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EVALUATION OF THE COMBINED BACTERICIDAL ACTIVITY OF ESSENTIAL OILS OF PLECTRANTHUS GLANDULOSUS AND ECALYPTUS PF1

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ABSTRACT

***Aim*:** The aim of this work was to test the sensitivity of 25 strains of *Escherichia coli*, *Staphylococcus. aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Salmonella sp.*, and *Klebsiella pneumoniae* to the essential oils of *Eucalyptus* PF1 and *Plecthranthus galandulosus*. ***Background*:** Plants of the *Plectranthus* and *Eucalyptus* genera are among the most used medicinal plants throughout the world. Their use constitutes one of the solutions to the treatment of bacteria resistant to current antibiotics. ***Objective*:** Identify which of the two essential oils or which of the mixture of the two essential oils or a mixture of the two oils makes it possible to maximize the diameter of the inhibition zone of the bacteria tested. ***Methods*:** The sensitivity of bacteria to essential oils was tested by the mixture design and the well diffusion method in three replicates. The regressions of the diameters of the inhibition zones were processed using the least squares method on Minitab. ***Results*:** Most of the strains tested were significantly inhibited by *Eucalyptus* PF1 and *P. glandulosus*. However, *Ecalyptus* oil was the most effective. Results allowed for validation of linear and quadratic regressions with 15 strains out of the 25 tested. Out of the fifteen optimizations carried out, eleven predicted maximum values of more than 14 mm with 100% *Ecalyptus* oil. ***Conclusion*:** The *Eucalyptus* PF1 essential oil was the best at killing strains of *P. aeruginosa*, *B. cereus*, and *Staphylococcus*.

KEYWORDS: Plectranthus, Eucalyptus, Bacterial, Inhibition, Mixture.

INTRODUCTION

One of the main global public health problems remains the development of new and potent antibiotics. It is true that a large number of bacterial strains have become resistant to the current antibiotics. There are enormous financial and health ramifications to this. Lack of adequate medications to combat resistant germs causes many people to pass away.[1] It is expensive for public authorities and taxpayers to provide ineffective treatment.

Among the world's best-known antibacterial agents are essential oils extracted from aromatic plants.[2] Since ancient times, these oils have been utilized in cooking, cosmetics, perfumes, and medicine. They can be taken as-is or combined with other substances to increase their effects. They are able to treat a wide range of viral, malignant, and metabolic disorders. Many species of bacteria, fungi, viruses, diseases, and protozoa are also susceptible to essential oils. Essential oils form a special area of medicine called aromatherapy.[3] Additionally, essential oils present business potential.[4] Considering their significance and low weight, these are rather expensive but simple to make. In the absence of light, they are both light and stable at ambient temperature. In international trade, a large variety of goods based on these materials play a significant role.

The examined bacterial strains cause a number of illnesses that can be fatal to people. Skin and internal organ infections are caused by *Staphylococcus*.[5,6] Food poisoning is caused by *Bacillus cereus*.[7–9] Foods contaminated with pathogenic strains of *Salmonella* can result in deadly foodborne infections and digestive difficulties.[10–12] Additionally causing more and occasionally dangerous infections are *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.[13–15]

This work is part of our program to develop phytomedicines based on Congolese plants. Indeed, our previous studies revealed that the effects of two-component essential oil blend interactions were greater than those of three or more component interactions, leading us to verify the bactericidal effect of mixtures of essential oils of *Plectranthus glandulosus* and *Eucalyptus* PF1. *Eucalyptus* PF1 is a hybrid cultivar of *Eucalyptus torelliana* x *citrodora* that naturally occurs in the Republic of Congo; *P. glandulosus* is a medicinal Lamicaceae cultivated in Central Africa.[16,17] The genera *Plectranthus* and *Eucalyptus* that are the subject in this study have numerous species that are used for various purposes, particularly in

medicine.[18–22] They are used in traditional and modern medicine.[23,24]

MATERIALS AND METHODS

Materials

It was made up on the one hand of *Plecthrantus glandulosus* and *Eucalyptus* PF1 essential oils, supplied by producers based respectively in Douakani and Dolisie in the southwest of the Republic of Congo, and six different genera represented by the 25 bacterial strains (Table 1) that were isolated and identified from the soils of the Sino-Congolese hospital center in Mfilou and the Bissita hospital center in Bacongo. The bacteria were then stored at -20 °C in an Eppendorf container holding 800 µL of liquid medium and 200 µL of glycerol. The bacteria were isolated for routine tasks in the Cellular and Molecular Biology Laboratory of the Faculty of Science and Techniques of Marien NGOUABI University.

Table 1: Bacteria strains tested.

No	Species	Gram Types	Abbreviations	Number of strains
1	*Bacillus cereus*	+	Bc	4
2	*Klebsiella pneumoniae*	-	Kl	3
3	*Pseudomonas aeruginosa*	-	Pa	4
4	*Salmonella* sp.	-	Sal	4
5	*Staphylococcus aureus*	+	Sta	6
6	*E.coli*	-	Ec	4
Total			5	25

METHODS

Design of experiments

The essential oil samples tested were prepared in accordance with a simplex centroid mixture design with two components: the essential oils of P.g. and E.PF1. The single total was set to 2 ml, and the lower and upper were set to 0 and 2 ml, respectively. The experiment was carried out in three replicates. This gave 15 tests per strain, for a total of 90 runs carried out.

Antibacterial tests

The strains were subcultured on various specific media and then incubated for 24 hours at 37 °C in an incubator (Thermosi SR1000) to obtain a young culture. Antibacterial activity was conducted to assess the oils' capacity to inhibit bacterial growth. The inoculum was prepared from a colony of the strain (E. coli, *S. aureus*, *P. aeruginosa*, *B. cereus*, *Salmonella* sp., and *Klesiella* sp.) with 5 mL of 0.9 % physiological water, and the optical density was adjusted to 0.1 using the spectrophotometer (Zuzi 4255/50) at a wavelength of 625 nm, a

value equivalent to 0.5 McFarland. Each inoculum was inoculated by swabbing onto Petri dishes containing previously poured Muller-Hinton agar. Wells of 6 mm diameter were made, in which a volume of 50 µL of each oil mixture was deposited. Sterile distilled water was used as a negative control and an antibiotic as a positive control. The Petri dishes were incubated at 37 °C in the oven for 24 hours. The bacterial growth inhibition diameters were measured.

Processing of data

Software called Minitab 17.3.1 was used to process the data. The least-squares method was used to examine the regressions. After an analysis, outliers were deleted whenever they appeared. The residual value plots and the coefficients of determination provided evidence for the validity of the regression assumptions. At the significance level of $\alpha = 0.05$, the Anderson-Darling coefficient verified the normality of the residual values. For p values higher than 0.05, the data were regarded as normally distributed. The models employed variance inflation factors (VIF) to characterize the significance of correlations as uncorrelated (FIV<1), moderately correlated (1<FIV<5), or strongly correlated (FIV>5). At the 5% significance level, the effects of the components and their interactions were assessed using analysis of variance (ANOVA). Regression models whose P values were less than the significance level of 0.05 were considered significant. They made it possible to optimize the response, which is the diameter of the inhibition zone. The inhibition diameter was to be maximized through optimization.

RESULTS

Diameters of inhibition zones

The diameter values were found to vary from 0 to 28 mm. The highest values were observed with the *B. cereus*, *P. aeruginosa*, and *S. aureus* strains and the lowest with the *Salmonella sp* and *K. pneumoniae* ones. According to the tests, these three strains seem to be themost sensitive to the essential oils. The essential oils of *P. glandulosus* and E. PF1 effectively inhibited the growth of strains of *B. cereus*, *E. coli*, *S. aureus*, and *P. aeruginosa*.

Table 2: Diameters of inhibition zones of *B. cereus* and *K. pneumoniae* inhibition zones.

Essais	*E.* PF	*P. g*	*B. cereus*				*K. pneumoniae*		
			B.c1	B.c	B.c3	B.c4	Kl1	Kl2	Kl3
1	0.00	2.00	6.00	16.50	5.00	7.00	3.0	0.0	0.0
2	0.00	2.00	6.50	16.00	5.50	7.50	3.0	0.0	0.0
3	0.00	2.00	7.50	15.50	4.50	7.00	3.0	0.0	0.0

4	0.50	1.50	4.00	18.00	0.00	0.00	0.0	0.0	5.0
5	0.50	1.50	4.50	18.50	0.00	0.00	0.0	0.0	4.0
6	0.50	1.50	5.00	18.00	0.00	0.00	0.0	0.0	4.0
7	1.00	1.00	5.00	26.00	0.00	0.00	8.0	10.0	0.0
8	1.00	1.00	5.50	26.00	0.00	0.00	8.0	9.0	0.0
9	1.00	1.00	5.00	26.00	0.00	0.00	7.0	7.0	0.0
10	1.50	0.50	4.00	26.00	3.00	2.00	0.0	0.0	0.0
11	1.50	0.50	5.00	26.00	4.00	3.00	0.0	0.0	0.0
12	1.50	0.50	6.00	26.00	4.00	2.50	0.0	0.0	0.0
13	2.00	0.00	0.00	21.00	0.00	4.00	7.0	0.0	3.0
14	2.00	0.00	0.00	22.00	0.00	4.50	4.0	0.0	0.0
15	2.00	0.00	0.00	22.50	0.00	4.60	4.0	0.0	3.0

Table 3: Diameters of inhibition zones of *P. aeruginosa* and *Salmonella sp.*

Essais	*E. PF*	*P.g*	*P. aerugionesa*				*sp*			
			Pa1	Pa2	Pa3	Pa4	Sal1	Sal2	Sal3	Sal4
1	0.0	2.0	22.0	0.0	10.0	6.0	0.0	0.0	0.0	0.0
2	0.0	2.0	23.0	0.0	12.0	4.0	0.0	0.0	0.0	0.0
3	0.0	2.0	21.0	0.0	11.0	4.0	0.0	0.0	0.0	0.0
4	0.5	1.5	10.0	0.0	20.0	20.0	0.0	0.0	4.0	0.0
5	0.5	1.5	13.0	0.0	21.0	21.0	0.0	0.0	4.0	0.0
6	0.5	1.5	14.0	0.0	19.0	22.0	0.0	0.0	4.0	0.0
7	1.0	1.0	18.0	0.0	10.0	28.0	0.0	0.0	0.0	12.0
8	1.0	1.0	16.0	0.0	11.0	27.0	0.0	0.0	0.0	9.0
9	1.0	1.0	14.0	0.0	12.0	26.0	0.0	0.0	0.0	10.0
10	1.5	0.5	18.0	0.0	15.0	22.0	10.0	0.0	0.0	0.0
11	1.5	0.5	17.0	0.0	16.0	23.0	10.0	0.0	0.0	0.0
12	1.5	0.5	16.0	0.0	17.0	21.0	10.0	0.0	0.0	0.0
13	2.0	0.0	20.0	6.0	26.0	27.0	10.0	0.0	5.0	10.0
14	2.0	0.0	21.0	4.0	25.0	26.0	10.0	0.0	4.0	9.0
15	2.0	0.0	22.0	3.0	26.0	25.0	10.5	0.0	4.5	8.0

Table 4: Diameters of inhibition zones *S. aureus* of and *E. coli.*

Essais	E. PF	P. g	*S. aureus*						*E. coli*			
			St1	St2	St3	St4	St5	St6	Ec1	Ec2	Ec3	Ec4
1	0.00	2.00	12.00	0.00	0.00	0.00	0.00	6.00	5.00	0.00	6.00	0.00
2	0.00	2.00	11.00	0.00	0.00	0.00	0.00	6.00	5.00	0.00	7.00	0.00
3	0.00	2.00	11.00	0.00	0.00	0.00	0.00	7.00	5.00	0.00	6.50	0.00
4	0.50	1.50	0.00	7.00	2.00	0.00	0.00	15.00	5.50	0.00	0.00	3.00
5	0.50	1.50	0.00	8.00	2.00	0.00	0.00	14.00	5.00	0.00	0.00	3.50
6	0.50	1.50	0.00	9.00	2.00	0.00	0.00	16.00	5.00	0.00	0.00	3.80
7	1.00	1.00	0.00	3.00	8.00	12.00	0.00	20.00	10.00	8.00	4.00	0.00
8	1.00	1.00	0.00	4.00	8.00	9.00	0.00	21.00	10.00	9.00	4.50	0.00
9	1.00	1.00	0.00	3.00	9.00	10.00	0.00	20.00	10.00	8.50	5.00	0.00
10	1.50	0.50	0.00	17.00	4.00	13.00	4.00	25.00	3.00	11.00	0.00	4.50
11	1.50	0.50	0.00	16.00	2.00	14.00	4.00	26.00	3.00	11.50	0.00	4.50
12	1.50	0.50	0.00	15.00	3.00	15.00	4.00	24.00	3.50	11.50	0.00	4.50

13	2.00	0.00	15.00	20.00	16.00	14.00	5.00	16.00	4.00	18.00	12.00	7.00
14	2.00	0.00	15.50	21.00	15.00	15.00	5.00	15.50	5.00	18.50	12.50	7.50
15	2.00	0.00	14.00	19.00	15.00	16.00	4.00	16.00	5.00	19.00	12.00	7.30

Modeling the diameters of inhibition zones based on the essential oil ratios

The summary of the regression analysis's findings for the growth inhibition diameter data for the bacterial strains under investigation is presented in Table 5. We note that the coefficients of determination R^2 ranged from 60 to 95% in all but one of the cases. This enabled us to state that the models obtained account for over 60% of the variability of the data. *S. aureus* strains produced coefficients greater than 87%. The models are able to predict the response values by more than 49%, according to the R^2(pred) values. Furthermore, the R^2 (adjust) values indicate that over 54% of the data are consistent with the developed models.

Models with p-values less than the significance level of 0.05 are the ones that were selected. Graphical analysis was done on other assumptions, like normality and independence of residuals. The correlation between the predictors was further verified by the variance inflation factors (VIF close to 1) and the normality of the residual values by the Anderson-Darling test (p AD >0.05). The combination of all the information above led us to validate models with 15 strains out of the 25 that were tested. Ten strains' diameter data, including those of *B. creus* (1), *K. pneumoniae* (3), *Salmonella sp* (3), *S. aureus* (1), and *E. coli* (2) did not fit into either of the two regression models. Table 5 provides the regression coefficients and the coefficients of determination. Table 5 provides the regression coefficients and the coefficients of determination.

Five of the models were linear, and the remaining six were quadratic. The models were written in the following forms

$Y = \beta_1 X_1 + \beta_2 X_2$ (1), for linear regressions and, $Y = \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2$ (2), for quadratic regressions.

Y=value of the inhibition diameter in mm;

B_1andB_2=regression coefficients of essential oils of *P. glandulosus* and of. PF1

B_{12}=coefficient of the interaction of the two oils

Optimization

The values Y_0, $X_{01,}$ and X_{02}. shown in Table 7 were predicted by the optimization, which aimed to maximize the diameter of inhibition of bacterial growth. Out of the twenty-five predictions that were computed, eleven of them were made using 100% (2 ml) of Eucalyptus

PF1 essential oil, two using combinations of the two oils, and the remaining two using 100% of *P. glandulosus* oil. Models predicted diameters of less than 7 mm with *Pg* oil and more than 14 mm with E. PF1 essential oil. In most cases, significant interactions were seen. In a strain of *P. aeruginosa and S. aureus*, the optimization only predicted the maximum diameter values with two mixtures of the two oils.

Table 5: Regression analysis of the inhibition diameters of bacterial strains according to the essential oils of *P. glandulosus* and E. PF1.

Strains	Regression	R2	R2 Prev	R2 adjusted	P_{value}	p_{AD}	Regression coefficients			Y_0	X_{01}	X_{02}
							β_1	β_2	β_{12}			
Bc 1	Linear	61.8	49.2	58.87	0.001	0.28	0.85	3.42		6,833	0.00	2,00
*Bc 2**	Linear	12.7	8.8		0.005	0.03	12.75	8.85		25; 5	2,00	0.00
Bc 3	Regression				0.129							
Bc 4	Quadratic	82.9	75.2	80.11	0.000	0.52	2.56	3.18	-5.88	6; 365	0.00	2,00
Kc 1	Regression				0.505							
Kc 2	Regression				1,000							
Kc 3	Regression				0.928							
Pa 1	Quadratic	73.3	59.8	68.45	0.000	0.95	10.71	10.6	-6.43	21,42	2,00	0.00
Pa 2	Quadratic	77.8	62.5	73.95	0.001	0.15	1.92	0.19	-2.48	3,838	2,00	0.00
Pa 3	Linear	40.2	24.6	35.60	0.011	0.31	10.90	5.83		21.80	2,00	0.00
Pa 4	Quadratic	85.7	79.2	83.30	0.000	0.07	11.89	3.15	10.2	27,10	1,43	0.57
Sal 1	Linear	75.4	69.6	73.48	0.000	0.08	5.05	1.02		10,00	2,00	0.00
Sal 2	Regression				1,000							
Sal 3	Regression				0.219							
Sa 4	Regression				0.133							
Sta 1	Quadratic	94.5	92.2	93.61	0.000	0.28	7.05	5.65	-14.9	14,11	2,00	0.00
Sta 2	Quadratic	82.9	76.2	80.11	0.000	0.52	10.20	0.60	-2.67	20.40	2,00	0.00
Sta 3	Quadratic	68.7	55.9	63.52	0.000	0.20	6.68	0.34	-2.57	13.35	2,00	0.00
Sta 4	Linear	87.5	84.2	86.54	0.000	0.13	8.33	-.47		19,10	2,00	0.00
Sta 5	Quadratic	86.2	80.0	83.91	0.017	0.04						
Sta 6	Quadratic	91.6	87.9	90.16	0.000	0.09	8.55	2.75	10.4	22,50	1,27	0.73
Ec 1	Régression				0,192							
Ec 2	Quadratic	95.8	94.1	95.10	0.029	0.55	9.29	-.38	-2.48	18.57	2.00	0.00
Ec 3	Quadratic	67.8	55.0	62.39	0.001	0.53	5.47	3.21	-8.09	10.94	2.00	0.00
Ec 4	Linear	62.8	55.0	59.88	0.000	0.02						

*Although the p-value of the Anderson-Darling test was less than 0.05, the regression of the

K. pneumoniae 2 strain was validated given the importance of the inhibition diameter values of this strain. optimal Diameter and Respective proportions of the E.PF1 and P.gessential oils that predict the optimal diameter.

CONCLUSION

Most of the strains that were investigated had their growth inhibited by the essential oils *of P. glandulosus* and E. PF1, as well as by their interactions. The obtained data, along with the adjusted values, showed a strong correlation with the prediction variables, which are the oil proportions. The coefficients of determination were over 90%. With the essential oil of E. PF1, the models predicted maximum inhibition diameters of more than 14 mm, and with *P. glandulosus* essential oil, less than 7 mm. It is important to note, however, that the significance of the bactericidal activity examined here is also determined by the various coefficients in addition to the measured inhibition diameter value. High coefficients combined with a low response value may indicate the presence of a very effective active ingredient, but in low quantity in the preparations. More thorough investigations are thus required to clarify this type of situation. Based on the information provided, it seems that the essential oil of the E. PF1 Congolese strain produced superior outcomes. Consequently, it could be chosen for formulations directed against the most susceptible strains of *P. aeruginosa*, *B. cereus*, and *S. aureus*.

CONFLICT OF INTEREST

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (Such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements) or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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