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Micrographic profiling and bio-optimisation of the antibacterial and antioxidant activities of *Solanum lycopersicum* L. (1753) by combining it with *Curcuma longa* L. (1753) extract

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ABSTRACT

Introduction

Oxidative stress and antibiotic resistance are currently a major public health problem; hence, the search for new sources of anti-infective agents is essential.

Purpose

The aim of the present study was to determine the micrographic profile of *Solanum lycopersicum* leaves and *Curcuma longa* rhizomes; and to evaluate the bio-optimization of the antioxidant and antibacterial activity of *Solanum lycopersicum* by association with *Curcuma longa* extract to increase the efficacy of the antioxidant and antibacterial action of *Solanum lycopersicum*.

Methods

The powder was observed using the Steimetz reagent, while antibacterial activity was assessed using the microdilution method in a liquid medium, and antioxidant activity was assessed using the free radical scavenging test with DPPH^{o} and ABTS.

Results

The study showed that the powders of these two species have characteristic elements such as starch grains, fragments of spiral vessels, etc. The antioxidant power of the combination of extracts was greater than that recorded with *Solanum lycopersicum* alone, at the same concentrations, by the two tests used. The antibacterial activity revealed that the synergy of extracts from these two plants was effective against most of the strains tested, even at very low doses in some cases.

Conclusion

These results provide a basis for validating combinations of different plant extracts in the pharmacopoeia of the Democratic Republic of the Congo.

INTRODUCTION

It is well established that oxidative stress and antibiotic resistance are currently a major public health problem; hence, the search for new sources of anti-infective agents is essential (Petithomme et al., 2023). However, the synthetic antioxidants commonly used in the food industry may be responsible for liver damage and carcinogenesis (Masengo et al., 2023), which is why scientists are looking for new alternatives that have fewer side effects, are self-administering, less expansive, and completely reversible (Kabena et al., 2021). Most of these properties are observed in drugs of natural plant origin. Many plants have antioxidant, antibacterial, antifungal, antihelminthic, anticancer, and other properties (Menga et al., 2021).

The World Health Organisation reports that around 80% of Africans rely on plants for their primary health needs (Kabena et al., 2020). The use of plants is not only a matter of choice but is also linked to the high cost of modern medicines, which makes them inaccessible to poor people in Africa (Kabena et al., 2018). The Democratic Republic of the Congo is endowed with immense biodiversity among the richest plants in the world and has a very high number of plants used as medicaments for therapeutic purposes (Ngbolua et al., 2021). These plants represent an immense reservoir of potential compounds attributed to secondary metabolites, which have the advantage of a wide diversity of chemical structures and a very broad range of biological activities (Kabena et al., 2021).

To make the most of the rich flora of the Democratic Republic of the Congo, we chose *Curcuma longa*, a species that is widely used in Congolese pharmacopoeia for its interesting biological properties, in particular as an antioxidant (Borguini et al., 2013), anti-allergic, anti-inflammatory, anti-microbial, anti-thrombotic, anti-atherogenic, healing, and anti-ulcerating (World Health Organisation [WHO], 2004). Although *Curcuma longa* is known for its interesting antioxidant properties, a limiting factor is linked to its low bioavailability (Vaquier, 2010); hence the association of its extracts with those of *Solanum lycopersicum*, a species with cardioprotective, vasodilatory properties, etc. (Balasundram et al, 2006), would optimise the antibacterial and antioxidant potential of the latter. The present study aimed to determine the characteristic elements of these two species and to evaluate the bio-optimisation of the antioxidant and antibacterial activity of *Solanum lycopersicum* by association with *Curcuma longa* extract.

The relevance of this work is clear, as it will enable us to validate the knowledge needed to formulate drugs derived from the combination of these plants.

METHODS

Materials

Plant material

The plant material consisted of *Curcuma longa* rhizomes and *Solanum lycopersicum* leaves. The *Curcuma longa* samples were purchased at the Zigida market from central Kongo (Democratic Republic of Congo), while the *Solanum lycopersicum* leaves were harvested at the Home 30 garden from plant growers in April 2022. This work required 1000 g of fresh *Solanum lycopersicum* leaves and 1000 g of *Curcuma longa* rhizomes.

Our samples were identified by botanical technician N'LANDU from the Herbarium in the Biology Department of the Faculty of Science at the University of Kinshasa.

Microbiological material

This consisted of three reference strains: *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923), and *Escherichia coli* (ATCC 25922) supplied by the Microbiology Unit of the Faculty of Pharmaceutical Sciences.

Methods

Preparation and conditioning of samples

The plant samples were oven-dried (HERAEUS) at a temperature of 34 to 37°C for two weeks. After drying, the plant material was crushed in a grinder and sieved using a USA Standard Testing Steve 1 mm diameter sieve to obtain a fine powder with a particle size of ± 1 mm. The powder obtained was stored in a glass container.

The various analyses were carried out simultaneously at the Food and Nutrition Analysis and Research Laboratory (LARAN) housed in the Faculty of Science, in the Biology Department, and at the Center for the Study of Natural Substances of Plant Origin (CESNOV) in the Faculty of Pharmaceutical Sciences, all at the University of Kinshasa.

Evaluation of powders by optical micrography

Microscopic analysis of the powders of the species studied was carried out according to the method used by Ngbolua et al. (2021).

The powders were observed using Steimetz reagent. For microscopic analysis, two drops of lactic acid reagent deposited on the slide were mixed with a small quantity of powder and then covered with a protective glass. The resulting microscopic preparation was heated to boiling (Inkoto et al., 2018). Observations and photos were made with the OLYMPUS microscope model CH10BIMF and photos were taken with the TECNO SPARK 7 Smart Phone.

Phytochemical screening

Phytochemical screening involves several qualitative analyses to identify the secondary metabolites (alkaloids, saponins, total polyphenols, flavonoid polyphenols, flavonoids, tannins, anthocyanins, leuco-anthocyanins, quinones, terpenes, and steroids) present in a given sample (Kabena et al., 2021). These chemical groups are detected by staining and precipitation reactions that occur with the addition of specific reagents. This phytochemical screening was carried out using the standard protocol modified by Kabena et al., 2020.

Estimation of phytomarker content

Samples for quantitative analysis were prepared from 40 mg of each extract dissolved in 40 mL of solvent (methanol).

Preparation of the Folin-Ciocalteu reagent

Ten grams (10 g) of sodium tungstate ($Na_2WO_4.2H_2O$) and 2.5 g of sodium molybdate ($Na_2MoO_4.2H_2O$) were dissolved in 70 mL of distilled water. Add 50 mL of 85% phosphoric acid (H_3PO_4) (d=1.71) and 10 mL of 36% concentrated hydrochloric acid (d=1.19). Boil under reflux for 10 hours, then add 15 g of lithium sulphate (Li_2SO_4), and a few drops of bromine and boil again for 15 minutes, cool and make up to 100 mL with distilled water.

The total polyphenol and flavonoid content were estimated using the protocol developed by Ngbolua et al. 2021.

Assessment of antibacterial activity

Determination of the minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was determined using the broth micro-dilution method as described in our previous research (Ngbolua et al., 2019).

Inocula of the microorganisms used were prepared from 24-hour-old broth cultures. The prepared microbial suspension was diluted (1/100) to obtain 106 CFU/mL. Stock solutions of the plant extracts were prepared in Tween 80 (Fisher chemicals) (3 mg/300 µL) and diluted to 2.7 mL with Mueller Hinton broth (MHB) to obtain a final Tween 80 concentration of 0.1%. This solution was transferred to 96-well plates (200 µL/well) and serially diluted twice with MHB to obtain final concentrations ranging from 1000 to 4 µg/mL.

Determination of the minimum bactericidal concentration (MBC)

The MBC was determined by inoculating 10 µL of the contents of the microplate wells onto a nutrient substrate of the contents of the microplate wells onto nutrient agar with no bacterial growth visible from the MIC. The inoculated plates were incubated for 24 hours at 37°C. The lowest concentration at which no growth occurred in the subculture was taken as the BMC.

Determination of bactericidal or bacteriostatic properties

The microdilution method in a liquid medium was used to determine the minimum inhibitory concentration (MIC). The minimum bactericidal concentration (MBC) was determined by growing the cultures obtained with the MICs on a solid medium.

These two variables were used to indicate whether the effect of our extracts was bacteriostatic or bactericidal against bacteria. When the MBC/MIC ratio is greater than 4 and less than 16, the product is said to be bacteriostatic; if the ratio is less than or equal to 4, the product is considered to be bactericidal. On the other hand, when it is equal to 1, the extract is said to be absolutely bactericidal (Sumalee et al., 2020). However, when this ratio is equal to or greater than 32, the bacteria are tolerant to the extract (Moroh et al., 2008).

Statistical analysis

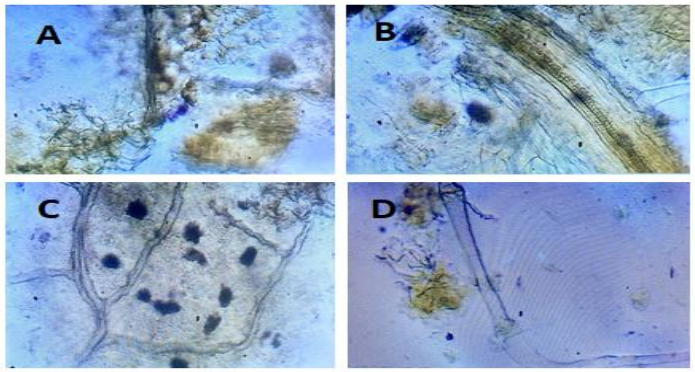
We used Grap Pad Prism 6.0 software for statistical analysis and determination of IC50s. The ANOVA test was used to determine the means, standard deviations, and comparison of sample means, followed by Tukey's pairwise multiple comparison test. The significance threshold was set at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Micrography

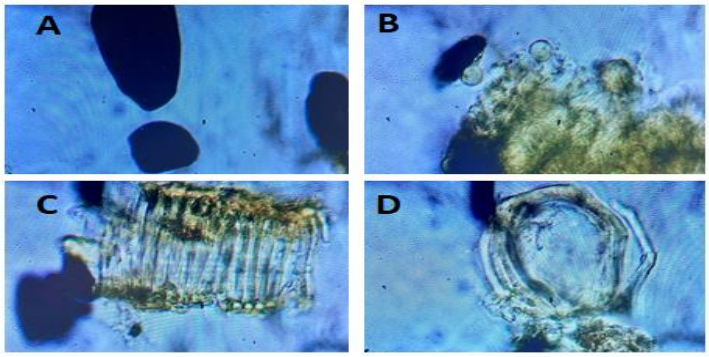
Microscopic analysis of the powders of the species studied revealed the presence of the following histological elements (**Figures 1 and 2**):

Figure 1:
Microscopic characteristics of *Solanum lycopersicum* powder (A, B, C and D) viewed under a light microscope (binocular). Total magnification = 250 X



Microscopic examination of the *Solanum lycopersicum* powder revealed the presence of the cells to be characterised (A), isolated starch grains stained black by the Steimetz reagent, filaments (B), starch grains grouped inside the polyhedral cells (C) and the tector hairs (D).

Figure 2:
Microscopic characteristics of *Curcuma longa* powder (A, B, C, and D) viewed under a light microscope (binocular). Total magnification = 250 X



Microscopic examination of *Curcuma longa* powder revealed the presence of polyhedral starch grains stained black by Steimetz reagent (A), numerous vessels (B), fragments of spiral vessels (C), and oil droplets (D).

The microscopic profiles of leaf powder from the plant species studied provide information that may be useful for plant authentication and quality control of raw materials.

Composition of secondary plant metabolites

The phytochemical screening enabled us to highlight the presence of tannins, alkaloids, and saponins, compounds likely to confer interesting biological properties to our samples. The *Curcuma longa* extract contained the two groups of tannins we were looking for (gallic and catechic), while the *Solanum lycopersicum* extract revealed only gallic tannins. Our results corroborate those of Mbadiko et al. (2019), who demonstrated the presence of tannins in *Curcuma longa* rhizomes, whereas Boukeria et al. (2019) reported the absence of tannins in *Curcuma longa* rhizomes.

Antioxidant activity

The results for antioxidant activity are presented in the **Table** below.

Table 4:
IC50 values expressed in µg/mL decoctate and percolate for ABTS and DPPH tests (mean ± standard deviation, n = 4)

N°	Samples	DPPH (IC50 in µg/mL)		ABTS (IC50 in µg/mL)	
		Decocted	**Percolate**	**Decocted**	**Percolate**
1	***Curcuma longa***	1620,349±0,044	89,163±0,298	16,34±2,17	3,437±2,352
2	***Solanum lycopersicum***	726,326±0,070	92,631±0,344	83,38±8,34	65,01±3,35
3	***C. longa + S. lycopersicum***	303,670±0,109	68,637±0,328	25,83±10,18	11,33±3,342
4	**Quercetine**	1,42 ± 0.04		3,21 ± 0.99	

The results presented in the table above show that the ABTS test has a high antioxidant power compared to DPPH. This difference is attributed to their reaction mechanism (Floegel et al., 2011), and on the other hand, percolation presented better antioxidant results compared to decoction; this could be caused by the fact that heat, when uncontrolled, can cause a modification in the structure of polyphenols, resulting in losses that vary greatly depending on the cooking method and the substance studied (Kadri, 2015).

The mixture of our two plants used in our study showed, for the decoctate, low antioxidant activity towards the DDPH radical (IC50 = 303.670±0.109 µg/mL) and good antioxidant activity towards the ABTS radical (IC50 = 25.83±10.18 µg/mL) compared with Quercetin taken as a control (1.42±0.04 and 3.21±0.99 µg/mL for DPPH and ABTS respectively).

The mixture of the decoctate and the percolate of *C. longa* rhizomes and *S. lycopersicum* leaves showed good antioxidant activity towards the DPPH and ABTS radicals compared to the results found with *Solanum lycopersicum* alone. It should be noted that the best bio-optimisation of antioxidant activity towards ABTS and DPPH was observed with the percolates. Based on these results, we can deduce that there is a significant correlation between the presence of polyphenols and flavonoids and the antioxidant activity of the extracts. The antioxidant power recorded suggests that the rhizomes of *Curcuma longa* and the leaves of *Solanum lycopersicum* can be considered antioxidant agents that have been particularly studied due to their use in traditional pharmacopoeia for their beneficial effects on health.

Antibacterial activity

The minimum inhibitory concentrations (MICs) of the control (Ceftriaxone) in relation to our strains are shown in the table below:

Table 5:
Minimum inhibitory concentrations (MICs) of the antibiotic (Ceftriaxone)

Strains	CMI µg/mL
E. Coli (ATCC 25922)	0,015
S. aureus (ATCC 25923)	4
P. aeregunosa (ATCC 27853)	32

The Table above shows that the MIC of the antibiotic was 0.15 µg/mL for the *E. coli* strain, 4 µg/mL for *S. aureus*, and 32 µg/mL for *P. aeregunosa*.

This means that all our strains are sensitive to the antibiotic used for the antibacterial test.

Table 6:
Minimum inhibitory concentrations (MICs) of plant decocts and percolates released in the presence of three microbial species

Extraits	E. coli (ATCC 25922)		P. aeruginosa (ATCC 27853)		S. aureus (ATCC25923)	
	CMI µg/ mL	CMB µg/mL	CMI µg/ mL	CMBµg/mL	CMI µg/mL	CMB µg/ mL
Percolate C. longa	3,906	7,813	62,5	500	3,906	125
Percolate S. lycopersicum	62,5	62,5	125	>1000	62,5	250
Percolate C. longa + S. lycopersicum	15,625	3,906	15,625	500	15,625	62,5
Decocted C. longa	15,625	500	250	>1000	62,5	125
Decocted S. lycopersicum	125	500	250	>1000	250	500
Decocted C. longa + S. lycopersicum	15,625	125	62,5	>1000	15,625	31,25

It can be seen from this table that the *C. longa* percolate was highly active against *E. coli* and *S. aureus* strains (MIC µg/mL= 3.906) and the *S. lycopersicum* percolate was highly active against *P. aeruginosa* strains (MIC µg/ mL=62.5). The results of the antibacterial activity of *S. lycopersicum* on *P. aeruginosa* strains corroborate those of (Okou et al., 2018), who demonstrated good antibacterial activity of another species belonging to the *Solanum* genus (*Solanum torvum*) against the same strain.

In light of these results, we can deduce that there was bio-optimisation of the antibacterial activity of *Solanum lycopersicum* extracts after combining them with those of *Curcuma longa* against our three strains.

This study showed that all these extracts had a bacteriostatic action on the *P. aeregunosa* strain. The CMB/CMI ratios give 2 for the action of the *Curcuma longa* percolate, making the extract bactericidal, 1 for the action of the *Solanum lycopersicum* percolate, and strictly less than 1, i.e. 0.249, for the action of the percolate of the synergy of two plants, so these extracts have an absolute bactericidal action on the *E. coli* strain, 32 for the action of the *Curcuma longa* decoctate, so the extract is tolerant, 4 for the action of the *Solanum lycopersicum* decoctate and 8 for the action of the two-plant synergy decoctate, so these extracts have a bacteriostatic action on the *E. coli* strain, 32 for the action of the *Curcuma longa* percolate, so the extract is tolerant, 4 for the action of the *Solanum lycopersicum* percolate and the combination of two plants, so these extracts have a bactericidal action on the *S. aureus* strain, 2 for the action of the decoctate of *Curcuma longa*, *Solanum lycopersicum*, and their synergy. These extracts had a bactericidal action on the *S. aureus* strain.

This work therefore shows that the use of *Solanum lycopersicum* leaves and *Curcuma longa* rhizomes as antibacterial agents in traditional environments is justified since the aqueous and organic extracts of these plants have antibacterial activity.

CONCLUSIONS AND SUGGESTIONS

This study aimed to optimise the antibacterial and antioxidant potential of *Solanum lycopersicum* (leaves) by combining it with *Curcuma longa* extract.

This study showed that the combination of our two plants had good antibacterial and antioxidant activity compared

with *Solanum lycopersicum* alone. In addition, our results showed that the mixtures prepared by percolation (cold extraction) had better antibacterial and antioxidant activity than those prepared by decoction (hot extraction); chemical analysis revealed the presence of tannins, alkaloids, saponins, anthocyanins, terpenes, iridoids, flavonoids, coumarins, and phenolic acids in the rhizomes of *Curcuma longa*. In the leaves of *S. Lycopersicum*, all these secondary metabolites were also present, except coumarins and terpenes; the powder in our samples contains a variety of characteristic elements, including starch grains, tector polishes, fragments of spiral vessels, and oil droplets.

Thus, we suggest in the future that phytochemical studies aimed at isolating and characterizing chemical compounds with antioxidant and antibacterial properties are needed to formulate herbal medicines to combat the problem of oxidative stress. In addition, microscopic profiles of leaf powder from the plant species studied provide relevant information that can be useful for plant authentication and quality control of raw materials.

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