Special section

XIII Meeting of the Pharmaceutical Sciences Graduate Program (PPGCF) of the Federal University of Rio Grande do Sul (UFRGS)

Porto Alegre, Brazil - December 6th and 7th, 2021

Presentation

Authorized by the Federal Education Board in 1973, the Pharmaceutical Sciences Graduate Program – UFRGS (PPGCF) (https://www.ufrgs.br/ppgcf/) was Brazil's first graduate course in medical drugs. In the 2010-2012 triennial evaluation carried out by CAPES, the program reached the highest grade (grade seven), because of the efforts made by the faculty, students, and staff in the consolidation of international-grade excellence standards. It means that the PPGCF is both a local and a global reference in pharmaceutical research and advanced studies.

Currently the Program has 39 professors that direct the development of PhD theses and/or MSc dissertations covering several research projects that branch off into three main research areas:

A1 - Development, quality evaluation and use of pharmaceutical and cosmetic services, raw materials, and products: from 34 projects, three present some of their results in the next pages.

A2 - Prospection, synthesis, and biological evaluation of molecules of pharmaceutical interest: from 30 projects, five present some of their results in sequence.

A3 - Investigation of therapeutic targets and biomarkers: from 18 projects, three woks present part of their results in sequence.

The annual meeting

The PPGCF was a pioneer among the Programs in the Brazilian Pharmacy area in the creation of an annual internal event, which began in 2009 with the purpose of bringing together all the staff of the Program to discuss the research results generated, beside to enrich the scientific and technological knowledge based on lectures given by national and international researchers.

Over the years, the meeting has addressed topics involving science, education, internationalization, technology, and innovation, always with the participation of professors and researchers from various national and international institutions.

The 12th edition was held during the COVID-19 pandemic, entirely in digital format and therefore the 1st online edition of the meeting. The 50th anniversary of the program was highlighted on the occasion.

The XIII Meeting of the Pharmaceutical Sciences Graduate Program of UFRGS with the theme: "THE ROLE OF PHARMACEUTICAL SCIENCES ON THE FRONTIER OF INNOVATION", was held on December 6th and 7th, 2021 (https://www.ufrgs.br/encontroppgcf2021/#sobre-o-xiiiencontroppgcf). The event was held remotely with live broadcast on YouTube.

Organizing Committee of the XIII PPGCF Meeting 2021:

Prof. Dra. Irene Kulkamp (Coordinator); Prof. Dr. Alexandre Fuentefria; Prof. Dra. Aline Zimmer; Prof. Dra. Renata Contri; Prof. Dra. Tiana Tasca; Dra. Gabriela Goethel; Dra. Gabriela Machado; Dra. Graciela Carlos; Dra. Luiza Abrahão Frank.

Speakers of the XIII PPGCF Meeting 2021

Prof. Dr. Luiz Carlos Dias (Unicamp, SP); Profa. Dr. Mellanie Fontes-Dutra (UFRGS); Prof. Dr. Everton Terres Cardoso (Unisinos, RS); Profa. Dr. Mairim Russo Serafini (UFSE, SE); M.Sc. Carlos Klein (Ventiur Startup).

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Development and Stability Assessment of Oral Liquid Pharmaceutical Forms Containing Baclofen for Hospital Use

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Research area: Development, quality evaluation and use of pharmaceutical and cosmetic services, raw materials and produtcts (A1)
Subject area: Quality control
Keywords: baclofen; liquid oral formulation; quality by design; stability; chiral drug.

Introduction

The lack of oral liquid pharmaceutical forms suitable for use in pediatric and adult patients with swallowing difficulties is a public health problem, especially in the hospital context (1-2). The chiral drug baclofen is marketed in Brazil only in the tablets form containing its racemate, which highlights the need to develop liquid formulations to facilitate dose adjustment, administration and swallowing (3). This study aimed to develop oral liquid formulations containing the raw material of the drug baclofen, free or not of sugar, as a therapeutic alternative for hospitalized pediatric or adults patients with swallowing difficulties, optimizing them through the quality by design approach (QbD) and assess its stability.

Experimental section

Preliminary stability studies were carried out with twelve pre-formulations containing baclofen (5 $mg.mL^{-1}$) stored at room temperature for 28 days, evaluating aspect, color, odor, pH, viscosity and sedimentation volume after preparation and every seven days. With the aid of the MOODE® 12.1 software, the QbD methodology was applied through experimental screening and optimization designs. High performance liquid chromatography analytical method (HPLC) was developed for the quantitative determination of baclofen in formulations. The formulations were stored in amber glass bottles at room temperature, refrigerated and in an oven and their physical-chemical and microbiological stability was evaluated 14 days after preparation.

Results and Discussion

The analytical method presented selectivity, linearity, detection and quantification limits, precision, accuracy and robustness, in addition to an indicative character of stability, being adequate for the intended purpose. In preliminary stability studies, the preformulations presented variations within the specified limits for the evaluated parameters. The QbD study led to two formulations that met the pre-defined critical quality attributes, one containing glycerin, potassium sorbate, citric acid, liquid flavor, purified water and simple syrup as a vehicle, and the other containing the same excipients, sucralose and sodium carboxymethylcellulose solution as a vehicle. Both formulations presented aspect, color, odor, pH, viscosity, contente and microbiological stability in accordance with the established limits, for 14 days, at the three temperatures evaluated.

Conclusions

The optimized formulations remained stable for a period of 14 days, when stored at room temperature, refrigerated and in an oven. Such formulations have a simplified composition and production process and may serve as a contribution to the preparation of liquid oral formulations containing baclofen in hospital routine, in addition to collaborating with the safety and adherence to the treatment of adult and pediatric patients.

Acknowledgments

Financial support from CNPq/Brazil, doctoral scholarship from CAPES/Brazil and Hospital de Clínicas de Porto Alegre.

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Quality evaluation of nanotechnological hydrogels containing tacrolimus for the treatment of psoriasis.

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Research area: Development, quality evaluation and use of pharmaceutical and cosmetic services, raw mat. ...(A1)
Subject area: Nanotechnology
Keywords: Lipid core nanocapsules; hydrogels; topical route

Introduction

Tacrolimus (TAC) is one of the main immunosuppressive drugs used to treat autoimmune diseases that affect the skin, such as psoriasis. However, therapy can be hampered due to its high lipophilicity, limiting permeation in regions with hyperkeratotic plaques. The use of lipid core (NC) nanocapsules containing encapsulated tacrolimus can be an alternative to increase its apparent solubility and promote its incorporation in the hydrogel, facilitating treatment adherence. In addition, studies on the use of nanoencapsulated drugs for topical administration have shown the importance of the semi-solid dosage form, such as pectin hydrogels, to improve drug permeation and retention and improve sensory characteristics in comparison to traditional presentations (1). It indicates that pectin hydrogels containing LNC to transport TAC via the topical route may become an alternative to existing presentations. Thus, the present work aims to develop pectin hydrogels as carriers of polymeric nanocapsules containing tacrolimus to treat psoriasis.

Experimental section

After obtaining by self-assembling using the solvent displacement method, the formulations with drug and without drug were incorporated into the pectin hydrogel (PEC/LNC-TAC and PEC/LNCb respectively). The parameters analyzed were particle diameter, SPAN, pH, and drug content. To study stability against storage at room temperature, pH and content were analyzed at times 0, 7, 14 and 21 days. The viscosity was determined using a rotational viscometer. The experiments were performed in duplicate.

Results and Discussion

The hydrogels showed a glossy, homogeneous, and white appearance, corresponding to the color of the LNC. The tacrolimus content in the hydrogel approached 100%. PEC/LNCb had a slightly larger diameter of 285±16.97nm, while PEC/LNC-TAC's mean diameters were 231.50±37.48nm. Furthermore, polydispersity (SPAN) was 1.68 ± 0.27 and 1.60±0.42, respectively. These indicated that the presence of the drug does not change physical properties related to particle size. Both hydrogels showed acid pH, 4.29±0.08 and 4.21±0.08, respectively. The slightly acidic formulations are most suitable for dermal use as there will be no disturbance of the acid mantle of normal human skin (2). As for stability, there were no significant changes in the period studied (content remained close to 100%, and the average pH around 4). These results agree with previous studies that studied the stability at room temperature of LNC with PCL and indicated the stability of hydrogels. Regarding the viscosity analysis, PEC/LNCb presented changes in viscosity as a function of speed and the hydrogels were classified as non-Newtonian fluids. Moreover, it was possible to observe increased viscosity in the pectin hydrogel containing the polymeric nanocapsules, this behavior characterizes shear thinning (or pseudoplastic) fluids (3). This increased viscosity can be related to the increased amount of polymer in the formulation containing the nanocapsules present (PEC/LNCb).

Conclusions

The hydrogel obtained showed quality characteristics suitable for topical application and stability for 21 days. Thus, this study opens possibilities for future efficacy evaluations against psoriasis in vitro and in vivo models.

Acknowledgments

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Development of nanoemulsion containing kojic dipalmitate and rosehip oil

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Research area: Development, quality evaluation and use of pharmaceutical and cosmetic raw materials and products (A1)
Subject area: Pharmaceutical technology and bioinformatics
Keywords: kojic dipalmitate; nanoemulsion; rosehip oil; skin whitening.

Introduction

Kojic dipalmitate (KDP) is a highly lipophilic molecule that-suffers hydrolysis in the skin, releasing kojic acid *in situ* (1). It is employed to treat skin hyperpigmentation disorders. Rosehip oil is rich in fatty acids and also has ascorbic acid, phenols and mineral salts (2). The oil has antioxidant activity and acts as healing agent. The objective of this study was to develop a nanoemulsion containing KDP and rosehip oil, evaluating different conditions aiming the most suitable formulation for skin whitening purposes.

Experimental section

An oil in water emulsion was prepared, the oil phase consisting of surfactant, rosehip oil and KDP, and the aqueous phase consisting of surfactant and water. The emulsion was submitted to a high energy method (Ultra-Turrax®) in order to obtain a nanoemulsion (3). Different conditions were employed aiming the most suitable formulation: processing temperature (room temperature or 60ºC), KDP concentration (0.1, 0.2 or 0.3%), total surfactant concentration (5.0 or 7.5%) and processing time (40 or 60 minutes). Formulations were evaluated regarding resistance to centrifugation (30 minutes, 3600 rpm). Samples that did not precipitate had their size distribution analyzed by laser diffraction (Mastersizer®2000, Malvern Instruments, UK).

Results and Discussion

Heating was needed to solubilize KDP (4), so the processing temperature was the first condition tested. Keeping the processing temperature (formulation with 0.1% KDP) at 60ºC allowed the obtainment of a stable formulation, while processing at room temperature led to precipitation. Therefore, heating was necessary. Higher concentrations of KDP were then tested. Incorporation of 0.1% and 0.2% of active did not form precipitate after centrifugation, while 0.3% did. Size distribution profiles of formulations with 0.1% and 0.2% of active showed profiles in the nanometric scale, with satisfactory average diameter (D4,3) and Span values. Hence, higher concentration of KDP was selected. The third parameter analyzed was the concentration of surfactant, which was initially used at 7.5%. Lowering it to 5.0% led to formation of precipitate. Finally, an attempt to reduce processing time was performed, changing the six cycles of 10 minutes each to four cycles. However, when analyzing size distribution profiles, after 40 minutes of processing, there was a micrometric peak. The selected formulation, which contained 5% of rosehip oil, 0.2% KDP, 7.5% of total surfactant, employing temperature of 60ºC during all processing time (60 minutes) in the dispersing equipment, was prepared in triplicate of batches. The developed nanoemulsion presented a homogeneous and opaque aspect, and particle diameter of 121 ± 3 nm with Span values of 0.894 ± 0.092.

Conclusions

A suitable nanoemulsion containing KDP and rosehip oil was developed by a high energy method, at 60ºC for 60 minutes. Surfactants were applied at 7.5% and it was possible to incorporate up to 0.2% of KDP. Perspectives include a full characterization of the formulation including KDP content and stability studies.

Acknowledgments

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8-Hydroxyquinoline analogues for combating difficult-to-treat fungi

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Research area: Prospection, Synthesis and Biological Evaluation of Molecules of Pharmaceutical Interest (A2)
Subject area: Medicinal Chemistry and Pharmacology
Keywords: 8-Hydroxyquinoline, clioquinol, nitroxoline, dermatophytes

Introduction

The number of patients affected by a fungal infection has grown in recent years and affects about 1.2 billion individuals worldwide[1]. Dermatophytes, they are opportunistic fungi related to infections that are difficult to treat due to resistance, since few drugs in a limited number of classes are available on the market. New therapeutic alternatives are being explored to combat the resistance and recurrence of these fungal infections[2]. 8-Hydroxyquinoline and its analogues, clioquinol and nitroxoline, represent important alternatives since they possess a variety of biological properties such as antibacterial and antifungal, all due to their chelating capacity for metal ions, since these metals have a very important function in biological processes[3]. Therefore, the objective was to evaluate the interaction of the clioquinol and nitroxoline in dermatophyte isolates

Experimental section

A total of 8 isolates were included in this study: *N. gypsea* (early classified *M. gypseum*) (2), *M. canis* (2), *T. mentagrophytes* (2) and *T. rubrum* (2). The minimum inhibitory concentrations (MICs) of the antifungal agents clioquinol and nitroxoline for the all isolates were determined by the broth microdilution method according to protocol M38-A2[4]. Checkerboard method was used to evaluate the interaction between antifungal agents. The concentrations tested varied from MIC/8 to MICx8 for each antifungal compound, resulting in 49 combinations[5,6].

Results and Discussion

The concentration range obtained in the susceptibility test was 1.0 - 2.0 µg/mL for nitroxoline and 0.5 - 2.0 µg/mL for clioquinol. Synergistic interaction was observed in 50% of isolates when clioquinol was associated with nitroxoline as shown in table 1.

Table 1: Minimal inhibitory concentration (MIC) of clioquinol (CQ) and Nitroxoline (NT) tested alone and in combination against dermatophytes.

ISOLATES	MIC (µg/mL)		**MIC combination (µg/mL): Nitroxoline + Clioquinol**			
	NT	**CQ**	**NT**	**CQ**	**FICI**	**INTER**
MCA HCPA 10	1.0	1.0	0.250	0.250	0.500	SYN
MCA HCPA 12	1.0	1.0	0.125	0.500	0.625	IND
MGY ATCC	1.0	2.0	0.125	0.500	0.375	SYN
MGY 42	2.0	2.0	1.0	0.0625	0.53125	IND
TME ATCC	1.0	0.5	0.250	0.250	0.750	IND
TME 2 HCPA	1.0	1.0	0.500	0.500	1.0	IND
TRU ATCC	1.0	1.0	0.250	0.250	0.500	SYN
TRU 45	1.0	1.0	0.250	0.250	0.500	SYN

FICI: fractional inhibitory concentration index; INTER: interaction; IND: indifferent; SYN: synergetic.
TRU: *Trichophyton rubrum*; TME: *Trichophyton mentagrophytes;* MCA: *Microsporum canis*; MGY: *Microsporum gypseum*

Authors have demonstrated in the literature the interactions between different antifungal agents and their benefits.[2,7] Our study demonstrated satisfactory results when antimicrobials are used in combination to treat or prevent dermatophytosis. However, more studies are needed to understand the mechanism of action of this combination. Pippi et al (2019) observed that clioquinol acts on the cell wall of dermatophytes but the mechanism of action of nitroxoline is still unknown.

Conclusions

The use of clioquinol in combination with nitroxoline in the treatment of dermatophytosis has been shown to be an important therapeutic strategy and in this way, problems related to therapeutic failure could be avoided.

Acknowledgments

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Secondary metabolites obtained from caatinga plants endophytic microorganisms and evaluation of antimicrobial and antibiofilm activity

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Research area: Prospecting, Synthesis and Biological Evaluation of Molecules of Pharmaceutical Interest (A2)
Subject area: Natural products
Keywords: endophytic microorganism; secondary metabolites; Caatinga biome; Bacterial biofilms.

Introduction

Endophytic fungi are those that live inside plant tissues, but without causing harm to the host(1), present themselves as promising natural sources in the search for molecules with bioactive potential against pathogenic bacteria of medical importance(2).

Experimental section

Two microorganisms endophytic, CAAT 004 and CAAT007, isolated from Caatinga plants, after fermentation and obtaining their crude extracts, mycelial extracts were selected based on previous antibacterial and anti-biofilm formation tests to proceed to fractionation in a C18 cartridge. The resulting semi-purified fractions were evaluated for antibacterial and anti-biofilm formation activity against *Staphylococcus aureus* Newman and *Pseudomonas aeruginosa.*

Results and Discussion

As a result, five fractions of CAAT 004 mycelium extract were active in inhibiting the formation of *S. aureus* Newman biofilm. Fraction of 30% MeOH inhibited 76% of the biofilm at the concentration of 24µg.mL^{-1} and antibacterial activity by 19%, the 60% MeOH fraction inhibited the biofilm by 91% at the concentration of 24µg.mL^{-1} and obtained antibiotic activity of 46%, the 70% MeOH fraction inhibited 92% of the biofilm with a concentration of 48µg.mL-1 and antibiotic activity of 13% for this same concentration, the 80% and 100% MeOH fractions exhibited antibiofilm activity of 76% at the concentration of 48µg.mL^{-1} and showed no antibacterial activity. The fractions did not show significant inhibition of *P. aeruginosa* biofilm. For the CAAT 007 mycelium extract, seven fractions were active against the biofilm of *S. aureus* Newman, they are: the 20% MeOH fraction inhibited the biofilm by 50% (48µg.mL^{-1}) and did not show antibiotic activity, the 30% MeOH fraction inhibited 69% (48µg.mL^{-1}) the biofilm and did not show antibiotic activity, the 40% MeOH fraction inhibited 61% of the *S. aureus* biofilm at a concentration of 24µg.mL^{-1} and 14% antibiotic activity at this same concentration, the 50% MeOH fraction inhibited the biofilm by 62% (48µg.mL^{-1}) and obtained 10% antibiotic activity (6µg.mL^{-1}), the 60% MeOH fraction inhibited the biofilm by 56% (24µg.mL^{-1}) and showed no antibiotic activity, the 70% MeOH fraction inhibited the biofilm by 57% (48µg.mL^{-1}) and 10% antibiotic activity and finally the fraction of 100% MeOH inhibited *S. aureus* biofilm by 50% at a concentration of 48µg.mL^{-1} and was not active in inhibiting bacterial growth. These fractions were not active in the inhibition of the biofilm of *P. aeruginosa*. However, this extract presented two fractions with antibacterial activity for *P. aeruginosa* of 39% and 40% of inhibition for the fractions of 40% MeOH and 60% MeOH, respectively, at a concentration of 48µg.mL^{-1} for the two fractions.

Conclusions

The results presented demonstrate the bioactive potential of secondary metabolites of endophytic microorganisms obtained from Caatinga plants in inhibiting the biofilm of important pathogens such as *S. aureus* Newman and *P. aeruginosa*.

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Drug repositioning for CD73 using a ligand-based virtual screening method

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Research area: Prospection, Synthesis and Biological Evaluation of Molecules of Pharmaceutical Interest (A2)
Subject area: Medicinal Chemistry and Pharmacology
Keywords: Drug Repositioning; Ligand-Based Virtual Screening; Medicinal Chemistry; Ecto-5'-nucleotidase.

Introduction

The ecto-5'-nucleotidase (CD73) converts AMP into adenosine, it is a promising anticancer target (1,2). The drug repositioning (DR) is a strategy to find new uses for drugs and it may help to overcome the challenges on finding new anticancer compounds. One of the DR methods relies on the chemical information of the compounds, which include similarity metrics and Ligand-Based Virtual Screening (LBVS). This work aims to identify several potential inhibitors of CD73 contained in DrugBank dataset through LBVS.

Experimental section

The LBVS was performed by the software SHAFTS (3). The compound AB680 was used as reference and the DrugBank dataset was used as query. The top 10 compounds had the score evaluated and one of them was chosen for testing on recombinant CD73 enzyme by malachite green-based assay. The compound was tested in 50, 75, 100 and 200 µM and compared to the standard inhibitor (AMPCP) at 1.25, 2.5, 5, 10 and 25 µM.

Results and Discussion

The LBVS performed by SHAFTS using AB680 revealed two purine analogs, two sulfonamides and some anti-inflammatories with high similarity to the reference compound (**Table 1**). Naproxen was chosen to be screened against CD73. Although the hybrid score showed a higher value than AMPCP, the compound did not show remarkable inhibitory activity on the tested concentrations (**Figure 1**). This may be related to the possible difficulty of naproxen to interact with important residues of CD73, which were not considered in the screening. Furthermore, the compound did not alter the expression of CD73 in glioma and in peripheral lymphocites (4).

Table 1 - SHAFTS results using AB680 as reference compound.

Rank	Name	HybScore	ShapeScore	FeatureScore
1	Cladribine	1.026	0.7133	0.3128
2	Sulfacytine	0.9948	0.7337	0.261
3	Clofarabine	0.9785	0.7231	0.2555
4	Suprofen	0.9687	0.6927	0.2761
5	**Naproxen**	**0.9684**	**0.6727**	**0.2956**
6	Parecoxib	0.9647	0.7082	0.2566
7	Sulfamethazine	0.9647	0.7226	0.2421
8	Clomifene	0.963	0.7066	0.2565
9	Erlotinib	0.9557	0.6783	0.2774
10	**AMPCP**	**0.9551**	**0.7148**	**0.2403**

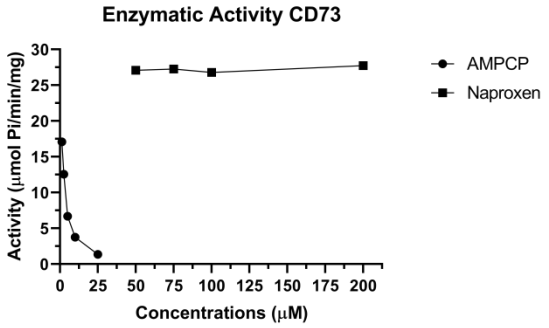

Figure SEQ Figura * ARABIC 1 - Enzymatic activity assay of naproxen in CD73

Conclusions

Although LBVS conducted by SHAFTS revealed RSK2 inhibitors (3), the screened compound naproxen did not show activity compared to AMPCP on the tested concentrations. These analyses may be considered exploratory, since they were based only on compounds' similarities and did not include target information (structural approach). Therefore, more studies and assays will be performed with other compounds and different approaches.

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Phytochemical profile and antioxidant activity of essential oils from *Piper regnelli* and *Piper xylosteoides*

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Research area: Prospecting, Synthesis and Biological Evaluation of Molecules of Pharmaceutical Interest (A2)
Subject area: Natural products
Keywords: Essential oils; antioxidant; *Piper regnelli*; *Piper xylosteoides*

Introduction

Medicinal plants for the treatment of various diseases have been widely used since ancient times, constituting a range of bioactive compounds, with great importance for chemical research and discovery of new drugs for the use of agents in therapeutics (1). Piperaceae family encompassing 12 genera and approximately 3700 species and are widely distributed in tropical and subtropical regions (2). One of the secondary metabolites in this genus are the essential oils that have been the target in search for new substances with antioxidant action. This study aimed the chemical characterization and antioxidant activity of the essential oil from *Piper regnelli* and *Piper xylosteoides* from Rio Grande do Sul.

Experimental section

The essential oils were obtained from crushed fresh samples by hydrodistillation in Clevenger apparatus for 4 hours. The chemical analysis was carried out by gas chromatography coupled to mass spectrometry (GC/MS). The identification of the constituents was based by comparison of both retention indices and mass spectra with authentic samples and data from literature (3). The protection of lipid peroxidation was evaluated by measuring the substances reactive to thiobarbituric acid method (TBARS). Samples of essential oils were tested at concentrations from 100 to 500 µg/mL using the BHT as positive control.

Results and Discussion

The essential oils obtained from *P. regnelli* and *P. xylosteoides* showed an average yield of 0.6±0.1% and 0.3±0.03%, respectively. Chemical composition of the essential oils demonstrated the predominance of apiole (69.2%) and dillapiole (15.4%) for *P. regnelli* and bicyclogermacrene (51.2%) and spathulenol (18.4%) for *P. xylosteoides*. In the antioxidant activity, the results showed that the samples tested at concentration of 500 µg/mL, showed an inhibition profile with 20.0% and 25.6% for *P. regnelli* and *P. xylosteoides*, respectively. Studies report that the compounds apiole and dillapiole are responsible for the biological activities attributed to *Piper* species, justifying the inhibition activity in *P. regnelli* (4). Bicyclogermacrene associated in lower proportions with spathulenol for example, may exhibit antioxidant activity observed in *P. xylosteoides* (5).

Conclusions

Therefore, the data presented in this study, showed apiol and dillapiole-rich essential oil as expected for some *Piper* species, as well as the presence of some sesquiterpenos, such as bicyclogermacrene, corroborating the pattern of compounds found in this genus. The antioxidant potential of essential oils was also determined and contribute with effective alternatives regarding the use of medicinal plants.

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Development of new mean of preservation of human corneas for transplantation with antifungal action spectrum

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Subject area: Medicinal Chemistry and Pharmacology
Keywords: antifungal agents; keratitis; *Fusarium*; combination

Introduction

Corneal transplantation is, in general, a very successful procedure. However, there is a growing incidence of very severe post-transplant infectious complications of fungal origin (keratitis and endophthalmitis), whose diagnosis and treatment can be complex. n this way, they are compromising the success of the procedure and the patients' vision. In view of the importance of this topic, as part of the general objective of the doctoral project, we determined the action potential of the triple combination of two antifungal agents already used together with a potential antifungal agent to be used against *Fusarium* strains, a genus commonly related to these infections.

Experimental section

The three-dimensional Checkerboard assay combining voriconazole, natamycin and clioquinol was performed according to Stein *et al*. (2015), with modifications. The wells of a microplate were filled with 100 µl of solution containing the three antifungal agents in different combinations of concentrations and 100 µl of the fungal inoculum. For each fungal strain and antifungal agent, the concentration ranges tested were selected from previously determined minimum inhibitory concentrations (MICs). The concentrations tested were: MICx2; MIC; MIC/2, MIC/4 and MIC/8 for each antifungal agent. The microplates were incubated at 32° C for 48 hours. The fractional inhibitory concentration index (FICI) for triple combination of antifungal agents was calculated following the formula:

$$FICI = \frac{MIC_A \text{ in combination}}{MIC_A \text{ alone}} + \frac{MIC_B \text{ in combination}}{MIC_B \text{ alone}} + \frac{MIC_C \text{ in combination}}{MIC_C \text{ alone}}$$

Results and Discussion

Voriconazole and natamycin are antifungal agents used in the treatment of eye infections, however, the therapeutic response is not always positive, even when combined. The triple combination (voriconazole, natamycin and clioquinol) showed action potential against *Fusarium* strains, since the synergistic interaction was observed in 60% of them (Table 1).

Conclusions

The combination of the usual antifungal agents (voriconazole and natamycin) with this potential antifungal agent (clioquinol) for the treatment of eye infections caused by fungi of the Fusarium genus represents a promising therapeutic option. The number of strains tested must be expanded and toxicity studies carried out to ensure the efficacy and safety of this combination.

Acknowledgments

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TABLE 1. Minimal Inhibitory Concentration (MIC) for voriconazole, natamycin and clioquinol tested alone and in combination against *Fusarium* strains.

STRAINS	**MIC (µg/mL)**			**MIC COMBINATION (µg/mL)**				
	VOR	**NAT**	**CLIO**	**VOR**	**NAT**	**CLIO**	**FICI**	**INTERACTION**
ATCC 36031	128	16	1	16	2	0.5	0.750	SYN
HCF46	4	16	1	1	4	1	1.500	IND
F9	128	16	1	64	2	0.25	0.875	IND
F21	128	16	1	8	2	0.5	0.687	SYN
F28	64	16	1	8	2	0.25	0.500	SYN

Fusarium solani: ATCC 36031, F28; *Fusarium oxysporum*: HCF46; *Fusarium falciforme*: F9; *Fusarium keratoplasticum*: F21.
MIC: Minimal Inhibitory Concentration.
VOR: voriconazole; NAT: natamycin; CLIO: clioquinol.
SYN (Synergism): FICI ≤ 0.75; IND (Indifference): 0.75 < FICI ≤ 4; ANT (Antagonism): FICI> 4.

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Evaluation of filter paper to transport inactivated Nontuberculous Mycobacteria for identification using the MALDI-TOF MS system

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Subject area: Therapeutic Targets and Biomarkers
Keywords: Nontuberculous Mycobacteria; Filter paper; MALDI-TOF MS; Means of transportation

Introduction

The identification of Nontuberculous Mycobacteria (NTM) is troublesome due to the complexity involved in the sample preparation process and to the time needed to obtain satisfactory results. Usually, the differentiation of species belonging to the *M. tuberculosis* complex from Non-Tuberculous Mycobacteria (NTM) is carried out at the State Center for Health Surveillance in Rio Grande do Sul state. However, the identification of NTM to species-level is only performed in the institution Professor Hélio Fraga in Rio de Janeiro state. Therefore, alternatives are needed to improve secure identification of the NTM regionally. The use of sterile filter paper previously impregnated with an inactivated bacterial biomass can be an option to transport microorganisms between laboratories (1). In addition, identification by MALDI-TOF MS would significantly reduce the time to obtain result (2). This study aimed to evaluate the use of filter paper to transport NTM for identification using MALDI-TOF MS.

Experimental section

A total of 60 isolates of NTM (41 *M. abscessus* complex, 11 *M. fortuitum* complex, 3 *M. avium* complex, 2 *M. smegmatis*, 2 *M. immunogenum*, 1 *M. intracellulare/M. chimaera*) identified by a reference method (PRA*hsp*65) were evaluated in this study. MALDI-TOF MS identification of each isolate was performed as follows: a) directly from original bacterial colony in solid media; b) after the extraction procedure recommended by Bruker®; and c) in filter paper after procedures of inactivation, impregnation and extraction (the paper filter was kept for 2 days in room temperature to simulate transport time). The score values were interpreted as follow: 2.00-3.00 - high-confidence identification (+++); 1.70-1.99 - low-confidence identification (+); 0.00-1.69 - no organism identification possible (-).

Results and Discussion

A total of 24 isolates were identified as (+++), 33 isolates as (+) and 3 isolates were not identified (-) directly from the original bacterial colony. Thirty-eight isolates were identified as (+++), 13 isolates as (+) and 9 isolates were not identified (-) after the extraction procedure. After filter paper disk transportation, a total of 49 isolates were identified as (+++), 10 isolates as (+) and 1 isolate were not identified (-). Considering the reliability of direct identification and after the extraction process, we obtained more high-confidence (+++) isolates after the extraction process, although 9 isolates were not identified after the extraction process, while only 3 isolates were not directly identified from original bacterial colony.

Conclusions

The mycobacterial cell wall, rich in mycolic acids, can make it difficult to directly identify these microorganisms by MALDI-TOF, so the manufacturer recommends the use of an extraction process to improve the sensitivity of the test. MNT present biohazard associated to their transport, as it has the ability to form biofilms and a safe method for identification is essential. Therefore, the inactivation of these microorganisms followed by impregnation in filter paper would make it possible for a large portion of MNT to be identified locally through reference laboratories that provide identification service using the MALDI-TOF MS. Moreover, inactivated MNT in filter paper does not present biohazard for transportation. Therefore, filter paper as a means to transport and storage of inactivated MNT can be considered a potential tool for faster, more accurate, biosafe and less expensive identification.

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"To evaluate the minimal inhibitory concentration (MIC) of clioquinol, cliclopyroxolamine, terbinafine, 8-hydroxyquinoline and dexamethasone, against dermatophytic strains pathogenic to humans and difficult to treat."

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Subject area:
Keywords: dermatophytes; synergism; clioquinol; 8-hidroxiquinoline; dexamethasone;

Introduction

Dermatophytoses or tineas are infections caused by dermatophytes, keratinophilic fungi that can infect the skin, hair and nails (1). The worldwide distribution of these infections and their causative agents vary according to geographic region and several factors, such as: population characteristics, lifestyle, migration of people, cultural practices and socioeconomic conditions, incidence of peculiar comorbidities and drug therapy (2). The knowledge about dermatophytes and their peculiarities are of fundamental importance in view of the numerous reports of resistance to current antifungal therapy (3).

Experimental section

Antifungal susceptibility was evaluated with five isolates (ATCC, TRU2, TRU 45, TRU 47 and TRU 51) of Trichophyton rubrum and five isolates (TME ATTC, TME 2, TME 32, TME 40 and TME 60) of Trichophyton mentagrophytes against cyclopyroxolamine (16 µg/mL), clioquinol (16 µg/mL), 8-hydroxyquinoline (32 µg/mL), terbinafine (0.5 µg/mL), and dexamethasone (2,000 µg/mL). This evaluation was carried out following what was proposed by CLSI, through the document M38-A2 for filamentous fungi.

Results and Discussion

The Minimum Inhibitory Concentration (MIC) achieved for Ciclopyroxolamine was 1 µg / mL against the TRU2, TRU45, TR47, TME32, TME40 and TME60 strains. Regarding Clioquinol, the MIC was 0.25 µg / mL for TRU45, while the MIC for 8-hydroxyquinoline was 0.25 µg / ml for TRU45, while the MIC for Terbinafine was 0.0078125 µg / mL and compared to dexamethasone, no MIC was obtained, as expected. This study aimed to evaluate the minimal inhibitory concentration (MIC) of clioquinol, cyclopyroxolamine, terbinafine, 8-hydroxyquinoline and dexamethasone, against dermatophytic strains pathogenic to humans and difficult to treat.

Conclusions

The drugs tested (clioquinol, 8-hydroxyquinoline, cyclopyroxolamine, terbinafine) are very promising, as they demonstrated concentration-dependent antifungal action against the strains of *Trichophyton rubrum* and *Trichophyton mentagrophytes* tested. And as expected, dexamethasone, as it is a corticosteroid, did not show any antifungal activity.

Acknowledgments

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Temporal Estimation of Latent Fingerprint Analysis: Development of Spectroscopic and Spectrometric Methods with Multivariate Approaches for Forensic Routine.

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Subject area:
Keywords: forensic science; aging fingermarks; infrared; mass spectrometry; chemometrics

Introduction

Fingerprints are a complex biological matrix and it composition can varies greatly depending on the donor and also extrinsic factors[1]. The age of latent is one of the most challenging problems in forensics[2]. The fingermark aging can be defined as the relative or absolute attribution of its age, consisting mainly of determining the time interval that separates the elements analyzed to the current date or determining the relative order of elements in the past, called chronology of events[3]. The development of suitable methods of detecting fingermarks aging may represent an improvement in criminal procedures[4]. In this sense, this doctoral research aim to develop a method using infrared spectroscopy with the use of chemometric tools and a mass spectrometry method with chemical imaging of samples for application in forensic routine.

Experimental section

Review Papers: A bibliographical survey on the subject was carried out, which resulted in two systematic review articles, the first focus on analytical methods applied to fingermark and the second focus on the most important components present fingermarks. The reviews analyzed papers from the last decade and were organized in tables by theme and relevance. **Spectroscopy Method**: Latent fingermarks were collected from three Caucasian female donors. The deposition protocol comprised the collection of the fingermark of the right thumb on a reflective microscope slide provided by Agilent Technologies, previously cleaned with ethanol, exerting a force between 1.0 and 1.5 kg for 15 seconds. Two samples were collected, one from the right thumb and other from the right index finger. The monitored times in the analysis were: hour zero, 3 days, 4 days, 5 days and 6 days. The analyzes were performed on the Cary 630 FTIR Spectrometer (Agilent Technologies) and the results were analyzed using Matlab® software. **Mass Spectrometry Method:** Latent fingermarks were collected from two donors, one male and one female, from the thumb of the dominant hand. The collection was carried out on the same day, repeating the procedure 8 times for each donor (16 analyses), with intervals of 30 minutes between collections. Analyzes were performed at 8 times (0, 1, 2, 7, 15, 21, 30, 50 days) with MALDI source in FT-ICR Mass Spectrometry equipment, (Bruker Daltonics), using 2 $mg.mL^{-1}$ CHCA as matrix.

Results and Discussion

Review Papers: The review was published in the Journal of Forensic Sciences[5] and the main results were: 62% of the selected articles used mass spectrometry as the principal or associated technique for fingerprint analysis, followed by 16% for spectroscopic methods. Also, it is possible to observe a growing use of chemical imaging in this field. The fingermark components review is in the final stage, but it can say that there are important components that can be found in this samples, such as squalene, cholesterol and other fatty acids, as well as contaminants that are strongly present and it can be an important accessory factor to help in fingermark aging surveys. **Spectroscopy Method:** Fingermark Analysis by Fourier Transform Micro Infrared Spectroscopy Using Chemometric Tools was published in the Brazilian Journal of Analytical Chemistry[6] and had as main results: even with different donors, the IR spectrum was similar, although humans did not secrete fatty acids in the hands, these are the most predominant signals on the spectrum; the chemometric analysis made it possible to differentiate sex

donors, as well as aged samples. **Spectrometry Methods:** The results are being processed for a better evaluation, but, it was possible to perform chemical imaging from the collected spectra data from the monitored ions until 50-day aged samples.

Conclusions

From the review studies, the development and advancement of analytical techniques for fingermark analysis is undeniable, a fact that was possible to prove in practice with the developed methods of FTIR and MALDI. The studies carried out allowed separating samples by time, enabling a technique suggestion for temporal estimation of this forensic evidence. These studies need to continue for to apply in the routine of forensic laboratories.

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