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Formulation and Evaluation of a Disinfectant Solution Based on Natural Products

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Abstract: Hands are the main mode of transmission of microorganisms. Hand hygiene is considered the most effective measure of general precautions in the prevention of microbial contamination. The hydroalcoholic solution containing a high concentration of alcohol can cause a burning sensation and marginal irritation of the skin. This is precisely the main reason for the need for a disinfectant solution but which does not have the disadvantages of the hydroalcoholic solution and it is the objective of our end-of-studies dissertation which is the formulation of a disinfectant solution based on essential oils by comparing its effectiveness with the hydroalcoholic solution. In order to measure the antibacterial effect, we performed the aromatogram technique on the hydroalcoholic solution as well as on ours based on essential oils, the effect of which is assessed by measuring the diameter of an area of inhibition. The result obtained allowed us to confirm that the hydroalcoholic solution is effective but it causes skin irritation, while our natural solution has proven to be very interesting for its antibacterial power and its moisturizing effect with aloe Vera.

Keywords: Hygiene, Antibacterial, Microorganism, Hydroalcoolic, Essential oils.

Introduction

Hand hygiene is one of the elements of daily hygiene. From an anatomical point of view, the hands are the gripping tool of Man and serve him to interact with his environment. This external environment is populated by bacterial or viral flora, but also by dirt and toxic elements. Having come into contact with and colonized by these agents, the hands participate in conveying these elements. With the spread of the COVID 19 pandemic since 2019, several manufacturers, in collaboration with researchers, have moved towards the marketing of adequate means of protection to fight against possible contamination.

The optimal practice of hand hygiene, whether by conventional washing with water and medical soap or not, or by hydro alcoholic friction, remains the first measure of prevention of these infections (Pittet et al.,). Unfortunately, people's compliance with this multi-daily gesture is very low, rarely exceeding 50%, mainly due to the dryness and irritation of the skin caused by the frequent use of SHA (Kampf et al., 2007).

Germs on the hands are divided into two groups: resident flora and transient flora. The resident flora consists of micro-organisms permanently anchored in the superficial layers of the skin. This bacterial flora varies qualitatively and quantitatively from one site to another in the same individual as well as from one individual to another. It renews itself regularly and is rarely the cause of infections. (Clin, 2001). The transient flora is most often composed of saprophytic bacteria, from the environment and sometimes of pathogenic bacteria from the commensal flora of treated patients. This flora is the main cause of cross infections. It is made up of Gram-

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negative bacteria (Enterobacteriaceae, Pseudomonas, etc.) and Gram-positive bacteria (Staphylococcus aureus, Streptococcus) (Errouane et al., 2016). Essential oils are defined as volatile and fragrant extracts, which are extracted from certain plants by steam distillation, pressing or incision of the plants they contain (Bruneton, 1999).

The mechanisms by which essential oils exert their antibacterial activity are poorly understood. Due to the complexity of their chemical composition, it is difficult to give a precise idea of the mode of action of EOs. It is likely that their antibacterial activity is not attributable to a single mechanism, but to multiple sites of action at the cellular level (Dorman, 2000).

Burt argued that the important characteristic of essential oils is attributed to the hydrophobicity of some of these components which allows them to easily cross the phospholipid bilayer of the cell membrane by altering its permeability and causing abnormal losses of ions, even macromolecules (Burt, 2004).

Table1. Essential oils

Essential oils	N	Mean
	Melaleuca alternifolia	Broad spectrum antibacterial, Antifungal, Antiviral, pest control
Clou de girofle (Clove)	Syzygium aromaticum, Eugenia caryophyllata	Antibacterial, antiviral, antifungal and antiparasitic, Cauterizing, Stomachic, Anesthetic, Antiseptic
Eucalyptus radié	Eucalyptus radiata	Antibacterial and antiviral stimulates the immune system
Ravintsara	Cinnamomum camphora cineoliferum	Antiviral, Antibacterial

Method

Microbiological Analysis of Essential Oils

The study of the antibacterial power of essential oils is carried out by the method of aromatograms on a Petri dish in order to confirm the antiseptic power of the selected essential oils. The first step consists of soaking the absorbent pads directly with the essential oil extracts and placing them on the culture medium: Muller Hinton. The bacterial strains used are: Escherichia coli, Staphylococcus aureus ATCC, Staphylococcus aureus MRSA, Pseudomonas aeruginosa, Klebsiella pneumonia, Enterococcus faecium, Enterococcus faecium.

Formulation

Nine formulas have been developed and are summarized in the table below:

Table 2. Formulas

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Arbre a the	0,5%	/	/	/	0,25%		0,125	0,5%	/
Clou de girofle	/	0,5%	/	/	/	0,25%	0,125%	0,5%	/
Eucalyptus	/	/	0,5%	/	/	0,25%	0,125%	0,5%	/
Ravintsara				0,5%	0,25%	/	0,125%	0,5%	/
Alcohol								35%	84%
Distilled water	89,5%	89,5%	89,5%	89,5%	89,5%	89,5%	89,5%	50%	9,5%
Aloe Vera	5%	5%	5%	5%	5%	5%	5%	5%	/
Solubilizing	5%	5%	5%	5%	5%	5%	5%	8%	/
Glycerol	/	/	/	/	/	/	/	/	1,5%
H_2O_2	/	/	/	/	/	/	/	/	5%

The same protocol was used for the preparation of the different formulas. Initially, mixture of aqueous excipients, then solubilization of essential oils, finally, mixing of the two solutions and filtration.

Characterization and Controls

All the solutions prepared have been checked on the physico-chemical and microbiological level. Organoleptic properties are determined by examining: color, appearance, clarity, homogeneity. The pH, the density as well as the refractive index were also measured. Stability tests were performed using centrifugation. A volume of 50 ml of each solution is introduced into an appropriate tube and subjected to ultracentrifugation for 3 minutes at 18000 rpm. The antiseptic and disinfectant activity of all the formulas prepared was evaluated by microbiological control using the following bacterial strains: Escherichia coli, Staphylococcus aureus ATCC, Staphylococcus aureus MRSA, Pseudomonas aeruginosa, Klebsiella pneumonie, Enterococcus faecium, Enterococcus faecalis.

Inoculation of the suspension on Mueller Hinton agar for 18 to 24 hours at 37°C. The evaluation of the antibacterial activity is carried out by aromatograms. The method is based on the diffusion of antiseptic solutions formulated in Mueller Hinton medium in a petri dish in contact with the bacterial strain. The effect is assessed by measuring an inhibition zone, and as a function of an inhibition diameter.

Results and Discussion

The result of the organoleptic examination of all the solutions prepared revealed clear, homogeneous solutions with a characteristic odor of each essential oil. The density value was between 0.80 and 1 and the pH value at 6. The refractive index averaged 1.35.

Microbiological Analysis

The result of the antibacterial activity of essential oils is summarized in Table 3. Essential oils have a broad spectrum of activity against microbial strains, except for Pseudomonas aeruginosa which has developed resistance against Clove, Eucalyptus, and Ravintsara extracts and has given poor results, while that in the case of tea tree, it fully inhibited the development of Pseudomonas aeruginosa and gave positive results for all strains. This is why the four essential oils should be combined to guarantee good antibacterial activity.

Table 3. Antibacterial activity of essential oils

Essential oils / Strains	Arbre à thé	Clou de girofle	Eucalyptus	Ravintsara
Escherichia coli	+++	+++	+++	+++
Staphylococcus aureus ATCC	+++	+++	+++	+++
taphylococcus aureus MRSA	+++	++	++	+
Pseudomonas aeruginosa	+++	-	-	-
Klebsiella pneumoniae	+++	+++	+	+++
Enterococcus faecium	+++	+++	+	+
Enterococcus faecalis	+++	++	+++	+++

The result of the antibacterial activity of essential oils is summarized in Table 4.

Table 3. Antibacterial activity of formulas

Formulas / Strains	F1	F2	F3	F4	F5	F6	F7	F8	F9
Escherichia coli	-	-	-	-	-	-	-	++	+++
Staphylococcus aureus ATCC	-	-	-	-	-	-	-	++	+++
Staphylococcus aureus MRSA	-	-	-	-	-	-	-	+++	++
Pseudomonas aeruginosa	-	-	-	-	-	-	-	+	+
Klebsiella pneumoniae	-	-	-	-	-	-	-	+	++
Enterococcus faecium	-	-	-	-	-	-	-	++	++
Enterococcus faecalis	-	-	-	-	-	-	-	+	+

The results obtained in this second step demonstrate that the first seven formulas did not inhibit the development of bacteria, and gave negative results against all bacteria, due to the fact that they are diluted and contain low concentrations of essential oils at 0.5%. While in the case of the eighth formula which contains four essential oils with a concentration of 2%, associated with 35% alcohol, has a broad spectrum of antibacterial activity, and it is more active and more marked against against gram positive bacteria such as Staphylococcus aureus.

For other bacterial strains, its activity is similar to the ninth formula which is the reference solution recommended by the WHO, we have found that the zone of inhibition is almost the same for gram negative bacteria such as Escherichia coli, and gram-positive Cocci such as Enterococcus, so we can conclude that the combination of essential oils with alcohol has produced good results.

Conclusion

Antiseptics are pharmaceutical products for external use with an essential role in the prevention and fight against infections. Their proper use can in some cases limit the use of antibiotics and limit the spread of resistant microbial strains, which poses a serious public health problem.

Dilutions of essential oils at 0.5% have not shown great antibacterial efficacy knowing that the threshold not to be exceeded for an essential oil solution for dermal use must be less than 2%. Secondly we made 4 other solutions including the 8th formula which was 2% essential oils with 35% alcohol, fully inhibited the development of bacteria, and gave positive results for all physicochemical controls with a pH around 6 which is ideal for dermal use.

Recommendations

In order to promote the formulation of disinfectant based on natural products, it is interesting to carry out more tests in order to optimize the quantities of essential oils in the formula and this in order to avoid the use of alcohol in order to increase the antibacterial effect.

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