proximal to the ileocecal valve. Material was mounted for tension recording and allowed to equilibrate for 1 - 2 h in 10 ml chambers containing Tyrode solution [composition (mM): 136.0 NaCl, 5.0 KCl, 0.98 $MgCl_2$, 2.0 $CaCl_2$, = 0.36 NaH_2PO_4, 11.9 $NaHCO_3$, and 5.5 glucose], pH 7.4 maintained at 37 °C and bubbled with air (5% CO_2 and 95% oxygen). In solution with elevated $[K]^+$, $[Na]^+$ was simultaneously decreased to maintain isosmolarity (Pochocha and Grampurohit, 2001). Concentration-effect curves for extracts were performed by cumulative addition to the bath. In experiments examining the relaxation of the basal tonus of the ileum, paired segments of ileum were set up; one piece exposed to the extract and the other receiving no treatment. Relaxation was taken to be the difference between the tonus of the control and test segments for recording the contractions using force traducers connected to a polygraph (Grass D) as previously described (Astudillo et al., 2004).

Measurement of contractile activity

After stabilization for 30 min, the test extracts were added to the bath. The extracts were dissolved in dimethylsulfoxide (Merck). In control preparations of dimethylsulfoxide, up to 100 µl were added to the organ bath to determine whether this vehicle alone was able to induced contractions. Next, the antispasmodic effect was investigated according to the following experimental schedule. (1) Hexane, chloroform, methanol and water extracts at concentrations of 50, 100, 200 and 300 µg/mL organ bath: 15 min contact period. (2) When stable submaximal responses to standard agonist histamine 3 X 10^{-7} M, acetylcholine 10^{-8} M, and barium chloride 10^{-4} M were obtained, the extracts were added into the bath (Van Den Broucke and Lemli, 1980). Percentage inhibitions of histamine, acetylcholine or barium chloride-induced contraction, in the presence of extracts, were calculated for each concentration (Begum et al., 2000). (3) The median inhibitory concentration (IC_{50}) was determined from the graph plotted of percent inhibition versus log dose.

Data analysis

The inhibition of ileal contractions by extracts were expressed as mean ± standard deviation (SD) of three replicates. Where applicable, the data were subjected to one way analysis of variance (ANOVA) and the differences between samples were determined by Duncan´s multiple range test using the Statistical Analysis System (SAS, 1999) programmer. P values < 0.05 were regarded as significant.

RESULTS

The concentration of extracts which inhibited 50% of response (median inhibitory concentration) IC_{50} was determined from the graph plotted of percent inhibition versus log dose. Addition of hexane extract of *B. ganeshii* (50-300 µg/mL) and *M. lacustris* (50 - 300 µg/mL) elicited a progressively increasing relaxation of the spontaneous tonus of the ileum with IC_{50} = 83.75 µg/mL (c.l.: 79 - 93 µg/mL, n = 6) for *B. ganeshii* and with IC_{50} = 57.67 µg/mL (c.l.: 40-68 µg/mL, n = 6) for *M. lacustris.* The inhibitory effects of hexane extract of *B. ganeshii* and *M. lacustris* on spontaneous contraction of ileum are shown in Figures 1 and 2. In a preliminary screening, the histamine induced contraction in rat ileum with IC_{50} = 22 µg/mL (c.l.: 12-28 µg/mL, n = 6), acetylcholine with IC_{50} = 27 µg/mL (c.l.: 15-30 µg/mL, n = 6), and barium chloride with IC_{50} = 48 µg/mL (c.l.: 25-52 µg/mL, n = 6). The IC_{50} for papaverine, used as a reference compound, were 3.4 µg/mL (c.l.: 1.2-4.5 µg/mL, n = 6), for histamine, 3.8 µg/mL (c.l.: 2.1-5.1 µg/mL, n = 6), for acetylcholine and 3.0 µg/mL (c.l.: 1.8 - 4.2 µg/mL, n = 6) for barium chloride-induced

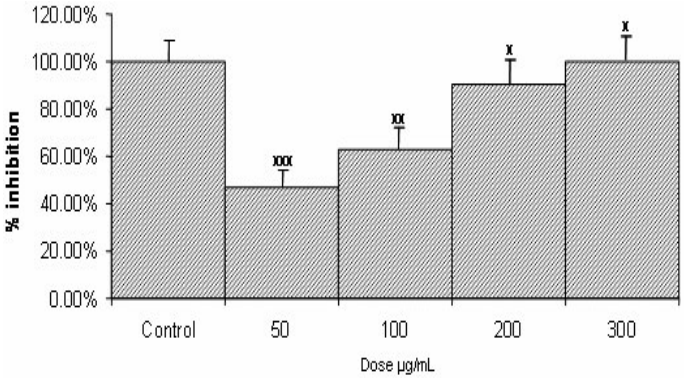

Figure 2. The inhibitory effects of the hexane extract of *M. lacustris* (50, 100, 200 and 300 µg/ml) on spontaneous contraction of isolated guinea-pig ileum. Control correspond to responses to papaverine (100 µM), was set as 100% of maximum relaxation. Results are expressed as mean ± S.E.M. *p < 0.05, **p < 0.01, ***p < 0.001 (n = 6).

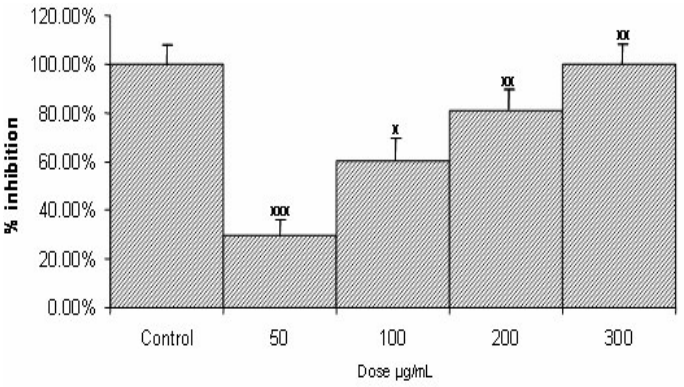

Figure 3. Concentration-response of the inhibitory effects of the hexane extract of *B. ganeshii* (50, 100, 200 and 300 µg/ml) on contraction induced by acetylcholine. Control correspond to respon-ses to papaverine (100 µM), was set as 100% of maximum rela-xation. Each bar represents mean ± S. E. M. *p < 0.05, **p < 0.01, ***p < 0.001 (n = 6).

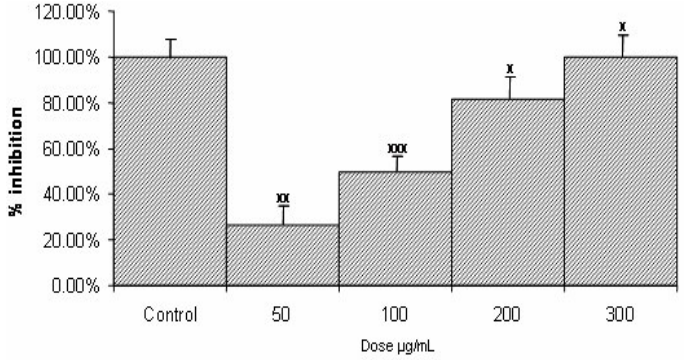

Figure 4. Effect of hexane extract of *B. ganeshii* (50, 100, 200 and 300 µg/ml) on contraction induced by histamine in isolated guinea-pig ileum. Contraction is expressed as a percentage against contraction induced by histamine in the absence of samples. Control correspond to responses to papaverine (100 µM), was set as 100% of maximum relaxation. Each value shows the mean ± S. E. M. *p < 0.05, **p < 0.01, ***p < 0.001 (n = 6).

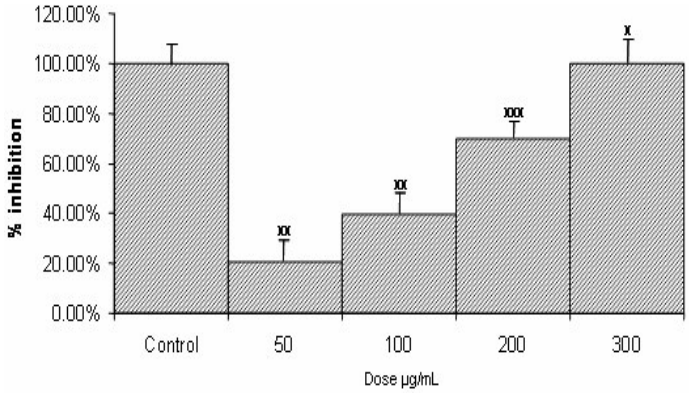

Figure 5. Effect of hexane extract of *B. ganeshii* (50, 100, 200 and 300 µg/ml) on contraction induced by $BaCl_2$ in isolated guinea-pig ileum. Contraction is expressed in % of the maximal contraction obtained in the same tissue before the administration of antispasmodic. Control correspond to responses to papaverine (100 µM), was set as 100% of maximum relaxation. Results are expressed as mean ± S. E. M. *p < 0.05, **p < 0.01, ***p < 0.001 (n = 6).

contractions respectively. The antispasmodic effects of *B. ganeshii* and *M. lacustris* are shown in the Figures 3 to 8.

The hexane extract of *B. ganeshii* showed a concentration-dependent inhibition of tone and the amplitude of spontaneous contraction of ileum with IC_{50} = 62.1 µg/mL (c.l.: 51-79 µg/mL, n = 7), acetylcholine with a IC_{50} = 79.79 µg/mL (c.l.: 63-86 µg/mL, n = 7), for histamine and barium chloride with a IC_{50} = 100.23 µg/mL (c.l.: 89-105 µg/mL, n = 7), IC_{50} = 127.05 µg/mL. (c.l.: 118-132 µg/mL, n = 7) respectively. Addition of hexane extract of *M. lacustris* induced relaxation of basal tone of the ileum with a IC_{50} = 49.86 µg/mL. (c.l.: 34-59 µg/mL, n= 7) with a IC_{50} = 57.67 µg/mL. (c.l.: 47-62 µg/mL, n = 7) for acetylcholine, IC_{50} = 69.02 µg/mL. (c.l.: 58-76 µg/mL, n = 6) for histamine, and IC_{50} = 109.14 µg/mL. (c.l.: 99-113 µg/mL, n = 7) for barium chloride. Methanol, chloroform and water extracts from *B. ganeshii* and *M. lacustris* did not show any inhibitory effects. The IC_{50} for hexane extracts are showed in Table 1.

Hexane extracts were found to antagonize contractions of the rat ileum, induced by acetylcholine, histamine and barium chloride in a concentration-dependent way.

DISCUSSION

The present study has shown that hexane extract from *B. ganeshii* and *M. lacustris* exerts reversible relaxant and antispasmodic effects on guinea-pig ileum. This is in accordance with our previous study indicating that both extracts relax the basal tone of this muscle with maximum amplitude similar to that of the well-charac-

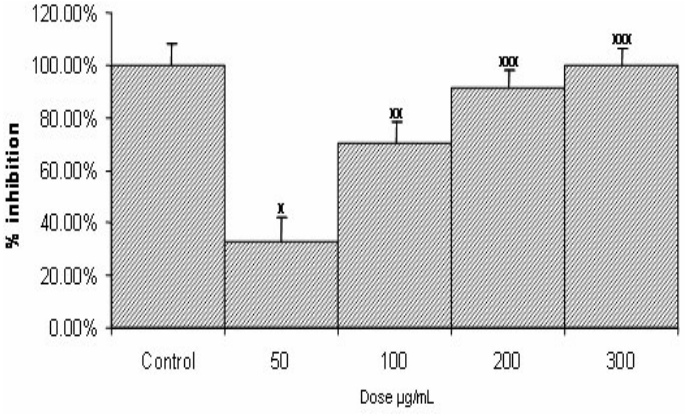

Figure 6. Concentration-response of the inhibitory effects of the hexane extract of *M. lacustris* (50, 100, 200 and 300 µg/ml) on contraction induced by acetylcholine. Control correspond to responses to papaverine (100 µM), was set as 100% of maximum relaxation. Each bar represents mean ± S. E. M. *p < 0.05, **p < 0.01, ***p < 0.001 (n = 6).

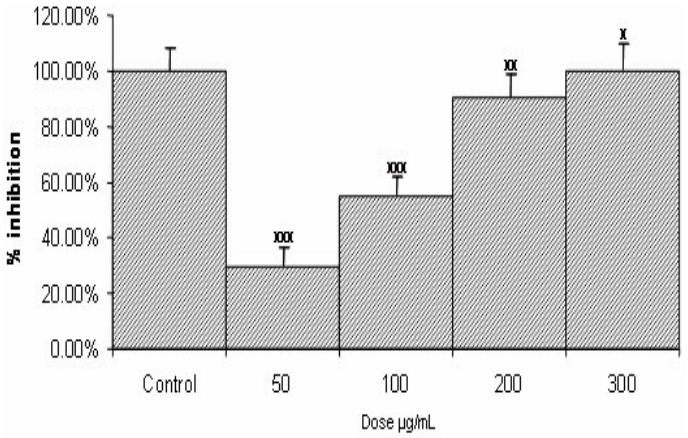

Figure 7. Effect of hexane extract of *M. lacustris* (50, 100, 200 and 300 µg/ml) on contraction induced by histamine in isolated guinea-pig ileum. . Contraction is expressed as a percentage against contraction induced by histamine in the absence of samples. Control correspond to responses to papaverine (100 µM), was set as 100% of maximum relaxation. Each value shows the mean ± S. E. M. *p < 0.05, **p < 0.01, ***p < 0.001 (n = 6).

terized smooth muscle relaxant, papaverine. Our current data show that extracts are also capable of inhibiting the response of a wide range of contractile stimuli, such as neurotransmitters acetylcholine and histamine, barium chloride a release bound (Ca^{2+}) although showing no obvious selectivity between contractile agents. Hexane extract from *B. ganeshii* and *M. lacustris* showed a direct action on the smooth muscles of the guinea-pig ileum. Present experiments demonstrated that both algae cause of spasmogens like acetylcholine, histamine and barium results confirmed that the crude extracts acted as a relaxant effect on ileum, but it also antagonized the effect chloride. The competitive antagonist of contractions

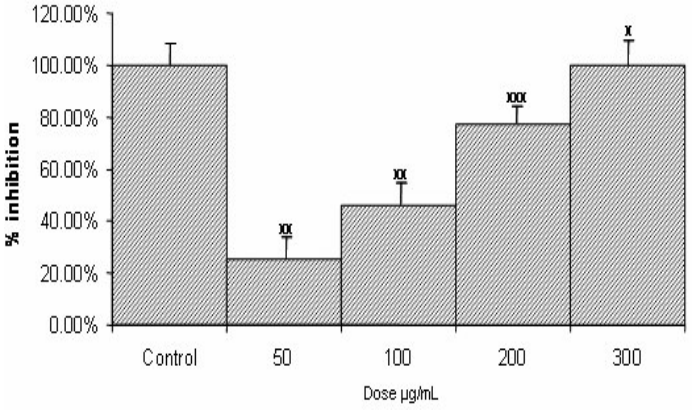

Figure 8. Effect of hexane extract of *M. lacustris* (50, 100, 200 and 300 µg/ml) on contraction induced by $BaCl_2$ in guinea-pig ileum. Contraction is expressed in % of the maximal contraction obtained in the same tissue before the administration of antispasmodic. Control correspond to responses to papaverine (100 µM), was set as 100% of maximum relaxation. Results are expressed as mean ± S. E. M. *p < 0.05, **p < 0.01, ***p < 0.001 (n = 6).

Table 1. Inhibition of contraction of hexane extract of *B. ganeshii* and *M. lacustris* expressed as IC_{50}.

Hexane extract	IC_{50} µg/ml	
	B. ganeshii	***M. lacustris***
Spontaneous contraction	62.7	49.86
Contraction by acetylcholine	79.79	57.67
Contraction by histamine	100.23	69.02
Contraction by barium chloride	127.05	109.14

induced by acetylcholine, histamine and barium chloride.

The antagonism against these spasmogens which have different modes of action suggests that the hexane extracts may act on the contraction mechanism. Acetylcholine, as is well known opens receptor-operated calcium channels and releases calcium from its storage sites, thus inducing phasic and tonic contractions (Bezerra et al., 2000). Since the extract inhibited acetylcholine and histamine-induced spasms, it could be concluded that the hexane extracts inhibited both muscarinic and histaminic receptors. The hexane extracts of the *B. ganeshii* and *M. lacustris* possess both anticholinergic and antihistaminic properties (Hayasi et al., 2002). The effect of the extract on transmitter release and receptor functions as evidenced by inhibition of contractions elicitted by acetylcholine and histamine suggest a neurotropic mechanism (Cunha et al., 2003, Aquino et al., 2001). Furthermore, as barium chloride (an agent that releases bound [Ca^{2+}]) induced spasms were also inhibited, the

extract appears to be also acting by the musculotropic route (Forster et al., 1980) and probably inhibit smooth muscle responsiveness, interfering with Ca^{2+} availability to contractile apparatus by inhibiting the release bound (Ca^{2+}) (Varagic et al., 1984). In conclusion, the data obtained have provided evidence to support the antispasmodic activity of cyanobacterial *B. ganeshii* and *M. lacustris*. Fractionation of the hexane extracts is in progress to identify the active fractions, to isolate and to characterize the actives compounds.

REFERENCES

Aquino R, Tortora S, Tetouani SF, Capasso A (2001). Saponin from roots of *Zygophyllum gaetulum* and their effects on electrically-stimulated guinea-pig ileum. Phytochem. 56: 393-398.

Astudillo A, Hong E, Bye R, Navarrete A (2004). Antispasmodic activity of extracts and compounds of *Acalypha phleoides* Cav. Phytother. Res. 18: 102-106.

Begum S, Sultana I, Siddiqui BS, Shaheen F, Gilani AH (2000). Spasmolytic constituents from *Eucalyptus camaldulensis* var. obtuse leaves. J. Nat. Prod. 63: 1265-1268.

Bezerra MA, Leal-Cardoso H, Coelho-deSouza AN, Criddle DN, Fonteles MC (2000). Myorelaxant and antispasmodic effect of the essential oil of *Alpinia speciosa* on rat ileum. Phytother. Res. 14: 549-551.

Carmichael WW, Azevedo SMFO, An JS, Molica RJR, Jochimsen EM, Lau S, Rinehart KL, Shaw GR, Eaglesham GK (2001). Human fatalities from cyanobacteria: Chemical and biological evidence for cyanotoxins. Environ Health Perspect 109: 663-668

Carmona J, Beltrán MY, Collado VL (2005). Taxonomy and distribution of freshwater *Blennothrix* (Oscillatoriaceae, Cyanophyceae) from central Mexico. Nova Hedwigia 80: 323-334.

Cunha KMA, Paiva LAF, Santos FA, Gramosa NV, Silveira ER, Rao VSN (2003). Smooth muscle relaxant effect of kaurenoic acid, a diterpene from *Copaifera langsdorfi*i on rat uterus *in vitro*. Phytother. Res. 17: 320-324.

Fogg GE, Stewart WDP, Fay P, Walsby AE (1973). The Blue-green Algae. Academic Press, London and New York

Forster HB, Niklas H, Lutz S (1980). Antispasmodic effects of some medicinal plants. Planta Med. 40: 309-319.

Hayasi J, Toshikazu S, Shigeyoshi D, Lin Q, Oiré S, Tsuchiya S, Yano S, Watanabe K, Ikegami F (2002). Phenolic compounds from *Gastrodia* rhizome and relaxant effects of related compounds on isolated smooth muscle preparation. Phytochem. 59: 513-519.

Pochocha N, Grampurohit ND (2001). Antispasmodic activity of the fruits of *Helicteres isora* Linn. Phytother. Res. 15: 49-52.

Skulger CM (2000). Microalgae as a source of bioactive molecules - experience from cyanophyte research. J. Appl. Phycol. 12:341-348

Valadez CF, Carmona J, Cantoral UE (1996). Algas de ambientes lóticos del estado de Morelos, México. Anales Inst. Biol. Univ. Nac. Autón. México, Ser. Bot. 67: 227-282.

Van Den Broucke CO, Lemli JA (1980). Antispasmodic activity of *Origanum compactum*. Planta Med. 38: 317-331.

Varagic VM, Mirovovanovic SR, Srkalovic G (1984). The effect of calcium-chanel blocking agents on the various types of smooth muscle activation of the isolated rat uterus. Arch. Int. Pharmacodyn. Ther. 270:79-81.