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## RESEARCH ARTICLE

## PURIFICATION AND BIOLOGICAL ACTIVITIES OF COMBINATION OF HEMI-SYNTHESIZED THIOSEMICARBAZONES OF *MITRACARPUS SCABER* ZUCC

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## ABSTRACT

The harvested *Mitracarpus Scaber* plants are identified in the national herbarium and registered under the number AA. 6252/HLB. During this work, three crude extracts are prepared from three organic solvents, namely dichloromethane; ethanol and hydro ethanol in 50/50 v/v proportion. The respective alkaloid extracts obtained from the corresponding crude extracts served as substrates for the hemi synthesis of thiosemicarbazone totals from thiosemicarbazides. The three hemi-synthesis products obtained were tested on eight (08) strains of germs, namely *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *E. faecalis* ATCC 22921, *P. aeruginosa* ATCC 27853, *C. albicans* ATCC 10231, *S. typhi*, *K. pneumoniae* and *Dermatophilus* 146. The ethanolic extract of thiosemicarbazones exhibited the best bioactive activity and was found to be the most selective. By a series of bio guided chromatographies: TLC thin layer chromatography; CPA atmospheric pressure chromatography and medium pressure liquid chromatography. MPLC (medium pressure liquid chromatography), separation on dextran gel: Sephadex® LH20). The use of available spectral data, cross-checked with that of the literature, made it possible to identify and purify three (03) molecules of thiosemicarbazones, making it possible to study the biological activity of their combination.

**Keywords:** Biological activities, chromatography, hemi synthesis, *Mitracarpus Scaber*, thiosemicarbazone.

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## INTRODUCTION

Herbal remedies are the precursors that continue to provide inspiration for modern medicine. Fortuitous discoveries and the transmission of information from generation to generation are at the origin of the choice of plants and their uses as remedies. Although the effectiveness of the various remedies used is not absolute, healing is rapid and the disease disappears. The recipe was often kept secret and was only transmitted in fragments. Initially considered as curiosities, medicinal plants have been valued by important botanical, biochemical, chemical and pharmacognosic research. Nowadays, the results are becoming more and more convincing, given the techniques and efficient equipment available to researchers<sup>1</sup>. In addition, and despite the competition

from synthetic products, certain medicinal plants whose active ingredients remain non-reproducible, are still of definite interest on the market. On the other hand, the interest of industrialized countries vis-à-vis natural substances could only encourage current research. Plants synthesize a very wide range of organic compounds which are traditionally considered primary and secondary metabolites, although the precise boundaries between the two groups may, in some cases, be somewhat ambiguous<sup>2</sup>.

Primary metabolites are the compounds that have essential roles related to the photosynthesis, respiration and plant growth and development. These include phytosterols, acyl lipids, nucleotides, amino acids and organic acids. The other photo chemicals, many of which accumulate in surprisingly high concentrations

in some species, are considered secondary metabolites. These have diverse and numerous structures and are distributed among a very limited number of species in the plant kingdom. The number of structures described exceeds one hundred thousand (100,000) and the real number in nature is certainly much higher because so far, only 20-30% of plants have been studied in phytochemistry<sup>3</sup>.

If ignored for a long time, their function in plants is attracting more and more attention because certain metabolites seem to have a key role in the protection of plants against herbivores and microbial infections, as attractants for pollinators. Secondary metabolites are also of particular interest due to their use as colorants, fibers, glues, oils, waxes, flavoring agents, drugs and perfumes, and they are considered to be potential sources of new natural medicines, antibiotics, insecticides and herbicides<sup>4</sup>.

Among the first natural products isolated from medicinal plants are the alkaloids. When they were first obtained from plant material during the early years of the 19th century, it was found that they contain nitrogenous bases which form salts with acids. The name of the alkaloid dates from the German pharmacist Meissner. They are chemically organic materials composed of carbon, hydrogen, nitrogen and oxygen<sup>5</sup>.

Due to their powerful biological activity, most of the known alkaloids, around 12,000, have been exploited as drugs, stimulants, narcotics, and poisons. Unlike most other types of secondary metabolites, the many classes of alkaloids have unique biosynthetic origins<sup>6</sup>.

Studies of combinations of bioactive chemicals in general are increasingly described in the literature<sup>7</sup>. This strategy is indeed of great interest with a view to potential clinical applications, since it makes it possible to reduce the possible side effects of current treatments by reducing the dose of compound used<sup>8</sup>, thus also limiting the development of resistance phenomena<sup>9</sup>. This approach would also reduce the essential quantity necessary for obtaining a therapeutic activity, this type of product is often known for having to be used at relatively high concentrations and to circumvent the problem of their low absorption in the intestinal level, which usually limits their applications in concerning the treatment of certain systemic diseases<sup>8,10</sup>. This type of study has already proven its effectiveness by showing a good correlation between the synergies demonstrated *in vitro* and the clinical results obtained in the treatment of certain bacterial infections<sup>11</sup>.

Thus, during this work, it was a question of carrying out hemi-synthesis reactions from alkaloid extracts prepared from the *M. Scaber* plant to study the combinations of the compounds isolated from three (03) categories of germs (gram + bacteria; gram- and yeast) in order to offer biologically active drug alternatives.

## MATERIALS AND METHODS

Present work aims to assess and compare the biological activities of thiosemicarbazones isolated from hemi-synthetic products, and study the combinations of biological activities of these products obtained

## Plant material

Fresh samples of *M. Scaber* were collected and identified under the number AA. 6252/HLB to the National herbarium of the University of Abomey-Calavi. The aerial part of the plant was cut and dried for 7 days in the absence of light, then reduced to powder using an electric grinder (Flour MILLS of Nigeria, El. MOTOR N° 1827) then the ground material is kept until use.

## Biological material

During the course of current study, 3 microbial strains and one parasitic strain were investigated.

The choice of the microbial strains to be subjected to the experiment is of capital importance. The selection criteria were as follows:

- They must represent a large number of bacteria from the same group;
- They must be available and easily accessible;
- They must lend themselves easily to culture in the laboratory.
- They must be pathogenic or resistant to common antibiotics.

To do this, the microbial support used consists of: *E. coli* ATCC 25922, *S. aureus*, ATCC 27853, *C. albicans* ATCC 10231.

## *In vitro* antimicrobial test

In order to assess the sensitivity of germs to different compounds the micro-dilution method in liquid medium on a 96-well microplate was used<sup>16,17,18,19,20</sup>.

The dilution microplates are incubated at 32°C. The results are observed after 2 days to 5 days for the strains of germs tested. The ICD (Maximum Inhibitory Concentration) is determined either as the concentration corresponding to the last well where microbial growth is absent (in the case of pure extracts and molecules), or as the concentration corresponding to the last well where bacterial growth reaches a maximum of 20% of control bacterial growth (in the case of antifungal). All the tests are repeated in duplicate on each plate, and at least in two separate experiments. The tests which gave different results were repeated until a reliable value was obtained.

## Bacteria and yeast test

### Preparation of test components

A stock solution of each compound is prepared by dissolving 100 mg in 5 mL of 1% DMSO i.e. a weight concentration of 20 mg/mL. This solution is subjected to a filtering filtration on millipore membranes with a diameter of 0.2 m (Acrodisc USA).

### Preparation of the microbial suspension

After having isolated the different strains on the solid Mueller Hinton agar medium, a platinum loop made it possible to sample a colony which was dissolved in 5 ml of 1% DMSO in a sterile tube. The tube is carried in the 37°C incubator for 2 hours in order to obtain 10<sup>6</sup> Colon Formation Units per milliliter (CFU/mL) (equal density scale 2 of MC Farland) from which the germs will be removed for carrying out the test.

### Description of the method

During this test two controls a negative control based on a compound and a positive control were used to control the growth of germs. The negative control (on

line A of the plate) consists of successive dilutions forming a geometric progression of reason 2 in 100L of Mueller Hinton Bouillon (MHB) containing phenol red 0.02g/L from 100 L of the solution. The positive control (on line B) is carried out in the same way as the negative control with the difference that the test solution is replaced by 100 L of the microbial suspension. In the wells of lines C and D the strains were incorporated into the test products by following the steps below-

- Put in each well 100 L of Mueller Hinton Bouillon (MHB) containing phenol red 0.02 g / L
- Carry out successive dilutions of reason 2 from 100 L of the prepared stock solution
- Add to the content of each well 100 L of the microbial suspension  $10^6$  CFU/mL prepared as indicated above.

The micro plate is then covered and incubated in the 37°C incubator for 24 hours after which time the minimum inhibitory concentration (MIC) is determined. It should be specified that the test is done in duplicate on the same plate on which it is test two extracts while keeping the same positive control.

Antimicrobial activity is expressed in MIC. The MIC is the Minimum Inhibitory Concentration i.e. the minimum concentration tested which completely inhibits the growth of microorganisms. It was determined visually by observation of the growth or absence of the bacterial strains by comparing the test wells with the control wells<sup>15,20</sup>. The wells which are not the same color as those of the positive control are the wells in which the compound has activity on the germ.

#### Purification methods

A series of chromatographic methods have made it possible to isolate active biomolecules from extracts of thiosemicarbazones. These are thin layer chromatography (TLC), preparative thin layer chromatography (TLC-prep), from the column to atmospheric pressure, MPLC (medium pressure liquid chromatography), and separation on dextran gel: Sephadex® LH20. Thin layer chromatography (TLC) It is a simple and quick method of analysis to study the composition of different plant extracts and fractions. Thin layer chromatography (TLC) is a frequently used technique for the analysis of plant extracts because it offers the advantage of its speed, its low cost and allows direct visualization of the separation of the compounds.

The fractions and extracts are analyzed by TLC in order to study their composition and to highlight the main classes of active ingredients using more or less specific reagents. Finally, the TLC analysis of the isolated products will give us an indication of their purity. In present work, Dragendorff's reagent for TLC analysis of the extracts, in order to identify compounds containing nitrogen (alkaloids and thiosemicarbazones). Sulfuric anisaldehyde was used to check the purity of nitrogen compounds isolated.

The plates used are normal silica plates and the detection of the bands of the various compounds was carried out first by direct visualization of the colored

bands, then under UV (254 and 366 nm) and finally using the aforementioned developers.

The analytical conditions are:

- **Stationary phase:** TLC Silica gel 60 F254S Merck®
- Quantity deposited: 100 µg
- **Phase mobile:** Hexane-Ethyl acetate (10-5; v / v)
- **Detection:** UV at 254 nm and 366 nm
- **Developers:** sulfuric anisaldehyde; Dragendorff reagent.

The developers used are prepared as follows:

#### Sulfuric anisaldehyde:

- **Preparation:** EtOH p-anisaldehyde-Acetic Acid (90-5-1)+5% H<sub>2</sub>SO<sub>4</sub> added immediately. After spraying, heating for 5 min at 105 °C.
- **Detection:** Colored spots (mauve-pink-blue) or under UV.
- Classes of molecules highlighted: terpenes, steroids, saponosides.

#### Dragendorff reagent

- **Preparation:** Solution a: 0.85 g of bismuth nitrate in 10 mL of acetic acid and 40 mL of water. Solution b: 8 g of potassium iodide in 30 mL of water. Stock solution: Solution a-Solution b (1: 1, V/V). Spray solution: 1 mL stock solution +2 mL acetic acid + 10 mL water.
- **Detection:** Yellowish spots
- Classes of molecules highlighted: alkaloids

#### Preparative thin layer chromatography (TLC-prep)

The TLC-prep method was used by Hougèbème 2015, when he studied phytochemicals of anti-infectious plants. Preparative TLC is a chromatographic technique used in the separation and isolation of various metabolites present in a slightly or not complex mixture. It derives from thin layer chromatography but in this case, the silica zones corresponding to the stains are recovered from the plate unlike the analytical TLC. To this difference is added the quantity of samples applied to the chromatographic plate (20 mg to 40 mg) as well as the thickness of the layer of silica gel on the plate. In this technique, the plates used generally are glass plates (Merck).

The extract to be separated is dissolved in a suitable solvent so as to obtain total dissolution. It is then deposited on the plate using a special comb. The plate is developed in a saturated tank containing the same eluent used in analytical TLC. As in the analytical TLC, the plate is dried at room temperature or with a hair dryer. The spots of the constituents are examined under UV light or a fringe of the plate is revealed by spraying with the appropriate reagent. Using a spatula, the compounds of interest fixed on the silica are recovered. The latter is then dispersed in a small amount of solvent, and then filtered under vacuum, to allow the recovery of the compounds.

#### Splitting methods

##### Column fractionation at atmospheric pressure

It is a method that allows the separation of the constituents of a mixture of substances using the properties that connect them to a given substrate. It is a classic technique based on the selective absorption of the components of a mixture on a solid stationary

phase, offering a large adsorption surface. In this case, the mobile phase composed by one or more solvents will allow differential elution as a function of the polarity of the compounds, first eluting the less polar compounds. During the entire elution process, a constant exchange phenomenon occurs between the adsorbent and the mobile phase. As method used it is as of Hounghè and al 2015 during this work. Silica gel (Merck) normal phase for liquid chromatography and polyamide gel to rid the extracts of pigments and chlorophylls. This method is less effective than MPLC or HPLC because it does not allow the use of stationary phase of fine grain size. However, this is a very important step in bio-guided fractionations because it makes it possible to carry out coarse fractionations and to identify in which fractions the most active compounds are found<sup>21</sup>. This technique is not without drawbacks. One of the major drawbacks is the slowness of the mobile phase. In addition, the bands of the various compounds can diffuse and widen on the column during their separation, which decreases the capacity of separation. There is an optimal elution speed to obtain good resolution. When the flow rate of the mobile phase is high, equilibrium is not reached and the separation of the compounds is not properly carried out.

During current work, the operating conditions were as follows:

- **Stationary phase:** Silica gel 0.062 mm-0.2 mm conditioned by methanol (i.e. 100 g of silica in 300 ml of MeOH for 24 h) poured into a glass column 2 cm in diameter and 22 cm in height of the gel.
- Quantity of extract deposited on column: varies according to the extract to be analyzed
- **Elution:** 300 mL of each mobile phase in the following order: 1). n-hexane; 2). Dichloromethane; 3). n-butanol; 4). Methanol.

**Medium Pressure Liquid Chromatography (MPLC)**  
MPLC columns are glass tubes filled with stationary phase generally composed of silica<sup>22,23</sup>. These columns can be prepared in the laboratory using normal or grafted silica. The separation principle is the same as in HPLC. The difference with HPLC lies in the size of the silica grains. In MPLC, the grains are larger and less regular; their size varies from 15 to 25  $\mu\text{m}$ . The pressure and the separation efficiency are therefore lower than in HPLC where the grain size varies between 2 and 8  $\mu\text{m}$ .

## RESULTS AND DISCUSSION

### Purification of thiosemicarbazones P2 extract (ethanolic extract)

#### Bioguided P2 fractionation

The proceeded to a column fractionation at atmospheric pressure (CPA). The results of said fractionation are expressed in the form of a yield, the values of which are given in Table 1.

Reading this table, it can be seen that the yields of the less or non-polar fractions are low while those of the polar fractions are higher, which can be explained by the polar nature of the P2 extract obtained by hemi synthesis of thiosemicarbazones. From total alkaloids from the crude ethanolic extract. Then conclude that

the fractionated extract would be richer in polar compounds.

### Toxicity and antimicrobial activities of the different fractions obtained

The results obtained following the larval and biological toxicity tests of the different fractions are shown in Table 2. All the fractions show a bacteriostatic action on the different microbial strains used (*E. coli*; *S. aureus*; *S. typhi*; *K. pneumoniae*) and a fungistatic action on *C. albicans*. For these fractions, the minimum inhibitory concentrations (MIC) vary from 0.3125 mg/ml to 2.5 mg/ml for *E. coli*; from 0.625 mg/ml to 5 mg/ml for *S. typhi*, from 0.625 mg/ml to 2.5 mg/ml for *S. aureus* from 1.25 mg/ml to 5 mg/ml for *K. pneumoniae* and for *C. albicans*.

At the end of this biological screening, only the fraction (F<sub>But</sub>) was found to be bactericidal and fungicidal on 3 microbial strains: *E. coli* (gram -), *S. aureus* (gram +) and *C. albicans* (pathogenic yeast). This fraction is therefore active on the maximum number of germs tested. The rest of current work focused on this F<sub>But</sub> fraction in order to isolate possible molecules of active thiosemicarbazones.

#### Purification of the butanolic fraction F<sub>But</sub>

For this section, given that it is a fraction and not an extract, we seek to identify by a simple and rapid method the compounds without participating in the fractionation process whose major drawback is the loss of certain molecules on the column. This is the bioautographic method for which we are interested in spots that inhibit the growth of microbial strains tested by delimiting an inhibition zone.

#### Identification of bioactive spots in the F<sub>But</sub> fraction

In this manipulation, the three germs are studied which of the fractions exhibits bactericidal and fungistatic activity. 100  $\mu\text{g}$  of the butanolic fraction and 30  $\mu\text{g}$  of reference antibiotic are deposited on a TLC plate, then the plate has been covered with a microbial suspension at the density of 10<sup>6</sup> CFU/mL of *E. Coli* (gram-), *S. aureus* (gram +) and *C. albicans* (yeast).

The plate was eluted with the hexane-ethyl acetate-methanol 10-5-2 system; V/V/V. The results obtained are expressed in the form of the diameter of the inhibition zone (Table 4).

By reading this table, it can retain that the butanolic fraction has an antimicrobial activity on *E coli*, *S. aureuse* and *C. albicans* but less marked compared to the controls that are tetracycline and gentamicin. Current study was continued on the basis of this butanolic fraction in order to isolate the bioactive molecules and this by re-passing the butanolic fraction for a fractionation on column at atmospheric pressure (CPA). Three butanolic sub fractions were obtained and deposited on a plate with two other fractions namely dichloromethane and ethyl acetate.

On reading this chromatographic profile, three spots of the initial butanolic fraction are noted with a respective R<sub>f</sub> of 0.4; 0.6 and 0.7; with the dichloromethane fraction, a drag is obtained as does the ethyl acetate fraction, on the other hand, with the P2 fraction, several spots are obtained, three of which come from feeder spots which are at the same R<sub>f</sub> as those of the butanolic fractions. We can deduce that these spots are

probably pure compounds which will be the subject of a structural determination by spectral methods. Identification, isolation of pure extracts C1; C2 and C3 of the butanolic fraction Identify and isolate C1; C2 and C3, C1 is recovered; C2 and C3 successively, and the purification of each of the compounds is carried out on a case-by-case basis. By <sup>1</sup>-H NMR spectral studies; <sup>13</sup> C-NMR; cross-checking of literature data and chem<sup>26</sup>. Draw Ultra 8.0 simulations; chemdraw office 2004 we have established the molecular structures of C1, C2 and C3 which are the following thiosemicarbazones of the following carbonyls: 4-methoxypropiophenone; 4 methylaceto phenone and 3, 4, 5-trimethoxyacetophenone.

#### Biological activities of the effects of combinations of compounds C1, C2 and C3

This type of study has already proven its effectiveness by showing a good correlation between the synergies demonstrated *in vitro* and the clinical results obtained in the treatment of certain bacterial infections<sup>11</sup>.

In this perspective, the compound C1 (thiosemicarbazone of 4-methoxypropiophenone) was tested in combination (serial dilution crossed), with successively the two other compounds namely C2 (thiosemicarbazone of 4-methylacetophenone and C3 (thiosemicarbazone of 3, 4, 5-trimethoxyacetophenone) on 3 strains: *E. Coli*, 25922 *S. Auréus* 25923 and *Candida albican* 10231. The results obtained are presented in the following table (Table 5). The concentrations fractional inhibitors (CIF) are calculated by dividing the MIC of the combination of the two products by the MIC of the products tested individually. The ICIF (CIF index), obtained by adding the two CIF values, were interpreted as follows- ICIF > 2: antagonistic association

- 0.5 < ICIF ≤ 2: additive or indifferent association
- ICIF ≤ 0.5: synergistic association

This choice is arbitrary because the limit values found in the literature are sometimes different (antagonism if the index is greater than 4<sup>11</sup>, greater than 1 additive<sup>8</sup>, synergy if the index is less than 1 rather than less than or equal to 0, 5<sup>24</sup> but allows the results obtained to be clearly distinguished. Minimum inhibitory concentrations (MIC, µg / ml), fractionated inhibitory concentrations and CIF index for combinations of the different purified compounds C1; C2 and C3 (Table 5). ICIF values ≤ 0.5 are shown in bold (synergy). On reading this summary table of the biological activities of the different combinations studied, the following observations emerge. The inhibitory concentration indices vary from 0.2 ≤ ICIF ≤ 4.5. Thus the effects observed are antagonistic; additive and synergistic. The combination of the thiosemicarbazones of 4-methoxypropiophenone and 4-methylacetophenone on strains of *E. Coli* bacteria has an additive effect, as does the combination of the thiosemicarbazones of 4-methoxypropiophenone and 3,4,5-trimethoxyacetophenone on *E. Coli*. against the combination of the thiosemicarbazones of 4-methylacetophenone and 3, 4,5-trimethoxyacetophenone have a synergistic action. It is also noted that the three successive combinations studied on *S. Aureus* presented a synergistic biological action with 0.2 ≤ ICIF 0, 0.3. Combinations of thiosemi-

-carbazones of 4-methoxypropiophenone and 4-methylacetophenone; 4-methylacetophenone and 3,4,5-trimethoxyacetophenone have all shown an antagonistic biological action on *Candida Albican*; only the combination of the thiosemicarbazones of 4-methoxypropiophenone and 3,4,5-trimethoxyacetophenone had a synergistic action on *C. albican* with an ICIF=0.5.

#### CONCLUSION

This study showed that we were able to purify three (03) thiosemicarbazones from extracts of synthetic hemi products obtained from extracts of ethanolic thiosemicarbazones. These extracts in a process of monitoring biological activities and guided biological fractionations made it possible to have the three thiosemicarbazones whose physicochemical studies have made it possible to know their structure which prove that of the three purified thiosemicarbazones only one comes from an already known carbonyl compound during the previous work of Bissignano in 2000 which had made progress on the knowledge of the phytochemical studies of the plant of *M. Scaber*. Two carbonyl precursor compounds of the identified thiosemicarbazones had not been discovered in the plant of *M. Scaber*. On the basis of these observations, this work has made it possible to confirm the concept which affirms that medicinal plants are libraries of several thousand molecules which we cannot stop exploring for new scientific discoveries. The combinations studied show particular attention to the different biological effects observed. The use of combined bioactive compounds must be the subject of prior scientific knowledge on informed therapeutic combinations.

#### CONFLICT OF INTEREST

The authors of this article declare that there is no conflict of interest.

#### CONTRIBUTION OF AUTHORS

All the authors have contributed to this work. BAMBOLA Bouraima, FAGBOHOUN Louis MEDEGAN Sèdami and HOUNGBEME Gouton Alban carried out the manipulations and wrote this manuscript; GBAGUIDI Ahokanou Fernand is the initiator of the work and is the Head of the analysis laboratories 1 and 2, he knew how to orient the work and approve the writing of this manuscript.

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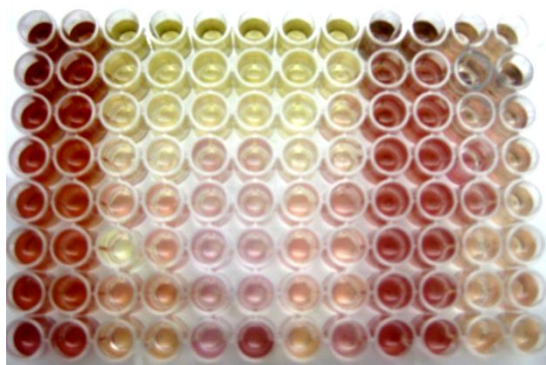


Figure 1: Antimicrobial activity, micro dilution well plate after incubation.

Table 1: Yield of the different P2 fractions

Fractions (0.139g deposited)	Mass obtained (mg)	Yield (%)
$F_{ET}$	13.74	9.88
$F_{DCM}$	19.70	14.17
$F_{ACE}$	42.31	30.43
$F_{But}$	51.40	36.97

$F_{ET}$ : petroleum ether fraction;  $F_{DCM}$ : dichloromethane fraction;  $F_{ACE}$ : ethyl acetate fraction;  $F_{But}$ : butanolic fraction.

Table 2: Antimicrobial activities and toxicity of P2 fractions for *E. coli*, *S. aureus* and *Salmonella typhi*

P2 fractions	Microbial strains												
	<i>E. coli</i>					<i>S. aureus</i>				<i>S. typhi</i>			
	LC <sub>50</sub>	MIC	IS	CMB	pa	MIC	IS	CMB	pa	MIC	IS	CMB	pa
F <sub>ET</sub>	0.12	0.312	0.02	-	-	2.5	0.02	-	-	0.625	0.08	-	-
F <sub>DCM</sub>	0.07	0.625	0.62	-	-	0.625	0.62	-	-	1.25	0.31	-	-
F <sub>ACE</sub>	0.18	0.312	1.12	-	-	1.25	0.28	-	-	2.5	0.14	-	-
F <sub>But</sub>	0.78	2.5	0.01	10	4	2.5	0.02	5	2	5	0.02	-	-

Table 3: Antimicrobial activities and toxicity of P2 fractions for *K. pneumoniae*, and *C. albicans*

P2 fractions	Microbial strains									
	<i>K. pneumoniae</i>					<i>C. albicans</i>				
	LC <sub>50</sub>	MIC	IS	CMB	pa	MIC	IS	CMB	pa	
F <sub>ET</sub>	0.12	1.25	0.04	-	-	5	0.024	-	-	
F <sub>DCM</sub>	0.03	1.25	0.312	-	-	2,5	0.012	-	-	
F <sub>ACE</sub>	0.18	5	0.36	-	-	1,25	0.144	-	-	
F <sub>But</sub>	0.78	2.5	0.016	-	-	2,5	0.312	5	2	

LC50: Lethal concentration 50% in mg/mL; MIC: Minimum inhibitory concentration (mg/ml) CMB: minimum bactericidal concentration (mg/ml), pa: antibiotic power; IS: selectivity index (IS = LC50/MIC); FET: petroleum ether fraction;

F<sub>DCM</sub>: dichloromethane fraction; F<sub>ACE</sub>: ethyl acetate fraction; F<sub>But</sub>: butanolic fraction

Table 4: Values of the diameter of the inhibition zones

Fraction/ Witnesses	Inhibition Diameter (mm)		
	<i>E. coli</i>	<i>S. aureus</i>	<i>C. albicans</i>
F <sub>But</sub>	2.2	3	5.2
Tetracycline	15	10	7
Gentamicine	13	9	11

Table 5: Minimum inhibitory concentrations (MIC, µg / ml), fractionated inhibitory concentrations and CIF index for combinations of the different purified compounds C1, C2 and C3.

Compound	<i>E. Coli</i>				<i>S. Aureus</i>				<i>C. Albicans</i>			
	MICs	MIC <sub>C1</sub>	CIF	ICIF <sub>I</sub>	MICs	MIC <sub>II</sub>	CIF <sub>II</sub>	ICIF <sub>II</sub>	MICs	MIC <sub>III</sub>	CIF <sub>III</sub>	I CIF <sub>III</sub>
C1	500	-	0.50	-	125	-	0.13	-	500	-	0.01	-
C2	1000	-	0.25	-	125	-	0.06	-	125	-	4	-
C3	125	-	0.25	-	31.25	-	0.13	-	31.25	-	0.5	-
C1+C2	-	250	-	0.8	-	15.65	-	0.2	-	3.91	-	4.0
C1+C3	-	250	-	0.8	-	7.825	-	0.3	-	500	-	0.5
C2+C3	-	62.5	-	0.5	-	3.91	-	0.2	-	15.8	-	4.5

ICIF values ≤ 0.5 are shown in bold (synergy)