

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

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Key words: *Costus spiralis*, *Vibrio cholerae*, antimicrobial, pathogenic, plant extract.

RESUMEN. La utilización de remedios de origen vegetal cubre una amplia variedad de enfermedades y constituye una vía alternativa a la terapia con antibióticos, que por otra parte, no parece ser promisorio a largo plazo, debido a la amplia diseminación de la resistencia a los antibióticos entre los microorganismos patógenos. Los principios activos que muestran actividad antimicrobiana pueden ser extraídos y purificados a partir de plantas para el desarrollo de nuevos medicamentos. Entre las diversas enfermedades que históricamente han afectado al hombre, el cólera ha sido potencialmente epidémico y una de las más sobresalientes. La bacteria *Vibrio cholerae*, el agente causal, puede ser eliminado mediante antibióticos, de modo que además del tratamiento tradicional de la enfermedad de rehidratación *via oral* o *intravenosa*, comúnmente son aplicados antibióticos tales como la tetraciclina, ciprofloxacina, norfloxacina o azitromicina. El efecto antimicrobiano *in vitro* de extractos de hojas de *Costus spiralis* (Roscoe) sobre varias cepas patógenas de *Vibrio cholerae* fue ensayado mediante la técnica de difusión en placas de agar. Hojas verdes de la plantas fueron colectadas, secadas en horno a 50 °C durante 48 h, molidas y finalmente, sometidas a extracción con etanol. Luego de secado, el material residual fue resuspendido en agua destilada a 100 mg/mL (p/v) y realizados los ensayos de actividad antimicrobiana. Aparentemente, las cepas patógenas que representan las pandemias del siglo xx: C7258 (O1, El Tor, Ogawa), C6706 (O1, El Tor, Inaba), O395 (O1, Clásica, Ogawa), CRC266 (O139) y 569B (O1, Clásica, Inaba) fueron matadas, a juzgar por la presencia de halos de inhibición de crecimiento en los ensayos. Adicionalmente, se determinaron las concentraciones mínimas inhibitorias de los extractos para las diferentes cepas. Los resultados anteriores fueron similares a los de la Ampicilina, lo que sugiere que *Costus spiralis* puede utilizarse como fuente de principios activos contra *Vibrio cholerae*.

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 may 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. 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*.

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.¹ Theoretically, active principles having antimicrobial

activity can be extracted and purified from plants for developing new drugs.^{2,3}

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.⁴ 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.⁵ The World

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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} The medicinal properties of *Costus spiralis* have been used for treating a wide variety of illnesses, mostly dealing with the urinary tract and kidney disturbances.⁸ This report intends to demonstrate the antimicrobial effect of *Costus spiralis* on Cholera. Here are the results of an *in vitro* antimicrobial assay from leaves extract of this plant on pathogenic strains of *Vibrio cholerae*, which represent the pandemics of the XX Century.

MATERIALS AND METHODS

Microorganisms

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

Media

The LB (Luria & Bertani) broth⁹ was the basal medium for growing cholera cells. For solid media, agar No. 3 (OXOID), 20 g/L was added.

Preparation of plant extracts

Fresh leaves from *Costus spiralis* were gathered and cut to small pieces and oven-dried for 48 h, at 50 °C. The material was powdered and 40 g ethanol-extracted (100 mL) at room temperature, for a week. After extraction and evaporation, the resulting solid material was picked, weighted and suspended in sterile water, up to 100 mg/mL (w/v) for further assays.

Antibacterial assays of extracts

The antimicrobial activity of extracts was determined by the agar diffusion test, with slight modifications.¹⁰ Cultures to be assayed were pre-grown in LB by shaking 200 r/min, at 37 °C, up to 1.0 absorbance λ 600 nm and 10⁶ FCU (forming colony units) plated on Petri dishes containing LB. Sterile filter paper discs (6 mm), soaked with 20 μ L of the extracts, were placed on the surface of the plates. After 24 h of incubation at 37 °C, the plates were checked and the diameters of clearing zones or halos of growth inhibition were measured in millimeters. The same protocol was applied to paper filter discs, soaked in 20 μ L ampicillin (1 mg/mL). The relative inhibition zones (RIZ) are expressed as follows:

$$RIZ (\%) = (IZD_{SAMPLE} / IZD_{AMPICILLIN}) \cdot 100$$

where:

IZD diameter of inhibition zones (mm).

For determining minimal inhibitory concentrations (MICs), 20 μ L of 10-fold serial dilutions from initial extracts were assayed.

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 gonorrhoea. 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

Concerning a possible antimicrobial activity of the plant, the antifungal principle methyl ester of paracoumaric acid was isolated from rhizomes of *Costus speciosus*.¹² Furthermore, a cytostatic effect of *Costus spiralis* aqueous, alcoholic and ketonic extracts was reported in *Neurospora crassa*.¹³

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 spiralis*¹⁴ to which otherwise their antimicrobial activity could be attributed.³ In this case, 20 μ L of the ethanolic extract was chosen for further assays. Indeed, a similar effect was observed when *Vibrio* cells were exposed to 20 μ L 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 $1.7 \cdot 10^8$ FCU/mL were detected after 2 h of incubation at 37 °C 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

Table 1. List of the pathogenic *Vibrio cholerae* strains, used in the assay.

Strain	Biotype	Origin
C7258	O1, El Tor, Ogawa	Perú, 1991.
C6706	O1, El Tor, Inaba	Perú, 1992.
O395	O1, Classic, Ogawa	Calcutta, India, 1964.
569B	O1, Classic, Inaba	Calcutta, India, 1945.
CRC266	O139	Bangladesh, 2000.

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

Strain	RIZ ² (%)	MIC ³ (mg/mL)
569B	87.5	5.0
CRC266	100	1.0
C7258	100	1.0
C6706	100	1.0
O395	71.4	1.0

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 °C, the plates were checked and diameters of halos of growth inhibition were measured in millimeters.

² Relative inhibition zones (RIZ) are expressed as follows:

$$\text{RIZ (\%)} = (\text{IZD}_{\text{SAMPLE}} / \text{IZD}_{\text{AMPICILLIN}}) \cdot 100$$

where:

IZD diameter of inhibition zones (mm).

³ MIC minimal inhibitory concentrations.

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,^{8,15} *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.

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