Abstract

We have harvested the Mitracarpus Scaber plants which have been identified at the National Herbarium and registered under No. AA. 6252 / HLB. During this work, we prepared three crude extracts from three (03) solvents, namely dichloromethane; ethanol and hydroethanol in 50/50 v / v proportion. The alkaloid extracts obtained respectively served us as substrates for the hemi synthesis of totnums of thiosemicarbazones from thiosemicarbazides. We obtained three products which we tested on eight (08) strains of germs. To do this, the microbial support used consists of: Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923, Enterococcus faecalis ATCC 29221, Pseudomonas aeruginosa ATCC 27853, Candida albicans ATCC 10231, Salmonella typhi, Klebsiella pneumoniae and Dermatophilus AT. Dermatophilus 146 Biological evaluation showed that the ethanolic extract of thiosemicarbazones exhibited the best bioactive and selective activity. By a bioguided series of 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). We have succeeded in using available spectral data, cross-checked with those in the literature, to identify the purification of three thiosemicarbazone molecules whose biological activity in their combination is being studied.

Keywords: Chromatography; Hemi sythesis Mitracarpus Scaber; Solvent; Thiosemicarbazone.

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.

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. 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 photochemicals, 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.
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.

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.

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.

Studies of combinations of bioactive chemicals in general are increasingly described in the literature. 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, thus also limiting the development of resistance phenomena. 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. 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.

Thus during this work we proposed to carry out hemi synthesis reactions from alkaloid extracts prepared from the Mitracarpus Scaber plant to study the combinations of the compounds isolated on three (03) categories of germs (bacteria gram +; gram- and yeast) in order to offer biologically active drug alternatives.

**MATERIAL AND METHODS**

Our work aimed at evaluating and comparing to extracts, the biological activities of thiosemicarbazones isolated from hemi-synthetic products, we used a varied range of materials and methods to achieve this. We mainly list the materials and the various methods of organic synthesis, triphytochemical, antimicrobial evaluation, and structural determination.

**Plant material**

Fresh samples of *Mitracarpus 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 our 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: Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 27853, Candida albicans ATCC 10231.

**Table 1**: Characteristics of the microbes studied

| Genus       | Species | Morphology                                      | Habitat                        | Powder Pathogenicity                          |
|-------------|---------|------------------------------------------------|--------------------------------|-----------------------------------------------|
| Escherichia | Coli    | Short coccobacilli, motile by peritrichous lashes | Intestinal tract               | Gastroenteritis, diarrhea, hemolitic synchrome and uremic |
| Staphylococcus | Aureus | Immobile bacterium, rounded, isolated or in clusters | Ubiquitous, skin and mucous | Suppuration, septicemia                       |
| Candida     | Albicans| Budding cell sometimes in filament and possessing blastoconidia and chlamidospores | Pharynx, gastrointestinal tract | Stomatitis, vulvovaginitis, anitis, urethritis, perionysis and onyxis, oral candidiasis, candidemias |

**In vitro antimicrobial test**

In order to assess the sensitivity of germs to different compounds, we used the micro dilution method in liquid medium on a 96-well microplate. The micro dilution plates 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 antifungals). 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.
**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 isolating the different strains on the Mueller Hinton agar solid medium, we sampled with the aid of a platinum loop 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 10^6 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, we produced two controls: a negative control based on a compound and a positive control 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 stock 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 microplate 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 we 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. 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

In order to isolate active biomolecules from extracts of thiosemicarbazones, we applied a series of chromatographic methods to them. These are thin layer chromatography (TLC), preparative thin layer chromatography (TLC-prep), Columnation at atmospheric pressure, MPLC (Medium Pressure Liquid Chromatography), 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 (Figure 3).

Figure 3: Diagram of TLC a tank

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 this work, we used 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
- Phasemobile: 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% H2SO4 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 Houngbèmè 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 we used are generally 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, 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 in 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.

We used the method of Houngbèmè et al in 2015 during our 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. 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 our work, the operating conditions are 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.

**MPLC (Medium Pressure Liquid Chromatography)**
MPLC columns are glass tubes filled with stationary phase generally composed of silica. 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 μm. The pressure and the separation efficiency are therefore lower than in HPLC where the grain size varies between 2 and 8 μm.

**RESULTS AND DISCUSSION**

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

**Bioguided P2 fractionation**
We 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 3 below:

| 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.

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. We can 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 4 below.

The results are summarized in Table 4 below.

| P2 | Microbial strains |
|----|-------------------|
All the fractions show a bacteriostatic action on the different microbial strains used (E. coli; S. aureus; Salmonella typhi; Klebsiella pneumoniae) and a fungistatic action on Candida 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 Salmonella typhi, from 0.625 mg / ml to 2.5 mg / ml for S. aureus from 1.25 mg / ml to 5 mg / ml for Klebsiella pneumoniae and for Candida albicans.

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

**Purification of the butanolic fraction FBut**

For this section, given that we have to do with a fraction and not an extract, we sought to identify by a simple and fast method the compounds without taking part 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 the microbial strains tested by delimiting an inhibition zone.

**Identification of bioactive spots in the FBut fraction**

In this manipulation, we studied only the three germs on which the fraction exhibited bactericidal and fungicidal activity.

We deposited on a TLC plate, 100 µg of the butanolic fraction and 30 µg of reference antibiotic, then the plate was covered with a microbial suspension at the density of 10^6 CFU / ml of E. coli (gram -), S. aureus (gram +) and Candida albicans (yeast). The plate is 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 5).

**Table 5:** values of the diameter of the inhibition zones

| Fraction / Witnesses | E. coli | S. aureus | Salmonella typhi |
|----------------------|---------|-----------|-----------------|
|                      | MIC     | IS        | CMB pa          | MIC     | IS        | CMB pa          |
| FET                  | 0.12    | 0.312     | 0.02            | 2.5     | 0.02      | 0.625 0.08      |
| FDCM                 | 0.07    | 0.625     | 0.62            | 0.625   | 0.62      | 1.25 0.31       |
| FACE                 | 0.18    | 0.312     | 1.12            | 1.25    | 0.28      | 2.5 0.14        |
| FBut                 | 0.78    | 2.5       | 0.01            | 10      | 4         | 2.5 0.02        |

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; FDCM: dichloromethane fraction; FACE: ethyl acetate fraction; FBut: butanolic fraction.

**Comment:** Quantities mentioned are in point or separated by coma?
Reading this table we can retain that the butanolic fraction has an antimicrobial activity on E coli, S aureus, and Candida albicans but less marked compared to the controls that are tetracycline and gentamicin. We then continued our investigations from this butanolic fraction in order to isolate the bioactive molecules and this by ironing the butanolic fraction for a column fractionation at atmospheric pressure (CPA). We obtained three butanolic subfractions which we deposited on a plate with two other fractions namely dichloromethane and ethyl acetate. The chromatographic profile is shown in Figure 4.

On reading this chromatographic profile, we note three spots from the initial butanolic fraction with respective Rf of 0.4; 0.6 and 0.7; with the dichloromethane fraction, one obtains a trail as does the ethyl acetate fraction, on the other hand, with the P2 fraction, several spots are obtained, three of which are from feeder spots which are at the same Rf as those of the butanolic fractions. We can deduce that probably these spots are 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

To identify and isolate C1; C2 and C3, we have recovered C 1; C2 and C3 successively and proceeded case by case thus to the purification of each of the compounds. By 1-H NMR spectral studies; NMR-13C; cross-checking data from Fatondji R. 2011 and chemDraw Ultra 8.0 simulations; chemdraw office 2004 we have established the molecular structures which are the following thiosemicarbazones of the following carbonyls: 4-methoxypropiophenone; 4-methylacetophenone 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. 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 S 25922 Auréus 25923 and Candida albicans 10231.

The results obtained are presented in the following table (Table 6). 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, is interpreted as follows (Puyn and Shin, 2006; Shin and Lim, 2004):
- 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, greater than 1 additive, synergy if the index is less than 1 rather than less than or equal to 0, 5 but allows the results obtained to be clearly distinguished.

Table 7: Minimum inhibitory concentrations (MIC, μg / ml), fractionated inhibitory concentrations and CIF index for combinations of the different purified compounds C1; C2 and C3. ICIF values ≤ 0.5 are shown in bold (synergy).

|       | MICs  | MICa  | CIF  | ICIF  | MICs  | MICa  | CIF  | ICIF  | MICs  | MICa  | CIF  | ICIF  | MICs  | MICa  | CIF  | ICIF  |
|-------|-------|-------|------|-------|-------|-------|------|-------|-------|-------|------|-------|-------|-------|------|-------|
| C1    | 500   |   -   | 0,5  |   -   | 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    |   -   |       |       |      |       |
| 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  |   -   |       |       |      |       |

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 the 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. We also observe that the three successive combinations studied on Staphylococcus Aureus presented a synergistic biological action with $0.2 \leq \text{ICIF} \leq 0.3$. Combinations of thiosemicarbazones of 4-methoxypropioophenone 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-methoxypropioophenone and 3,4,5-trimethoxyacetophenone had a synergistic action on Candida albican with an ICIF=0.5.

CONCLUSION
This study showed that we were able to purify three (03) thiosemicarbazones from extracts of hemi synthetic products obtained from extracts of ethanolic thiosemicarbazones. These extracts in a process of monitoring biological activities and guided biological fractionations allowed to have the three thiosemicarbazones whose physicochemical studies have served to know their structure which prove that of the three purified thiosemicarbazones only one comes from a carbonyl compound already known during the previous work of Bissignano in 2000 which had made prowess on the knowledge of phytochemical studies of the plant of Mitracarpus Scaber. Two carbonyl precursor compounds of thiosemicarbazones identified had not been discovered from the plant of Mitracarpus Scaber. Thus we confirm that medicinal plants are libraries of several thousand molecules that we cannot stop exploring for new scientific discoveries. The combinations studied indicate attention for the different biological effects observed.

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 Sédami 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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