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Phytochemical profile and microbial sensibility of thiosemicarbazone extracts hemi synthesized of *Mitracarpus scaber* Zucc.

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Abstract
The phytochemical screening of *Mitracarpus scaber* powder showed the presence of alkaloids, catechic and Gallic tannins, flavonoids, saponosides, mucilages, reducing compounds, steroids and coumarins. The extracts (dichloromethane, water-alcohol (50:50 v/v) and alcohol) and their total alkaloid extracts were prepared. The alkaloid extracts were used for the hemi synthesis reaction of thiosemicarbazones. Hemi-synthesis products showed interesting bacteriostatic and fungistatic activities on the growth of the germs tested with the exception of *Escherichia coli* ATCC 25922 which remained insensitive to the various extracts (MIC > 10 mg/ml). The alkaloid extract from the alcohol crude extract and its hemi-synthesis product showed the strongest inhibitions on the growth of *Dermatophilus congolensis* with the minimum inhibitory concentration (MIC) equal to 1mg/mL and 0.5mg/mL respectively. However, the antimicrobial activity exhibited by the alkaloid extracts and their hemi-synthesis products remains lower than that of the control antibiotics: gentamicin ($0.031 \leq MIC \leq 0.125$ mg/ml) and tetracycline ($MIC = 10^{-3}$ mg/ml). The hemi synthesis have permitted to improve the antimicrobial activity of the total alkaloids derived from the crude extracts of *M. scaber*.

Keywords: *Mitracarpus scaber*, hemi synthesis, thiosemicarbazone, antimicrobial activity

Introduction
The health system in Africa and Benin in particular faces many challenges related to the poor performance of available preventive and curative services, the high cost of services in hospitals, the heavy external dependence on supply of essential drugs, poor medical coverage and sociocultural constraints to disease management and prevention [1, 2]. With these difficulties, traditional medicine is a significant alternative for medical care in response to the growing health needs of the population [2]. For example, the aqueous extract of *Mitracarpus scaber* is used in the treatment of dermatoses, either by direct application (eczema) or as an ointment [3]. It is used in traditional Senegalese medicine to treat syphilis [4]. Several studies have already been done on this plant such as the identification of substances responsible for some biological activities. The literature showed that the naphthoquinone derivatives identified in the plant have antibacterial and antifungal activities [5]. Similarly, it was isolated from the plant in 1999, 2-azaanthraquinone which exhibited inhibitory activity on some AIDS-related pathogens [6]. Other studies have shown that the alcoholic extract of the aerial part of *Mitracarpus scaber*, as well as certain isolated chemical principles (2-azaanthraquinone, oleanolic acid and ursolic acid) are active on *Dermatophilus congolensis* [7, 8]. In addition, many of some molecules identified in the plant contain one or more carbonyl groups and exhibit very marked antimicrobial activities. We can cite, among others, naphthoquinone, pentalongin with antimicrobial and antifungal activities [9]; 4-methoxyacetophenone and 3, 4, 5-trimethoxyacetophenone, which inhibit the growth of *Candida albicans* [10]; some flavonoids, namely kampferol-3-O-rutinoside and rutin, possess antibacterial and antimycotic activities [10].
The thiosemicarbazones are known for their broad spectrum of biological activities [11]. These include their antiviral [12], antibacterial [13-15], antimalarial [16], antitumor [17-22], anticonvulsant [23]. According to the importance of thiosemicarbazones in the field of health and the proven antimicrobial potential of the aerial part of *Mitracarpus scaber*, it would be beneficial to continue investigations on the plant by exploring other lines of research on the subject. Influence of the blocking of the carbonyl functions of the molecules present on the plant biological activity. It is the context that the present work, whose main objective is to carry out an *in situ* hemi synthesis of thiosemicarbazones from the alkaloid extracts of *Mitracarpus*

scaber and to compare the antimicrobial activities of the product obtained with those of the starting extracts, in order to estimate the therapeutic interest of the hemi-synthesis reaction.

Material and Methods

Material

- Fresh samples of *Mitracarpus scaber* were harvested and identified as No AA. 6252/HLB to the National Herbarium of the Abomey-Calavi University. The aerial part of the plant was spread out and dried for 7 days in the dark. The leaves were removed and reduced to powder using an electric grinder (*MILLS of NIGERIA Flour, El. MOTOR No. 1827*) and the powder is stored until use.
- The microbial support used for the tests contain 8 strains: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 22921, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 10231, *Salmonella typhi*, *Klebsiella pneumoniae* and *Dermatophilus congolensis* ATCC 14637.

Methods

Phytochemical screening

The different chemical groups were identified in *Mitracarpus scaber* using the classical method of Houghton [24] and routinely used by Houngbeme *et al.* [2], when they highlighted the broad classes of compounds in plants used to treat sexually transmitted infections and HIV-AIDS. Thus the Mayer and Dragendorff tests are used for alkaloids, the Fehling test for reducing and glycoside compounds, the Liebermann-Burchard test for triterpenoids and steroids, the frothy test for saponins, the Shinoda tests and sodium hydroxide for flavonoids, the ferric chloride test for tannins, the Guignard test for free cyanogenic derivatives and the Borntrager test for anthraquinones.

Preparation of crude and alkaloid extracts

100 g of powder were mixed with 500 ml of solvent (dichloromethane, alcohol and alcohol-water: 50/50, V/V) and left stirring for 72 h. The mixture is filtered 3 times consecutively on hydrophilic cotton and evaporated to dryness under reduced pressure using a rotavapor (*Heidolph Laborota 4000 efficient*) coupled to a water cooler (*Julabo FL 300*). The dry residue obtained constitutes the crude extract which was weighed to determine the yield.

In addition 1 g of each crude extract obtained is dissolved in 300 ml of water acidified with 99% sulfuric acid (pH = 2) and extracted 3 times with 60 ml of n-hexane (*SIGMA-Aldrich*). The aqueous solution is basified with NaOH 1 N to pH = 11 and extracted with 3x60 mL of dichloromethane (*SIGMA-Aldrich*). The dichloromethane fraction is recovered and evaporated to dryness in a rotavapor at 40 °C and the residue obtained constitutes the alkaloid extract.

In situ hemi synthesis of thiosemicarbazones

0.175 g of each crude and alkaloid extract is dissolved in 15 ml of 95° ethanol (SIGMA-ALDRCH) in an Erlenmeyer flask. In another Erlenmeyer flask containing 5 ml of the 1N hydrochloric acid solution (R.P. PROLABO), 1.82g of thiosemicarbazide (SIGMA-ALDRCH) are dissolved and stirred gently until complete dissolution. The contents of the Erlenmeyer flasks are then mixed in a 100 ml flask kept on a non-heating magnetic stirrer (BIOBLOCK AM 3000 D). The reaction is allowed to continue for 45 to 60 minutes. After filtration under vacuum, the crystals obtained are dried and recrystallized in ethanol at 95°. The hemi synthesis reaction is showed below on figure 1.

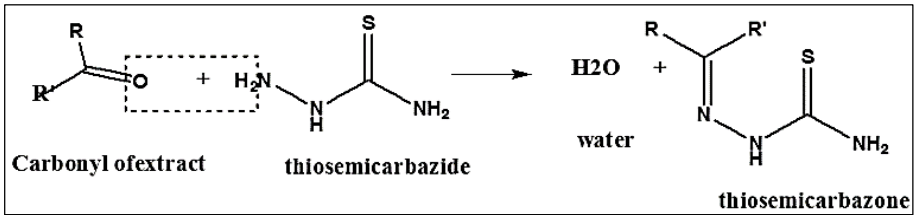

Fig 1: Equation of hemi synthesis reaction

In vitro antimicrobial test

The evaluation of the antimicrobial activity of the extracts was carried out by the method of micro dilution in a liquid medium on a 96-well microplate, widely described in the literature [25]. The tested extract solutions were prepared in acetone at 20 mg/ml as initial concentration. The bacterial suspensions were prepared according to the McFarland scale 2, i.e. a microbial density of 106 CFU/ml of LB medium (Luria Bertani) for *Escherichia coli* and *Staphylococcus aureus*, of Tryptone-yeast medium for *Pseudomonas aeruginosa* and *Enterococcus faecalis*, of Mueller Hinton Broth for *Klebsiella pneumoniae*, *Salmonella typhi* and *Candida albicans*.

100µl of the alkaloid extract solution and hemi-synthesis product were added to 100µl of distilled water from the first well of each line and successive dilutions of reason 2, were carried out in distilled water until the last well where 100µL of the mixture is discarded. Then 100µL of microbial suspension was added and the plate is incubated at 37 °C. After 18 hours of incubation, 40 µl of a solution at 0.2 mg/ml of violet p-iodonitrotetrazolium (p-INT) were added to each well and the plate was incubated again for 1 hour. P-INT is reduced by mitochondrial enzymes and colored in red; thus marking the presence of a life and enzymatic activity in the medium. The minimum inhibitory concentration (MIC) is the concentration of the non-stained red well in which there is the smallest amount of extract. The reading is done in comparison with the control wells. The positive control was achieved with gentamycin at concentrations equivalent to those in the wells.

The antimicrobial activity on *Dermatophilus congolensis* was carried out according to the method of dilution in a solid medium (National Committee for Clinical Laboratory Standards, 1990) used by Gbaguidi *et al.* [8]. This method consists of determining the minimum inhibitory concentration (MIC) of the various extracts using tetracycline (Sigma-Aldrich) as a positive control. The microbial suspension was prepared in BH (Brain heart or Tryptone Broth-Oxoid enriched nutrient broth medium) overnight at a density of 103-104 CFU. The strains were then seeded by streaking in Muller-Hinton Agar medium contained in a petri dish and containing the extracts at the concentration range of 1000 to 2µg/ml and 64 to 0.5µg/ml for tetracycline. The dishes were

incubated for 4 days at 37 °C and then read to determine the MIC. The tests were done in duplicate to ensure the accuracy of the results.

Results and Discussion

Extraction yield and chemical composition

The yields obtained during the various extractions carried out are respectively 16.13%, 21.09% and 19.23% for the crude extracts and 2.48%, 5.08% and 4.44% for the alkaloids of these extracts in this order.

Phytochemical screening showed the presence of alkaloids, catechin and Gallic tannins, flavonoids, saponoside, mucilage, reducing compounds, steroids and coumarins. These results recall those of Bisignano *et al*. (2000) and are similar to those of Gbaguidi [26].

Physical characteristics of hemi synthesis products

The first crystals appeared in the medium after 10 to 15 minutes. The final crystals obtained at the end of the reaction, are greenish color possibly due to the presence of chlorophyll in combination with these products. After recrystallization by ethanol at 95°, the dry residue was weighed and the various weight of products obtained are summarized in Table 1 below:

Table 1: weight of hemi synthesis products

Product	Wight (g)
P_1	0,204
P_2	0,207
P_3	0,236

P_1, P_2 and P_3 respectively denote the hemi-synthesis products derived from the total alkaloids from the hydroethanolic, ethanolic and dichloromethane extracts.

Antimicrobial activities of extracts and hemi-synthesis products

The results of the antimicrobial tests are shown in Table 2 below:

Table 2: Values of minimum inhibitory concentration (MIC) of the extracts

Extracts and antibiotics	MIC (mg/ml)							
	E. coli	*S. aureus*	*S. tiphy*	*K. pneumoniae*	*E. faecalis*	*C. albicans*	*P. aeruginosa*	*D. congolensis*
P_1	>10	0,625	5	5	1,25	2,5	1,25	1
P_2	>10	1,25	2,5	1,25	1,25	2,5	>10	0,5
P_3	>10	2,5	2,5	5	2,5	5	>10	>1
E_1	>10	2,5	5	5	2.5	5	>10	>1
E_2	>10	2,5	2,5	2,5	>10	2,5	>10	1
E_3	>10	>10	5	10	>10	5	>10	>1
Tétracycline	-	-	-	-	-	-	-	10^{-3}
Gentamicin	0,125	0,0312	0,125	0,125	0,125	0,0625	0,125	-

E_1: Alkaloid extract of dichloromethan crude extract
E_2: Alkaloid extract of alcohol crude extract
E_3: Alkaloid extract of water-alcohol crude extract
P_1: Hemi synthesis product from E_1
P_2: Hemi synthesis product from E_2
P_3: hemi synthesis product from E_3

The reading of this table shows that the various extracts tested proved to be inactive on *Escherichia coli* (MIC> 10 mg/mL). In contrast, hemi synthetic products obtained from total alkaloids showed antimicrobial activity with MICs ranging from 0.625 to 2.5 mg/mL for *Staphylococcus aureus*; 1.25 to 2.5 mg/mL for *Enterococcus faecalis*, 1.25 mg/mL for *Pseudomonas aeruginosa*, 1.25 to 5 mg/mL for *Klebsiella pneumoniae*, 2.5 to 5 mg/mL for *Salmonella tiphy* and *Candida albicans* and 0.5 to 1 mg/mL for *Dermatophilus congolensis* These extracts of thiosemicarbazones were more active on *Staphylococcus aureus* (MIC = 0.625 mg/ml for P_1) and on *Dermatophilus congolensis* (MIC = 0.5 mg/mL for P_2). This high sensitivity observed is due to the Gram-positive nature of these bacteria and their non-acid-fast nature [2, 26] On the other hand, the resistant nature of *E. coli* to the various extracts tested (alkaloids and hemi-synthesis products) is related to the complexity of its membrane composition, being a gram-negative bacterium. Furthermore, the results also show that the alkaloid extracts that served as a substrate for the hemi-synthesis reaction are bacteriostatic (2.5≤ MIC≤ 10 mg/ml) and fungistatic (1 ≤ MIC ≤5 mg/ml) on the strains explored with the exception of *E. coli* which remained resistant.

According to the MIC values, we can be deduced that the alkaloid extracts tested are fungistatic and bacteriostatic. Even better, it is generally noted that the microbial sensitivity becomes more and more high when alkaloid extracts are passed to the thiosemicarbazone extracts in this case for the P_2 extract of thiosemicarbazone on *Dermatophilus congolensis* (MIC = 0.5 mg/mL) which is 2 times more active than the corresponding E_2 substrate (MIC = 1 mg/mL). The alkaloid extract E_2 comes from the alcohol crude extract which according to the literature, is active on *Dermatophilus congolensis* with a minimum inhibitory concentration of 1 mg/ml [8].

Our work has confirmed this activity which is better for the alkaloid fraction and the extract of its thiosemicarbazone. The inhibitory activity demonstrated by hemi-synthesis products is justified by the antibacterial properties recognized by thiosemicarbazones [13-15].

Moreover, the activity exhibited by the hemi-synthesis products is inherent to the presence of the sulfur atom which has an essential function in the antibacterial activity of thiosemicarbazones [16].

The hemi-synthesis reaction thus reveals a capital interest that of enhancing the antimicrobial activity of the extracts used as basic substrates.

Conclusion

This study showed that the synthesized thiosemicarbazone hemi-extracts have a more marked antimicrobial activity on the multiplication of the germs used with the exception of E. coli, compared to the corresponding alkaloid extracts representing the substrates of the reaction. The alkaloid extract which come from the alcohol crude extract (E_2) and its hemi synthetic product (P_2) showed the strongest inhibitions

on the growth of *Dermatophilus congolensis* with MIC equal to 1 mg/ml and 0.5 mg/ml respectively. According to these results the most resistant strain to the tested extracts is *E. coli*, while *Dermatophilus congolensis* is the most sensitive. Further works are needed to continue investigations of the hemi synthetic product P_2 by identifying active molecules of thiosemicarbazones and to test *in vivo* and *in vitro* their toxicity in order to assess their selectivity on the germs.

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References

1. Nikiema P, Wendpagnagde R. Propriétés pharmaco chimiques de *Calotropis procera* Ait. récolté au Mali: étude préclinique des effets anti-inflammatoires et antimicrobiens des extraits des écorces de racines. Thèse de Doctorat, Université de Bamako. 2005, 162.
2. Houngbeme AG, Gandonou C, Yehouenou B, Kpoviessi SDS, Sohounhloue D, Moudachirou M *et al*. Phytochemical analysis, toxicity and antibacterial activity of Benin medicinal plants extracts used in the treatment of sexually transmitted infections associated with HIV-AIDS. Int. J Pharm Sci. Res 2014; 5(5):1739-1745.
3. Neuwinger HD. African traditional medicine: a dictionary of plant use and application, 2000.
4. Kambu K. Elément de Phytothérapie Comparée, 1990.
5. Ogundaini. Antimicrobial agent from some Nigerian plants. Publié dans Niger. J Nat. Prod. Med. 1999; 3:26-27.
6. Okunade AL, Alice MC, Charles DH, Babajide OO. Azaanthraquinone: An Antimicrobial Alkaloid from *Mitracarpus scaber*. Planta Medica, 1999, 447-448.
7. Ali-Emmanuel N, Moudachirou M, Akakpo AJ, Quetin-Leclercq J. Activités antibactériennes *in vitro* de *Cassia alata*, *Lantana camara* et *Mitracarpus scaber* sur *Dermatophilus congolensis* isolé au Bénin. Élev. Méd. vét. Pays. 2002; 55(3):183-187.
8. Gbaguidi F, Accrombessi G, Moudachirou M, Quetin-Leclercq J. HPLC quantification of two isomeric triterpenic acids isolated from *Mitracarpus scaber* and antimicrobial activity on *Dermatophilus congolensis*; J Pharm Biomed Anal. 2005; 39(5):990-5.
9. Pialat. Synthesis and extraction of pentalongin, a naphthoquinone from *Mitracarpus scaber*. Publié dans Fr. Nat. Prod. Lett. 1998; 12(1):23-30.
10. Bisignano G, Sanogo R, Marino A, Aquino R, D'Angelo V, Germano MP *et al*. Antimicrobial activities of *Mitracarpus scaber* extract and isolated constituents. Lett. Appl. Microbiol. 2000; 30(2):105-108.
11. Fatondji HR, Kpoviessi S, Gbaguidi F, Bero J, Hannaert V, Quetin-Leclercq J *et al*. Structure-activity relationship study of thiosemicarbazones on an African trypanosome: *Trypanosoma brucei brucei*. Med Chem. Res. 2013; 22(5):2151-2162.
12. Garcia CC, Brousse BN, Carlucci MJ, Moglioni AG, Martins AM, Moltrasio GY *et al*. Inhibitory effect of thiosemicarbazone derivatives on Junin virus replication *in vitro*. Antivir Chem. Chemother. 2003; 14:99-105.
13. Sau DK, Butcher RJ, Chandhuri S, Saha N. Spectroscopic, structural and antibacterial properties of copper (II) complexes with bio-relevant 5-methyl-3-formylpyrazole N (4)-benzyl-N(4). Methylthiosemicarbazone. Mol Cell Biochem. 2003; 253(1-2):21-22.
14. Rebolledo AP, De Lima GM, Gambi LN, Speziali NL, Maia DF, Pinheiro CB *et al*. Tin (IV) complexes of 2-benzoylpyridine N (4)- phenylthiosemicarbazide: spectral characterization, structural studies and antifungal, 2003.
15. Kasuga NC, Sekino K. Ishibawa. Synthesis, crystal structures and antibacterial activity of monomeric 7-cordinate bismuth (III) Med Chem. Res. 2003; 22:2151-2162.
16. Klayman DL, Scovill JP, Bruce J, Bartosevich JF. 2-Acethylpyridine thiosemicarbazones derivatives of methylisoquinoline as potential antimalarial agents. J Med Chem. 1999; 27:84.
17. Quiroga AG, Perez JM, Lo´pez-Solera I, Montero EI, Masaguer JR, Luque A *et al*. Novel tetranuclear orthometalated complexes of Pd (II) and Pt (II) derived from p-isopropylbenzaldehyde thiosemicarbazone with cytotoxic activity in cis-DDP resistant tumor cell lines. Interaction of these complexes with DNA. J Med Chem. 1998; 41(9):1399-1408.
18. Perez JM, Matesanz AI, Martin A, Navaro P, Alonso C, Sousa P. Synthesis and characterization of complexes of p-isopropylbenzaldehyde and methyl 2-pyridyl ketone thiosemicarbazones with Zn (II) and Cd (II) metallic centers. Cytotoxic activity and induction of apoptosis in Pam-ras cells. J Inorg Biochem. 1999; 75:255-261.
19. Easmon J, Purstinger G, Heinisch G, Roth T, Fiebig HH, Holzer W *et al*. Synthesis, cytotoxicity, and antitumor activity of copper (II) and iron (II) complexes of (4) N-azabicyclo [3.2.2] nonane thiosemicarbazones derived from acyl diazines. Med Chem. 2001; 44:21-64.
20. Hall IH, Lachey CB, Kistler TD, Durham RW, Jouad EM, Khan M *et al*. Cytotoxicity of copper and cobalt complexes of furfural semicarbazone and thiosemicarbazone derivatives in murine and human tumor cell lines. Pharmazie. 2000; 55(12):937-941.
21. Kovala-Demertzi D, Demertzis MA, Miller JR, Papadopoulou C, Dodorou C, Filousis G. Structure of bis (2-acetylpyridine 3-hexamethylene iminyl thiosemicarbazone) palladium (II), a potential antitumor complex. J Inorg. Biochem. 2002; 92:137.
22. Afrasiabi Z, Sinn E, Rath N, Fok J. A appended 1, 2-nathoquinone as anticancer agents Synthesis, structural, spectral and antitumor activities of ortho-Antivir. Chem. Other. 2004; 14:99-105.
23. Pandeya SN, Aggarwal N, Jain JS. Evaluation of semicarbazones for anticonvulsant and sedative-hypnotic properties. Pharmazie. 1999; 54:300.
24. Houghton PJ. Raman A. Laboratory handbook for the fractionation of natural extracts. Chapman ET Hall, London, 1998.
25. Houngbeme AG, Ganfon MYH, Medegan S, Yehouenou B, Bambola B, Gandonou C *et al*. Antimicrobial activity of compounds from *Acanthospermum hispidum* DC and *Caesalpinia bonduc* (L.) ROXB: Beninese plants used by healers against HIV-associated microbial infections. J App Pharm Sci. 2015; 5(08):073-081.
26. Gbaguidi AF. Identification, purification, isolement et quantifications de principes actifs de *Mitracarpus scaber* et évaluation de leurs activités antimicrobiennes sur *Dermatophilus congolensis*. Thèse de doctorat, Université d'Abomey-calavi et Université Catholique de Louvain, 2005, 176.