Antibacterial effect of Costus spiralis leaves extract on pathogenic strains of Vibrio cholerae

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ABSTRACT. The use of remedies from plant origin covers a wide variety of maladies and constitutes an alternative way to antibiotic therapy, which otherwise seems to be no longer promising due to antibiotics widespread resistance among pathogenic microorganisms. Active principles having antimicrobial activity can be extracted and purified from plants for developing new drugs. Among several illnesses that have historically scourged man, Cholera has been potentially epidemic and one of the most outstanding. It is characterized by watery stools, often accompanied by vomiting, and one of the most outstanding. It is characterized by life-threatening secretory diarrhea with voluminous water up to 100 mg/mL (v/v) and realized the essays of actividad antimicrobiana. Among the pathogenic strains representing the pandemics of the 20th Century: C7258 (O1, El Tor, Ogawa), C6706 (O1, El Tor, Inaba), O395 (O1, Classic, Ogawa), CRC266 (O139) and 569B (O1, Classic, Inaba) were apparently killed, as judged by halos of inhibition of growth in the assays. Adicionalmente, se determinaron las concentraciones mínimas inhibitorias de los extractos para las diferentes cepas. Los resultados anteriores fueron similares a los de la Ampicillina, lo que sugiere que Costus spiralis pude utilizarse como fuente de principios activos contra Vibrio cholerae.

INTRODUCTION

Knowledge on medicinal properties of plants has extensively been applied all over the World to cure or ameliorate suffering of mankind. The use of remedies from plant origin covers a wide variety of maladies and constitutes an alternative way to antibiotics, which is otherwise no longer promising due to the generalized antibiotic resistance among pathogenic microorganisms. Theoretically, active principles having antimicrobial activity can be extracted and purified from plants for developing new drugs. Among several illnesses that have historically scourged man, Cholera has been potentially epidemic and one of the most outstanding. It is characterized by life-threatening secretory diarrhea with voluminous watery stools, often accompanied by vomiting, and resulting in hypoglycemic shock and acidosis. The bacterium Vibrio cholerae, the causal agent, can be eliminated with antibiotics so that besides the traditional treatment of rehydration via oral or intravenous, antibiotics such as tetracycline, ciprofloxacin, norfloxacin or azithromycin are commonly applied. The antimicrobial effect of Costus spiralis (Roscoe) leaves extracts on various pathogenic strains of Vibrio cholerae was assayed in vitro by means of the agar plate diffusion technique. Fresh leaves from this plant were picked up, oven-dried for 48 h at 50 °C, powdered and finally ethanol-extracted. After drying, the remaining residue was suspended in distilled water up to 100 mg/mL (w/v) and the antimicrobial assays carried out. The pathogenic strains representing the pandemics of the 20th Century: C7258 (O1, El Tor, Ogawa), C6706 (O1, El Tor, Inaba), O395 (O1, Classic, Ogawa), CRC266 (O139) and 569B (O1, Classic, Inaba) were apparently killed, as judged by halos of inhibition of growth in the assays. Furthermore, minimal inhibitory concentrations (MICs) of the extracts for the various strains were also attempted. The above results were similar to those from Ampicillin, suggesting that Costus spiralis may be used as a source of active principles against Vibrio cholerae.

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Health Organization recommends antibiotics treatment only in cases of severe dehydration.6

The risk of the illness is currently higher in underdeveloped areas, with no enough sanitary conditions, unsuitable or non-existing water purification systems and elimination of residual waters. Although cholera has effectively been treated everywhere and even vaccination developed, plant medicines must be considered, mostly in such areas where the above strategies might not be available. In fact, there are several reports elsewhere concerning the matter.3,7

Concerning a possible antimicrobial activity of the plant, the antifungal principle methyl ester of para-coumaric acid was isolated from rhizomes of Costus speciosus.13 Furthermore, a cytostatic effect of Costus spiralis aqueous, alcoholic and ketonic extracts was reported in Neurospora crassa.19

The epidemic strains of Vibrio cholerae were used throughout this study. Table 1 shows the corresponding phenotypes.

RESULTS AND DISCUSSION

Inhibition of growth of Vibrio strains

Costus spiralis (Jacq.) Roscoe, belonging to the botanical family Zingiberaceae, has been traditionally known by its anti-inflammatory effect on the urogenital tract, kidney, bladder and venereal diseases such as syphilis and gonorrhea. The plant has been also applied for the treatment of numerous illnesses including diabetes, rheumatism and heart disorders. It has been also confirmed the anti-urolithiatic activity of aqueous extracts of Costus spiralis in rats.11

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The results of inhibition of growth of the various strains of Vibrio cholerae (Table 2) clearly indicates that ethanolic extract from leaves of Costus spiralis should contain a compound or compounds, effective against the bacterium. Although aqueous extracts of the plant (not shown) were also effective, ethanol must be better for extraction, according to the organic chemical nature of such compounds in plants. For instance, it has been reported the presence of flavonol glycosides from leaves of Costus spiralis14 to which otherwise their antimicrobial activity could be attributed.13 In this case, 20 μL of the ethanolic extract was chosen for further assays. Indeed, a similar effect was observed when Vibrio cells were exposed to 20 μL of the antibiotic ampicillin (1 mg/mL), soaked in filter paper discs, for comparison (Table 2).

Moreover, the antimicrobial activity of extracts was checked by suspending O395 cells in LB liquid medium containing the extract at 100 and 1 mg/mL. No survival of 1.7 · 10^6 FCU/mL were detected after 2 h of incubation at 37 ºC when such cultures were plated on LB, suggesting a vibriocidal effect of the extract, at least in this case. Further assays must be carried out to the rest of the strains, in order to confirm this result.

Determination of minimal inhibitory concentrations (MICs).

The response was similar for all strains (MIC of 1 mg/mL) except for 569B, where a MIC of 5 mg/mL was observed (Table 2). This behavior might reveal a difference in the nature of this strain.

Although the exact nature of compounds having antimicrobial effect in Costus spiralis leave extracts
is unknown, these results might be useful for further assays concerning purification of active principles.

Since few folk applications concerning intestinal disorders have been suggested for this plant, Costus spiralis may be considered as an alternative way to antibiotic therapy for cholera. The above results must be supported by further in vivo studies in experimental animal models involving pathogenic strains of Vibrio cholerae. In principle, this phytotherapy could be assumed as highly effective, mostly in those cases where the malady becomes epidemic and sanitary conditions were not promptly available. Additional research must be done, in order to elucidate the actual chemical composition of the compounds involved in the possible vibriocidal effect of this plant.

CONCLUSIONS

The ethanolic extracts from leaves of Costus spiralis must contain active principles showing antimicrobial activity against pathogenic strains of Vibrio cholerae. Costus spiralis may be considered as an alternative way to antibiotic therapy for cholera, mostly in those cases where the malady becomes epidemic and sanitary conditions were not promptly available.

ACKNOWLEDGMENTS

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Table 2. Antimicrobial effect of Costus spiralis leaves extracts on Vibrio cholerae.1

<table>
<thead>
<tr>
<th>Strain</th>
<th>RIZ2 (%)</th>
<th>MIC3 (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>569B</td>
<td>87.5</td>
<td>5.0</td>
</tr>
<tr>
<td>CRC266</td>
<td>100</td>
<td>1.0</td>
</tr>
<tr>
<td>C7258</td>
<td>100</td>
<td>1.0</td>
</tr>
<tr>
<td>C6706</td>
<td>100</td>
<td>1.0</td>
</tr>
<tr>
<td>O385</td>
<td>71.4</td>
<td>1.0</td>
</tr>
</tbody>
</table>

1 The antimicrobial effect was determined by the agar diffusion method. Paper filter discs, soaked with 20 μL of the extract or ampicillin were placed on LB plates, previously seeded with Vibrio cells. After 24 h of incubation at 37 ºC, the plates were checked and diameters of halos of growth inhibition were measured in millimeters.

2 Relative inhibition zones (RIZ) are expressed as follows: RIZ (%) = (IZD SAMPLE / IZD AMPICILLIN)100

where: IZD diameter of inhibition zones (mm).

3 MIC minimal inhibitory concentrations.

BIBLIOGRAPHY


