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Antimicrobial effects of aqueous extract of mixture from *Aframomum melegueta* (K Schum) – *Citrus aurantifolia* (Christm and Panzer) and *Sterculia setigera (*Delile*)* on five respiratory pathogenic germs

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Abstract

More and more people resort to Traditional Medicine recipe to treat cough, symptomatic of respiratory tract infections in Benin. Thus, improving Traditional Pharmacopoeia in order to value it becomes a necessity. The objective of this study is to evaluate the antimicrobial activity of aqueous extracts of mixture from *Aframomum melegueta - Citrus aurantifolia and Sterculia setigera* on five pathogen germs of respiratory tract or coughing. A phytochemical study followed with calculating the Minimum Inhibitory Concentrations (MIC), the Minimum Bactericidal Concentration (MBC) and the antibiotic power of these extracts has enabled us to reach this aim. The phytochemical study revealed potentially active groups of chemical substances: alkaloids, tannin and essential oils. The presence of these groups in the plants show their biological activities. *Micrococcus luteus* is the most sensitive germ on the aqueous extract of the mixture *Aframomum melegueta - Citrus aurantifolia*, and *Escherichia coli* the most resistant microorganism. The mixture *Aframomum melegueta - Citrus aurantifolia* is the most active extract on all strains of bacteria studied.

Keywords: activity-antimicrobial-aqueous extract- pathogen germs.

Introduction

Infectious diseases are the most common health conditions in Benin. Among these diseases, respiratory infections (infectious rhinitis, pneumonia, bronchitis), malaria attacks, diarrheal diseases come first (MSP / Benin, 2004).

Nowadays, a large proportion of the world population uses herbal-based medicines for the treatment of diseases (Martson et al, 1993). According to World Health Organization (2003) statistical data, 80% of the African population relies primarily and sometimes exclusively on Traditional Medicine to solve their health problems. This medicine provides the basic information that help to carry out new drugs (Farns et al, 1995).

The renewed interest currently being experienced by traditional medicine has led us to show great deals of interests in order to prepare effective extracts for the treatment of some infectious and parasitic diseases that affect our sportsmen.

Indeed, the proliferation of pathogenic microorganisms due to improper and inappropriate use of antibiotics currently poses a public health problem. Antibiotic resistance sometimes makes the therapeutic treatment expensive and ineffective (Cimanga et al., 2002). Owing to diseases care requirements, less expensive traditional treatments based on medicinal plants are offered. Plants stand as a source of new molecules endowed with antimicrobial activity (Burt, 2004). Sportsmen confronted with traumatisms, inflammations and respiratory diseases resort to these plants to treat themselves. Indeed, germs are responsible of some respiratory diseases. It is therefore necessary to study the antimicrobial activity of some of these plants that are used for traditional treatments. Through this study, the emphasis is put on three plants traditionally used in the treatment of respiratory tract infections especially cough: *Aframomum melegueta; Citrus aurantifolia and Sterculia setigera.*

The objective of the study is to evaluate the antimicrobial activity of aqueous extracts of the mixture *Aframomum melegueta* - *Citrus aurantifolia* and *Sterculia setigera* on five respiratory pathogens or even cough.

Materials and Methods

MATERIAL

Plant material

These are extracts from dry seeds of *Aframomum melegueta* and leaves of *Sterculia setigera* and *Citrus aurantifolia.*

Microbial strains

The antibacterial and antifungal activity was evaluated on reference stump ATCC (American Type Culture Collection) and IP (Institut Pasteur of Strasbourg) (table 1)

Table 1: Reference stump

STUMP	REFERENCE	GRAM
Micrcoccus luteus	ATCC 10240	+
Staphylococcus aureus	ATCC 29213	+
Pseudomonas aeruginosa	ATCC 27853	-
Escherichia coli	ATCC 25922	-
*Candida albicans**	IP 4872	

METHODS

This is an experimental cross-sectional study that was carried out over a period of 5 months (May-September) and in three stages:

Harvesting, drying, crushing of plant material

Dried berries containing *Aframomum melegueta* seeds, leaves of *Sterculia setigera* and those of *Citrus aurantifolia* are dried in the shade. Seeds removed from the envelopes are ground into thin powder and stored at a temperature below 4°C.

Preparation of aqueous extracts

Aframomum melegueta was collected in Adjarra, locality in the Department of Ouémé in Benin. The harvest of the sheets and fruits of *Citrus aurantifolia* was collected at Ouando locaty in the same department. The identification was made by the National Herbarium of the University of Abomey-Calavi of Benin. Each specimen was deposited at the University (Voucher N° AA6374/HNA for *Aframomum melegueta* (Roscoe) K. Schum, N° AA6375/HNB for *Citrus aurantifolia* (Christm. and Panzer) Swingle and N° AA6376/HNB for *Sterculia setigera*). The total aqueous extract was prepared by mixing 125g of *Aframomum melegueta* seeds with the filtrate obtained from *Citrus aurantifolia* 125g of leaves and 290g of fruits in 1 liter of distilled water.

Sterculia setigera extract is obtained from 60g of leaves powder decoction in 1L of distilled water. The processes which follow are performed as before.

After 5 hours of stirring, the mixture was filtered, centrifuged and lyophilized (Huet, 1993; Houghton and Amala, 1998).

Phytochemical profiling

Phytochemical screening is based on differential reactions (coloration and precipitation) of major chemical compounds of groups contained in the plants according to Houghton and Amla method (1998).

Experimental phase

It's to do with studying in vitro extracts antimicrobial potency. It consists in testing germs sensitivity: *Micrococcus luteus; Staphylococcus aureus; Pseudomonas aeruginosa; Escherichia coli* and *Candida albicans,* with the various extracts by microdilution method.

Determination of minimum inhibitory concentrations (MIC)

The Minimum Inhibitory Concentration (MIC) is the lowest concentration where there is no visible growth to naked eye. We obtained stock solutions by dissolving 10 mg of each extract in 1 mL of nutrient broth or Sabouraud broth. After isolation, a group of germs is subcultured in 10 µL of sterile distilled water. From this stock solution, we perform a series of dilution with sterile distilled water. Each dilution obtained is cultured on nutrient broth or Sabouraud broth. After 24 or 48 hours of incubation at 37 ° C for bacteria and 20 ° C for Candida albicans, one counts the number of Colonies Forming Units (CFU). The dilution which is chosen for antimicrobial testing is the one that gives us a concentration of 10^6 germs / ml for bacteria and 10^7 germs / ml for Candida albicans.

- We share 100 µL of nutrient broth into each shaft of the microship except those of the first two columns.

- We add 100 µL of the stock solution of each aqueous extract in each of the 6 last shafts of the first 3 columns. From the 3rd column we then perform serial dilutions in progressive series from 2 to 2, shaft by shaft, column by column up to the last column, and the remaining 100 µL, is thrown away.

- We finally inoculate all the shafts except the one in the first row and the last six of the 1st column by introducing 100 µL of the suspension at 10^6 germs / ml for bacteria and germs at 10^7 / ml for *Candida albicans.*

The microplate is covered and incubated at 37 ° C for 24 hours when it is bacteria and 72 hours at 20 ° C for *Candida albicans.* The reading is done by comparing between control shafts and test shafts.

While reading, when one notices a turbidity or a color change it indicates a bacterial growth.

The MIC is the lowest concentration where there is no visible growth.

Minimum bactericidal concentration (MBC)

It is performed together with the MIC. Once the MIC determined, shafts where germs were inhibited, starting from the shaft that was used to fix the MIC and moving to the high concentration, are taken and transplanted on nutrient agar. We then carry out a control agar. The seedlings are made using a platinum loop, previously heated to red by striae.

If any visible growth, one compares germs proliferation with those of the control agar. MBC is the lowest concentration for which there was at most 0.01% of surviving germs.

Statistical analysis

The data obtained were processed with Excel in Windows Vista Office 2007

Results

Phytochemical profile

The phytochemical analysis' results are presented in Table 2. Table 2 shows that the two extracts contain almost the same chemical groups except of catechin tannins and steroids contained in *Sterculia setigera* et absent in the *Aframomum melegueta - Citrus aurantifolia* mixture, essential oils absent in *Sterculia setigera* and in the *Aframomum melegueta - Citrus aurantifolia* mixture.

Table 2: Phytochemical screening results

CHEMICAL GROUP		OBSERVATIONS	
		Sterculia setigera	***Aframomum melegueta - Citrus aurantifolia***
Alkaloides	Alkaloides	+	+
Polyphenolic compounds	tanins catechic tanins tanins galliques Flavonoids	+ + + +	+ – + +
Saponosides	Saponosides	_	_
triterpenoides et Stéroids	triterpenoides Stéroids	_ +	–
Cardénolides		_	_
Anthocyanosides		+	+
Leuco anthocyanes		+	+
Mucilage	Mucilage	+	+
Reducing compounds	Reducing compounds	+	+
Coumarines	Coumarines	–	–
Quinon derivatives	Quinon derivatives	+	+
Anthracene Derivatives	Fre Combined	– _	– –
Essential oils		_	+

"+" indicates the presence of excess chemical compounds group, and "-" a negative reaction so an absence.

Antimicrobial activity of aqueous extracts

Minimum inhibitory concentrations (MIC)

The results of the MIC are displayed in Table 3.

The analysis of this table reveals that:
- The extracts studied show at various degrees, inhibitory activities on different studied germs.
- The MIC of different extracts varies between 2.5 and 10 mg / Ml
- *Micrococcus luteus* is the most sensitive germ of the aqueous extract of the mixture *Aframomum melegueta* - *Citrus aurantifolia* (MIC = 2.5 mg / mL), while *Staphylococcus aureus* is the most resistant microorganism (MIC = 10 mg / mL).
- Only the stock solutions have activity against *Candida albicans*.

Table 3: Results of determination MIC (mg / ml) of various extracts

Aqueous extracts	**MIC (mg/mL)**				
	Staphylococcus aureus	*Micrococcus luteus*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Candida albicans*
Aframomum melegueta – Citrus aurantifolia	10	2,5	5	10	10
Sterculia setigera	10	5	5	2,5	10

Minimal Bactericidal Concentration

The results are noted down in Table 4. It globally emerges that: the studied extracts had no fungicidal activity on *Candida albicans* (MBC> 10mg / mL). *Sterculia setigera* extract has bactericidal activity *Staphylococcus aureus* only (CMB = 10mg / ml).

The aqueous extract of *Aframomum melegueta* - *Citrus aurantifolia* mixture shows bactericidal activity on 4 of the 5 germs tested.

Table 4: Result of determination of the CMB (mg / mL) of the various extracts

Aqueous extracts	MBC (mg/mL)				
	Staphylococcus aureus	*Micrococcus luteus*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Candida albicans*
Aframomum melegueta – Citrus aurantifolia	10	5	5	10	ND
Sterculia setigera	10	ND	ND	ND	ND

ND: Not determined

Plant extracts antimicrobial power

Table 5 shows the relationship between MBC and MIC of each plant extract compared to incoming germs in this study. The MBC/CMI ratio classifies antibiotics in "bactericidal" when the quotient is less than or equal to 4 and "bacteriostatic" when the quotient more than 4.

Considering MIC and MBC results, it emerges that *Micrococcus luteus* is the most sensitive germ of the aqueous extract of *Aframomum melegueta* - *Citrus aurantifolia*. *Escherichia coli* is the most resistant microorganism and, moreover, the mixture *Aframomum melegueta* - *Citrus aurantifolia* is the extract the most active on all studied strains of bacteria.

Table 5: Results of CMB / CMI reports

Aqueous extracts	MBC/MIC				
	Staphylococcus aureus	*Micrococcus luteus*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Candida albicans*
Aframomum melegueta – Citrus aurantifolia	1 Bactericidal	2 Bactericidal	1 Bactericidal	1 Bactericidal	ND
Sterculia setigera	1 Bactericidal	ND	ND	ND	ND

ND: Not determined

Discussion

The current study consisted in searching antibacterial properties of aqueous extracts of medicinal plants: *Aframomum melegueta* - *Citrus aurantifolia* and *Sterculia setigera* mixture, on five germs with respiratory tropism. These are: *Staphylococcus aureus; Micrococcus luteus; Escherichia coli; Pseudomonas aeruginosa* and *Candida albicans.*

The analysis of plants phytochemical screening results, shows that their chemical families are: Alkaloids, polyphenolic by-products (catechin tannins, gallic tannins, leuco anthocyanin, flavonoids), mucilage, anthocyanins and essential oils. Referring to the biological properties (Bruneton, 199) of these chemicals we can say that these plants have antibacterial properties. The majority of chemical groups present in recipes like tannins, saponins, flavonoids, coumarins, anthocyanosides, mucilage and essential oils can be extracted with water. Water seems to be the best solvent to extract the majority of chemical components responsible for different pharmacobiologic activities, demonstrating the relevance of the way majority of traditional remedies are used.

To study the antimicrobial properties of aqueous extracts of plants, micro dilution method in liquid medium combined with the spread on agar medium containing no extract seems more sensitive and more specific than those using biodiscs, the solid medium or macro dilution method in liquid medium dilution (Hmamouch, 1992; Kambu, 1989, Murengezi, 1993; Ndounaga, 1991). This is due to the fact that not only do we use fewer products, but again, the germs are completely in contact with the chemical constituents of extracts.

As far as the Minimum Inhibitory Concentrations are concerned, plants aqueous extracts all showed inhibitory activities as regard to the different seeds used.

These results are similar to those of Kamtchouing (2002) to *Aframomum melegueta - Citrus aurantifolia* extracts mixture.

About Minimum Bactericidal Concentrations, although the studied extracts revealed inhibitory activity on all tested germs, they do not yet have bactericidal activities on all these as shown in Tables 4 and 5.

Indeed, this result is explained by the fact that aqueous extracts are generally bacteriostatic compared with essential oils that are bactericidal (Lacmata et al., 2012). This strong activity about essential oils is related to their high content of oxygenated compounds that have antimicrobial activity.

The fact that *Aframomum melegueta - Citrus aurantifolia* extracts mixture are the most active on all studied strains of bacteria would likely be due to the presence of essential oils that are known for their antimicrobial power.

Micrococcus luteus (Gram +) is the most sensitive germ of the aqueous extract of *Aframomum melegueta - Citrus aurantifolia, Escherichia coli* (gram -) mixture - the most resistant microorganism. This difference in sensitivity of these two germs is due to their membership of gram. Indeed, gram – bacteria' wall is thicker than that of Gram + thereby preventing from a quick penetration of substances inside thereof.

At the end of this study, extracts different antimicrobial activities have been proven, which thus enables us to justify and objectify therapeutic indications of our various medicinal plants based traditional preparations.

Conclusion

Considering the results of MIC and MBC, it emerges that *Micrococcus luteus* is the most sensitive germ of the aqueous extract in the *Aframomum melegueta - Citrus aurantifolia* mixture. *Escherichia coli* is the most resistant microorganism and, furthermore, the mixture *Aframomum melegueta - Citrus aurantifolia* is the most active on all studied strains of bacteria.

The different antimicrobial activities of the extracts have been proven, and to justify and objectify the therapeutic indications of traditional preparations of our various plants.

Our results can be used for purifying the active principles of plants used and the preparation of improved forms of effective remedies based on these plants against respiratory pathogens.

We hope, through this work, having set the starting point for the development of an improved traditional medicine indicated in respiratory tract infections, after the deepening of some aspects of the study and the carrying out of clinical trials.

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