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Possible Prophylactic Alternatives in Infection with Clostridioides / Clostridium Difficile in Vitro Inhibitory Effect of Linalool and Terpineol

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Abstract

Objective: Clostridium / Clostridioides difficile infections are a current health problem, as evidenced by epidemiological data reflecting the increasing trend in the incidence and severity of cases. Prophylactic measures are a major concern as long as a tendency in bacterial antibiotic resistance becomes the ultimate challenge in the future infectious diseases worldwide. In our study we tested the inhibitory effect of some plant extracts (Linalool and Terpineol) on the development of C. difficile colonies in culture, observing an inhibitory effect of both components, more obviously for Linalool, and dependent on the interaction time for both components. . The results obtained by us agree with data from the literature that support the efficacy of phytobiological compounds as a therapeutic or prophylactic alternative in bacterial infections, which would limit the use of antibiotics in the future.

Methods: Clinically isolated strains of C. difficile were used, cultured on RFC (Reinforce Clostridium) medium. The inhibitory effect of Linalool and Terpineol as phytobiological compounds extracted from plants on C. difficile development was measured by reading the spectrophotometric values at 48 and 96 hours of incubation, and comparing them with the values obtained for the control samples. C. difficile cultures were incubated at 37 ° C in anaerobiosis using RFC medium with Gentamicin added to limit sample contamination.

Results: Statistically significant differences in the development of C. difficile colonies were observed in the Linalool and Terpineol samples compared to the control samples, with the mean spectrophotometric values of 0.1140, p = 0.02 (95% CI) for Linalool and respectively 0.0952, p = 0.049 (95% CI) for Terpineol. There is a slight difference between the two extracts, with Linalool appearing to be more effective than Terpineol in bacterial inhibition. The inhibitory effect persists for 96 hours, but its intensity decreases to values of no statistical significance.

Conclusions: The antibacterial activity of phytobiological compounds has been previously demonstrated, with the advantage of not creating secondary dysbiosis, as in the case of the use of antibiotics. In the case of C. difficile infections, the use of Linalool and Terpineol for prophylactic or therapeutic purposes could be an effective alternative in terms of biological safety and maintaining the balance of the normal microbiota. Similar results obtained in vitro in animals in other diarrheal syndromes of infectious etiology suport the feasibility of our proposal. Further extended in vivo studies are needed to confirm the therapeutic / prophylactic efficacy of Linalool and Terpineol in C. difficile infection and to determine the optimal doses or possible side effects.

Epidemiological context of C. difficile infections and current therapeutic possibilities

Characterization of Linalool and Terpineol with application in the field of microbiology

Description of experiments - inhibitory effects of Linalool and Terpineol on the growth and development of C. difficile colonies in vitro

Introduction

Epidemiological context of C. difficile infections and current therapeutic possibilities

C. difficile is a Gram-positive, anaerobic sporulating bacillus, with significant resistance under hostile conditions in the external medium. It is the number one etiological agent of

nosocomial infections, causing a broad-spectrum diarrheal syndrome, from mild, self-limiting digestive manifestations to pseudomembranous colitis or lingering diarrheal syndromes with multiple relapses or extreme manifestations such as colitis fulminant, with perforation of the colon and death. Among the pathogenic factors involved in the etiology, the use of antibiotics is considered the main trigger of clinical manifestations by inducing a state of intestinal dysbiosis which contributes by insufficiently elucidated mechanisms to the activation of sporulated forms of the bacterium becoming vegetative toxinogenic forms with destructive effect on the intestinal wall.

A recent meta-analysis of 13 relevant studies on the incidence of nosocomial infections with C. difficile reported an average of 8.3 cases per 10,000 patient-days, compared to a hospitalization period ranging from 3 to 21.6 days. (Marra A.R., 2020) Epidemiological studies prior to the COVID-19 period reported an increase in the incidence of C. difficile infection as a whole, along with increased antibiotic resistance, the emergence of hypervirulent strains, and an increase in the number of community infections. The number of C. difficile infections in the context of the COVID-19 pandemic is not yet officially reported, except for isolated studies showing a slight increase in the incidence of cases, but a possible increase in susceptibility to C. difficile infection is taken into consideration due to the coincidence of the vulnerable population segment in both pathologies (elderly patients with comorbidities) and extended use of broad-spectrum antibiotics in patients with COVID-19. Also, the impact of digestive disorders observed in patients with COVID-19 in generating a dysbiotic condition as a starting point for activating C. difficile infections in nosocomial conditions has not been established [1].

There is currently insufficient evidence to support the usefulness of C. difficile infections prophylaxis by concomitant administration of probiotics with antibiotics, unlike other infectious etiologies, the decision to administer these pharmaceuticals for this purpose remaining at the discretion of the physician. As a therapeutic regimen, in the first episode of RDI, the mild, moderate or severe clinical form, it is recommended to use Vancomycin or Fidaxomycin and in their absence, Metronidazole can be tested. In fulminant forms, the dose of Vancomycin can be increased and administrated by enem. In case of relapse, Vancomycin may be used in the same doses as in the first episode or prolonged regimen, with gradual dose reduction. In case of multiple relapses, prolonged treatment with Vancomycin is recommended, followed by Rifaximin or Fidaxomycin or fecal transplantation. New therapeutic alternatives are Beztoxumab-type monoclonal antibodies, which bind to toxin B, vaccines against C. difficile, non-toxinogenic C. difficile strains or oral β-lactamase SYN-004-ribaxamase, which is thought to protect the gut microbiota by lowering the concentration of antibiotics in the intestinal lumen.

Characterization of Linalool and Terpineol with application in the field of microbiology

In the general trend of the medical field to use increasingly evolved therapeutic byotehnological resources, we sustain exploring of more accesible and simple therapeutic alternatives, with proven biological activity and safety in animals and humans, like phytobiological compounds, such as components of plant pollen extracted as essential oils, which have already been shown to be effective in addressing various microbiological pathologies. Of these compounds we chosed to evaluate the inhibitory effects of Linalool and Terpineol on C. difficile development in vitro for their previously demonstrated antibacterial effects [2].

Linalool (C10H18O), 3,7-dimethyl-1,6-octadien-3-ol, a monoterpenic alcohol, the dominant component of essential oils obtained from plants, especially lavender and coriander. This substance has been shown to be bioactive, being recognized for its beneficial effects in inflammatory conditions, cancers, pain syndromes, anxiety, depression and neuroporotection. Bioactive properties are amplified by nanotechnology-based management systems [3].

In infectious diseases, an inhibitory effect on dental plaque pathogenic bacteria, such as Candida albicans, Lactobacillus casei, S. aureus, Streptococcus sobrinus, Porphyromonas gingivalis and Streptococcus mutans, has been shown to be included as an ingredient in various oral hygiene preparations. The in vitro inhibitory effects of Linalool appear to be selective, depending on the tested species with inhibitory effects on E. coli but not on Salmonella thyphimurium strains. Other applications in the field of microbiology studied so far are related to the possibility of reducing bacterial density in respiratory diseases and synergistic effects of Linalool in combination with other essential oils on Plasmodium falciparum [4].

Terpineols are unsaturated monocyclic mono-terpenoid alcohols found in various plants in varying amounts depending on the species, with 5 most common isomeric forms: α, β, γ, δ and terpinen-4-ol, of which α-T and T-4-ol are the main commercial products and are found in large quantities in essential oils, unlike β, γ, and δ, which are rarely found in nature. A-T enantiomers, which are found, for example, in lemon and lavender oils, are widely used in the cosmetics industry. Apart from these applications, compounds of this type are distinguished by a number of biological properties, such as protective effects on the cardiovascular and hypotensive system, antioxidant activity, anticancer activity, anti-nociceptive, gastric protection, anticonvulsant and sedative activity and anti-inflammatory effects. at the level of the bronchial tree [5].

Description of experiments - inhibitory effects of Linalool and Terpineol on the growth and development of C. difficile colonies in vitro

To test the inhibitory effects of Linalool and Terpineol on the growth of C. difficile in culture we performed an experimental series, using 10 different C. difficile strains, clinical isolates provided by the National Institute for Medical-Military Research-Development "Dr. I. Cantacuzino ", originally grown on RFC (Reinforce Clostridium) medium with the addition of Gentamicin to limit the development of other bacterial species. From the obtained cultures we incubated 1800µl culture in 2ml tubes, without any added substances as control samples and the other

with the addition of 100µl of pollen extract - Linalool and Terpineol, these being then incubated at 37˚C, with the first spectrophotometric reading at 48 hours and the second at 96 hours. From the spectrophotometric values obtained for each sample, we extracted the value obtained in the simple RFC medium control sample with Gentamicin [6-8].

The values obtained by spectrophotometric reading at the wavelength λ of 620nm, for each sample at 48h and 96h were summarized in the tables below.

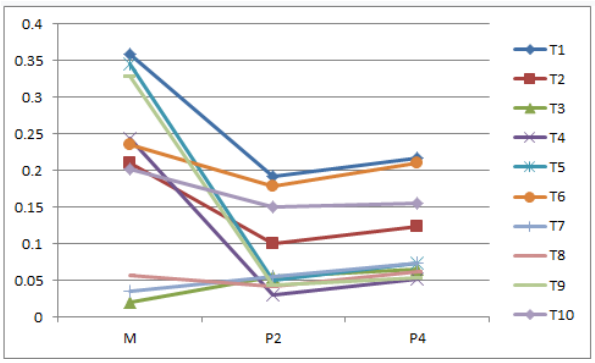

Figure 1: The spectrophotometric values demonstrating the inhibitory effect of Lianalool (P2) and Terpineol (P4) extracts on the 10 different strains of C. difficile cultivated on RFC medium with Gentamicin, after 48h of incubation compared with the values obtained for control samples.

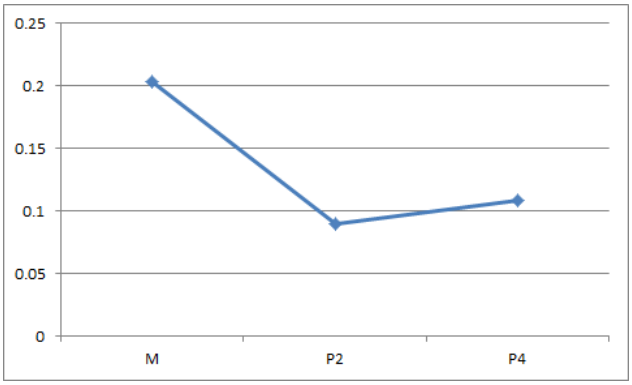

Figure 2: The spectrophotometric medium values demonstrating the inhibitory effect of Lianalool (P2) and Terpineol (P4) extracts on the 10 different strains of C. difficile cultivated on RFC medium with Gentamicin, after 48h of incubation compared with the values obtained for control samples.

The spectrophotometric values at 48 hours reflect the development of C. difficile in all samples used. There is an inhibitory effect of Linalool on the development of C. difficile colonies, with a difference between the mean values of 0.1140 between the values obtained for the control samples (without the addition of Linalool) and those to which Linalool was added, p = 0.02 (CI 95%) and a mean value difference of 0.0952 between the control samples and those containing Terpineol, p = 0.049 (95% CI). Both substances showed inhibitory capacity on C. difficile development, with a slight difference in the intensity of the effect, with Linaloolbeing more efficient in producing inhibiotion than Terpineol [9].

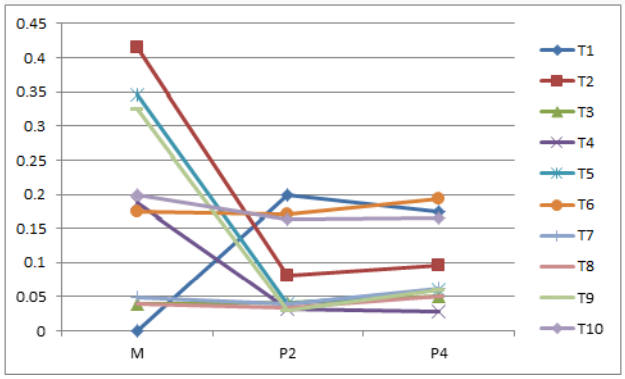

Figure 3: The spectrophotometric values demonstrating the inhibitory effect of Lianalool (P2) and Terpineol (P4) extracts on the 10 different strains of C. difficile cultivated on RFC medium with Gentamicin, after 96h of incubation compared with the values obtained for control samples.

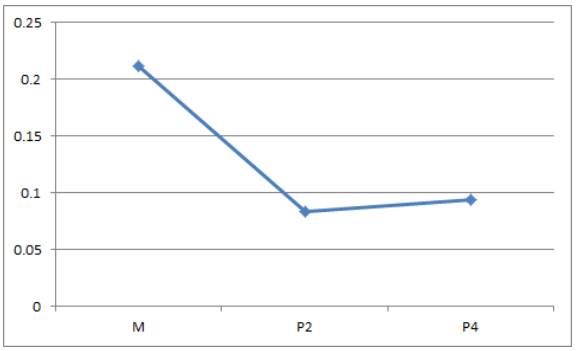

Figure 4: The spectrophotometric medium values demonstrating the inhibitory effect of Lianalool (P2) and Terpineol (P4) extracts on the 10 different strains of C. difficile cultivated on RFC medium with Gentamicin, after 96h of incubation compared with the values obtained for control samples.

Reading the results at 96 hours reflects the maintenance of the inhibitory effect of the two substances added to the environment on the development of C. difficile colonies, following the pattern observed at 48 hours (Linalool has a slightly greater inhibitory effect than Terpineol), but the differences between the average values in the three series have no statistical significance: 0.0937 between the control values and the spectrophotometric values after the addition of Linalool, p = 0.08 (95% CI) and, respectively, the difference of 0.0085 between the mean values in the control samples and the samples in which Terpineol was added, p = 0 .93 (95% CI) [10].

Results and discussion

An inhibitory effect of pollen extracts, for both Linalool and Terpineol, was observed on the development of C. difficile colonies on RFC medium, with a more obvious effect for Linalool in this experiment. The results obtained at 48 hours reflect differences between the mean values between the control samples and those with Linalool of 0.11140, p = 0.02 (95% CI) and 0.0952 respectively between the control samples and those with the addition of Terpineol, p = 0.049 (95% CI). The effect

persists for 96 hours, but the results are not statistically significant, which probably reflects a gradual decrease in the inhibitory effect proportional to the duration of the interaction between the bacterial cultures and the extracts used.

Currently, the management of C. difficile infections in terms of their prophylaxis is focused on measures to limit the spread of spore contamination in hospitals and institutions, the identification of patients at high risk of infection, and use of antibiotics, such Vancomycin selectively administrated in patients with increased risk of infection.

Our study proposes as a prophylactic measure the use of biologically active compounds, pollen extracts, Linalool and Terpineol, as an alternative, in patients at high risk of infection, instead of using antibiotics and probiotics. These compounds are already in therapeutic use in various pathologies, having a biological safety profile already demonstrated. A possible additional advantage of Terpineol is its gastroprotective effect, demonstrated in laboratory animals (ulcer models induced by the administration of indomethacin in rats), with positive effects obtained by cytoprotection, and not by anti-secretory activity and maintaining the gastroprotective effect at subsequent use of compounds from the class of nonsteroidal anti-inflammatory drugs, already demonstrated etiological factors of ulcer disease. This gastroprotective effect of Terpineol could limit the use of antacids (proton pump inhibitors) in critically ill patients who are at increased risk of digestive complications, doubling the protective effect against C. difficile infections, both by replacing the factor risk represented by the use of proton pump inhibitors, as well as the additional intrinsic effect of C. difficile inhibition.

Inhibitory effects similar to those observed in our study are supported by in vivo studies performed in pigs, by the use of citrus essential oils which tried to limit the use of antibiotics for the prophylaxis of digestive infections caused by Escherichia coli (ETEC) after weaning. Resistance to digestive infections when administered with such compounds is due to a variable antipathogenic effect depending on the bacterial species involved (inhibitory effects on S. aureus, Bacillus subtilis, Pseudomonas aeruginosa, Listeria monocytogenes, Salmonella enterica have already been demonstrated), which, unlike antibiotics, has the advantage of the relative resistance of probiotic-type bacterial strains, such as Lactobacillus, for which the inhibitory dose is higher than for pathogenic species, which could be equivalent to a probiotic effect. As far as we know, such phytobiological compounds are the only substances that combine both a selective antibiotic and probiotic effect, being ideal candidates in prevention and treatment of digestive disorder as they posses the valuable quality of not creating intestinal dysbiosis while acting on pathogenic bacterial strains. Possible antiinflamatory additive effects of essential oils could contribute in modulating the pathological intestinal processeses involved in digestive infectious dissorders.

Conclusions

The current epidemiological context of antibiotic-resistant bacterial infections brings to the fore the concern for alternative solutions. At present, research in microbiology has focused on two distinct directions - one aiming at advanced technological solutions and the second at exploiting easily accessible resources, with therapeutic or prophylactic potential still untapped. This includes pollen extracts, such as Linalool and Terpineol, which have an already demonstrated a spectrum of biological activity with multiple applications in the medical and cosmetic fields. With regard to C. difficile infections, sustained efforts to limit the spread of cases and the prospect of expanding antibiotic resistance require, from our point of view, a distinct approach, focusing on prevention rather than treatment per se, changing the vision by correcting triggers in the vulnerable patients segment, and expanding acquisitions in the field to infectious diseases whose therapeutic spectrum is increasingly limited by extensive antibiotic resistance, and this aproach will change the overall infectious pathology picture in the future.

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