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Original Research Article

Analysis by HPLC of organic extracts of *Tapinanthus bangwensis* used for the conservation of stocks of *Zea mays* (Linné) and *Vigna unguilculata* L. Walf against two principal devastating insects (*Sitophilus zeamais* and *Callosobruchus maculatus*)

El Hadji Gorgui DIOUF[1, 2], Abdoulaye SAMB[1], Moussoukhoye DIOP[1], Aboubacary Sène[1], Sam Gueye[2]
[1]Department of Chemistry, University Cheikh Anta Diop, Faculty of Sciences and Technolocals, Laboratory of Natural Products, Dakar, Senegal
[2]Regional Centre for Ecotoxicology Studies and Environment Security (CERES-Locustox), Dakar, Senegal

***Corresponding author**
El Hadji Gorgui DIOUF
Email: goorgui82@yahoo.fr

Abstract: The objective is to find the alternate ones with insecticides of synthesis, the extracts of plants are used more and more by the peasants to protect stocks from harvest against the devastating insects. Thus, a chemical analysis by liquid chromatography high efficiency (HPLC) is carried out on the organic extracts, of *Tapinanthus bangwensis*, tested on *Sitophilus zeamaïs* and *Callosobruchus maculatus*. The results of the analysis by HPLC show that the extracts contain polar compounds. These results are corroborated by the phyotochimic tests with the identification of molecules (alkaloids, flavanoides, tannins…) likely to be responsible for this insecticidal activity.
Keywords:extracts, *Tapinanthus bangwensis*, *Sitophilus zeamais*, *Callosobruchus maculatus*, stored food products, maize, niébé.

INTRODUCTION

In sub-Saharian Africa, the losses post-harvest of cereals, before transformation, are estimated at 10-20% and approximately 4 billion dollars. These losses account for 13.5% of the total bill of the cereal production of these countries. The losses post-harvest are estimated in the 22 member countries CORAF with 10% for the cereals [1,2]. These difficulties of storage are related to several factors among which one can quote the attack of the devastating insects of maize stock and of niébé of which most frightening are *Sitophilus zeamaïs* and *Callosobruchus maculatus* respectively. Losses due to the insects strongly penalizing the commercial value of the productions and the products stored create deficits mainly filled by expensive imports of foodstuffs.

To fight against the devastating insects of stocks, the most used method is the use of synthetic insecticides (organophosphates and organochlorines). These pesticides of long persistence ensure the seed protection since the storerooms until in the fields after sowings as well as young seedlings against the insects and the diseases. This causes not only problems of resistance in the devastating insects but, would involve also adverse effects on the environment and the human health.

In the search for alternative methods of fight, the vegetable kingdom offers many possibilities. Many studies currently develop to insulate or identify secondary metabolites extracted from plants which have an insecticidal activity, repulsive or anti appetizing with respect to the insects [3]. It is accordingly that this study is registered.

The Objective of this work is to analyze by liquid chromatography high efficiency (HPLC) of organic extracts of *Tapinanthus bangwensis* used for the conservation of stocks of *Zea mays* (Linné) and *Vigna unguilculata* L. Walf against two principal devastating insects (*Sitophilus zeamais* et *Callosobruchus maculatus*)

MATERIALS AND METHODS

Material

Technical Material

For this study, classical equipment made it possible to have access to the plants and to et al. make samplings while referring of work of Nguessan [4]. Drying was made in the shade and safe from the light before using

an electric crushing for pulverization. An electronic balance is used to carry out the various weighings. A rotary evaporator, regulated with less 50°C to avoid the artifacts, is used for evaporations. This equipment also comprised spatulas, for the taking away of the powders of drug, of the absorbent cotton used like filter, a hood of protection against the powders ejected during the pulverization of drugs, a rod of trituration and grips besides the glassmaking. For thin layer chromatography (TLC) of the plates out of Silica glass are used for support, of the tanks and the pipettes Pasteur, a lamp UV (ultraviolet) for the observation of the spots. For the biological tests, limp of Petri of diameter 90mm and of the pipettes Pasteur of brand LABMATE Software were used.

Plant material

It comprises various types of drugs (barks of stem, sheets) which were crushed and used for the extractions with solvents of gradient of increasing polarities.

Solvents

The various solvents used for the extraction, the tests of identification, the biological tests and the TLC are:

- Ethyl acetate PA-ACS-ISO; Mininum assay (G.C.): 99, 5%; Identity: IR p/t.; Density at 20/4: 0, 9000-0,902;
- Acetic acid PA-ACS-ISO; Mininum assay (G.C.): 99, 5%; Identity: IR p/t.; Density at 20/4: 1, 05;
- Cyclohexane, (Reag. USP, Ph.Eur.) PA-ACS; Mininum assay (G.C.): 99, 0%; Identity: IR p/t.; Density at 20/20: 0, 659-0, 663;
- Chloroform (Reag. USP, Ph.Eur.)PA-ACS-ISO; Mininum assay (G.C.): 99, 5%; Identity: IR p/t.; Density at 20/4:1, 48;
- Dichmoromethan stabilized with amylene PA-ACS-ISO; Mininum assay (G.C.): 99,5%; Identity: IR pt.; Density at 20/4: 1, 323-1, 325;
- Methanol (Reag. USP, Ph.Eur.)PA-ACS-ISO; Mininum assay (G.C.): 99, 5%; Identity: IR p/t.; Density at 20/4: 0, 791-0, 792.

Reagents

For the identification and the description of the various chemical groups present in each extract, several types of reagents and witnesses were used while taking as a starting point the work by Békro *et al* [5]. For the identification and testing of different present chemical groups in each sample, several types of reagents and controls were used. For tannins, we used as control tannic acid and ferric chloride at 20% as revelator. Flavonoids and polyphenols were determined using Vitexin as control and Aluminium Chloride as reagent. The alkaloids were revealed through the reagent of Draggendorf using Cinchonine as control. The reagent of Draggendorf is prepared starting from a solution made up of 0,85g of basic nitrate of bismuth and 10g of tartaric acid in 40 ml of water (solution A) and a solution containing 16 g of KI in 40 ml of water (solution B). Extemporaneously to mix 5 ml of A, 5 ml of B, 100 ml of water and 20 g of tartaric acid.

Biological material

Animal material relates to *Sitophilus zeamais* and *Callosobruchus maculatus* obtained by mass rearing.

METHODS

Harvest, drying, extraction and breeding of mass

Harvest and drying

The specimens are collected in the rural community of Keur Balla, locality located in the department of Mbour which is a subsidy of the region of Thiès ranging between the latitudes 14°02' and 15°27' Northern and Western longitudes 16°09' and 17°12'. Thus, thanks to the assistance of an old tradipratician, we collected the sheets of *Tapinanthus bangwensis*. Vegetable material obtained was dried in the shade using the light during two weeks.

Extraction

The method used for the extractions is the maceration with solvents of gradient of increasing polarity (Cyclohexan, Administers chloroform to and Methanol). The extract obtained is concentrated using a rotary evaporator before being dried at the room temperature and safe from the light.

Mass rearing

That relates to *Sitophilus zeamais* and *Callosobruchus maculatus*. We found maize infested at the laboratory and niébé infested at the market with which one operated sorting, recovered the insects and launched thebreedings. The breedings are launched in jars out of glass of 500 ml of volume approximately. Inside the jars, one put a number from 20 to 25 insects and impregnated absorbent water cotton to create the conditions of moisture necessary for a good reproduction of the insects. These jars are perforated and covered with fabric of mosquito net to make it possible the insects to breathe. The breedings are made in the shade and the room temperature (25°C approximately). At the end of 17 to 28 days, we observed emergences. The tests of insecticidal activity are carried out on insects of first generation this be-with-to say which are old between 0 and 24 hours.

Photochemical screening

The highlighting of different families of chemical compounds in *Tapinanthus bangwensis* is made by Thin Layer Chromatography (TLC) and by staining tests and precipitation. For the identification of different chemical groups by thin layer chromatography (TLC), we relied on the course of [6] Bassene on lipid extraction. For the identification of tannins, we used as eluent a mixture of ethyl acetate, methanol and water in the proportions of 40 ml, 5 ml and 8 ml respectively. For this purpose, we used as the stationary phase glass plates covered of silica gel. The brown color of spots indicates the presence of tannin in the extracts. For

flavonoids, the eluent used was a mixture of ethyl acetate and water (15%). The stationary phase was glass plates covered with cellulose. The revelation was made with Aluminum Chloride and observation under UV at 254 nm. The yellow coloring indicates the presence of flavonoids. Alongside the identification of flavonoids, may be that of the polyphenols with a UV exposure without direct use of reagents. Thus, there may be several luminescences with various colorations. To find saponins, we paid in a test tube, 10 ml of aqueous total extract. The tube was agitated for 15s and allowed to stand for 15 minutes. Height persistent foam than 1 cm indicated the presence of saponins.

Biological test

The tests relate to three extracts (cyclohexanic, chloroformic and methanolic) of *Tapinanthus bangwensis* and two types of insects (devastating *Callosobrucus maculatus* of niébé and devastating *Sitophilus zeamais* of maize) and two speculations (niébé and maize). Each extract is tested on the two types of insect. Starting from each dry extract, we prepared five solutions of different amouns (100mg/ml, 2: 50mg/ml, 3.4 and 5). Solution 5 is obtained by taking 1g of dry extract which one dissolves in 10 ml of solvent. Solution 4 is obtained by piping 5 ml of the solution 5 which one supplements to 10 ml with solvent. With the same process, we obtained solution 3 starting from solution 4.2 from the 3 and 1 from the 2. The biological tests are carried out in limp of Petri of diameter 90mm. In each limps, one put 20g speculation (niebe or maize). The tests are carried out by pulverizing 500 µl of each solution in limp of Petri thanks to a pipette Pasteur. The test is repeated five times for *Sitophiluszeamais* and four times for *Callosobruchus maculatus*. The whole is then left with the free air during 20 mn to allow the evaporation of solvent. The insects are introduced thereafter into each limp. On the whole, we used 1215 experimental units to evaluate the toxicity of all the extracts on the insects is 135 units per extract (135× 9 = 1215). The insects are introduced thereafter into each limp. The dead insects are sorted and recovered using aluminium grip. The number of died, alive and emerged insects are then counted. The formula of Aboth: Mc = (Mo-MT)/ (100-MT) *100; (with Mc: calculated mortality, Mo: mortality observed and MT: mortality in the pilot batches) is used to correct mortality observed.

STATISTICAL ANALYSIS

For the data of the biological tests with the extracts of the plant, the measured variables are the number of died insects, the number of surviving insects and the number of emerged insects. Calculated mortality was obtained by applying the formula of Aboth (1925): Mc = (Mo-MT)/(100-MT) *100; (where Mo = mortality in the treated batches, MT = mortality in the witness and Mc = calculated mortality). The variables many died insects, number of the surviving insects and number of the emerged insects are subjected to a variance analysis, model fixed with three factors (extracted, amounts and time). Variable mortality rate underwent a transformation arcsin (X = mortality rate, N = size of the population; n= 1999) in order to standardize the population and to stabilize the variance. The method General Linear Model in Minitab 17 was used for the statistical analysis of the collected data. The variables many surviving insects and many insects emerged as for them underwent a transformation square root in order to standardize the population and to stabilize the varian. The curves and the tables are used to have the result of the analysis.

Analysis by liquid Chromatography high efficiency HPLC UV of the active extracts of *Tapinanthus bangwensis*

Material

The material consists of:
- Cyclohexanic extract of *Tapinanthus bangwensis*;
- Chloroformic extract of *Tapinanthus bangwensis*;
- Méthanolic extracts of *Tapinanthus bangwensis*;
- Methanol;
-Distilled water quality HPLC;
- Acetonitrile;
- Column RPC18, 15×3, 2mm, 5µm;
- Syringe, vials, agitator;
- HPLC of brand JASCO

Methods

A liquid chromatography Jasco high efficiency was used. It is provided with a pump (PU 2080 More) and is coupled with a system of detection with absorption UV (MD 2015 Plus) with bar of diodes (UV-DAD). The unit is controlled by a computer provided with and the acquisition operating software of the Chromnav data.The system of introduction of the samples is consisted a loop rheodyne® of 20 µL which is filled with the sample using a syringe.

A column of the type Kromasil 100 C18 (25 X 0.46 cm, 5µm, Tecknokroma, Barcelona, Spain) allows the separation of the molecules and elution in isocratic mode was made by a binary mixture composed of acetonitrile and water with 60 and 40% respectively with a flow of 1 mL/min. Under these conditions, the duration of the analysis is of 20 min. the compounds were identified according to their time of retention and their spectrum UV.

RESULTS

Extraction Results

From64.154gofpowder of the plant (*Tapinanthus bangwensis*), we completed threecyclohexane,chloroformandmethanol extracts. The results of theextractionswere confinedin the following table:

Table 1 : Results of extractions

Extracts	Aspect	Mass (g)	Yield
Cyclohexanic	Powder	2.097	3.27%
Chloroformic	Powder	1.414	2.20%
Methanolic	Pasty	6.742	10.51%

Phytochemical study

The table of phytochemicaltestsshowed the presence ofalkaloids incyclohexanicandchloroformicextractsof the plant.Flavonoidswere presentinthe methanolic extract. The three extracts (cyclohexanic,methanolicandchoroformic)containall polyphenols. As for tannins,they were identified only with chloroformicandmethanolicextracts. The research of saponins is positive inthe aqueous extract of*Tapinanthusbangwensis*.

Table 2: Results of Phytochemical tests

Extracts	Alkaloids	flavonoids	Polyphenols	Tannins	Saponins
Cyclohexanic	+	-	+	-	-
Chloroformic	+	-	+	+	-
Methanolic	-	+	+	+	-
Aqueous	-	-	-	-	+

+ : presence - : absence

Table 3: Result of variance analysis of observed parameters

	Mortality			Emergency		
Source of variation	DL	F	P	DL	F	P
Extracts	2	12, 94	0, 000	2	28, 86	0, 000
Doses	4	2, 23	0, 064	4	0, 80	0, 522
Insects	1	352, 09	0, 000	1	12, 82	0, 000
Time	1	470, 61	0, 000	1	0, 04	0, 850
Time doses	4	3, 16	0, 013	4	0, 58	0, 677
Time extracts	2	9, 18	0, 000	2	21, 40	0, 000
Time insects	1	32, 53	0, 000	1	15, 43	0, 000
Doses extracts	4	0, 67	0, 719	8	1, 01	0, 421
Doses insects	1	1, 21	0, 303	4	0, 52	0, 724
Extracts insects	2	20, 18	0, 000	2	19, 72	0, 000
Time doses extracts	8	1, 89	0, 057	8	1, 23	0, 279
Time doses insects	4	2, 31	0, 056	4	0, 54	0, 708
Temps extraits insects	2	21, 53	0, 000	2	25, 99	0, 000
Doses extraits insects	8	2, 39	0, 015	8	0, 53	0, 833
Time doses extracts insects	8	2, 81	0, 004	8	0, 88	0, 529
Error	975			330		
Total	1299			439		

Figure 1 show the pace of the curve of mortality according to time, the amounts, the extracts and the insects. The cyclohexanic, chloroformic and methanolic extracts of *Tapinanthus bangwensis* are effective on *Sitophilus zeamaïs*.The curve of mortality according to time shows that mortality rate is high with short (1^{e}, 4^{th}, 5^{th} and 7^{e}j) and with long terms (17^{th} and 18^{e}j).

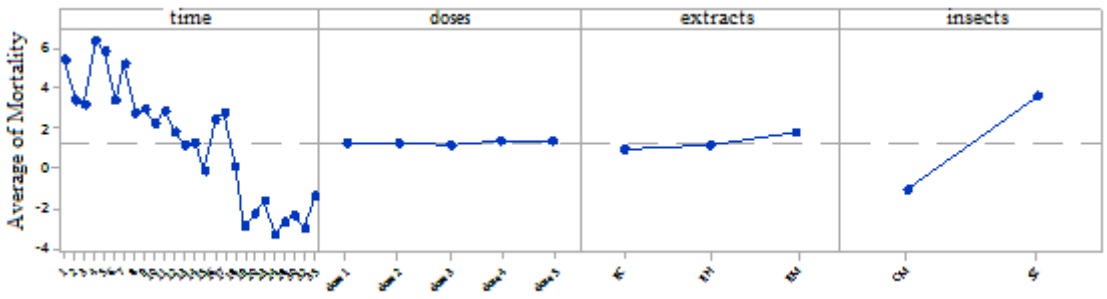

Fig 1: Curve of mortality in function of doses, extracts, insects and time. EC: cyclohexan extract. EH: chloroform extract. EM: methanolic extract. Dose 1: 6. 25g/l. dose 2: 12. 5g/l. dose 3: 25g/l. dose 4: 50g/l. dose 5: 100g/l.

Figure 2 give the shape of the curve of the insects emerged according to time, the amounts, the extracts and the insects. The cyclohexanic and the methanolic extracts of *Tapinanthus bangwensis* reduce considerably the number of insects emerged on *Sitophilus zeamaïs* in the long run.

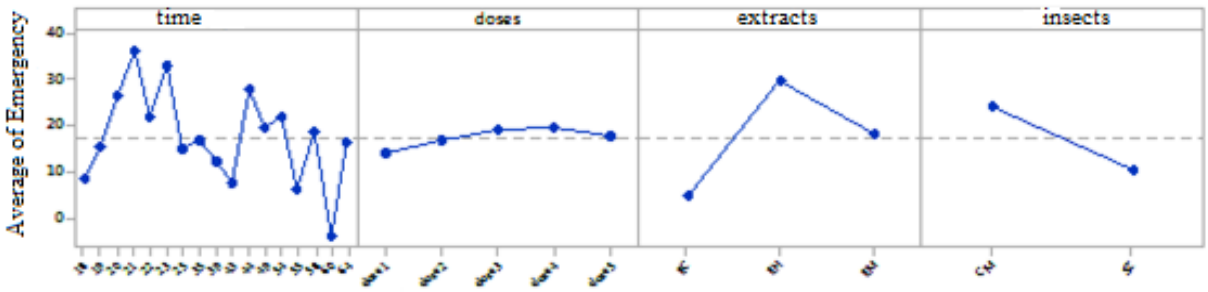

Fig 2: Curve of insects emerged in function of extracts, doses, insects and times of *Tapinanthus bangwensis*

Results of the chemical analyses by liquid chromatography high efficiency HPLC-UV of the active extracts of *Tapinanthus bangwensis*

Figure 3 give the chromatogram and the results of the analysis by HPLC of the chloroformic extract of *Tapinanthus bangwensis*. The chromatogram contains 19 peaks with times of retention ranging between 0 and 17.2 mn. The most important peak is $N^0$4 (tr =1,967) come then the peaks $N^0$8 (tr=3,242mn), $N^0$13 (tr=6,808) and $N^0$5 (tr =2,225) with respectively of surfaces of integration of 1,958, 3,242, 6,808 and 2,225mm2. The results show that the chloroformic extract of *Tapinanthus bangwensis* contains polar molecules (TR between 0 and 5 mn), of average polarity (tr between 5 and 10 mn) and of the non-polar substances (tr between 10 and 17.5 mn).

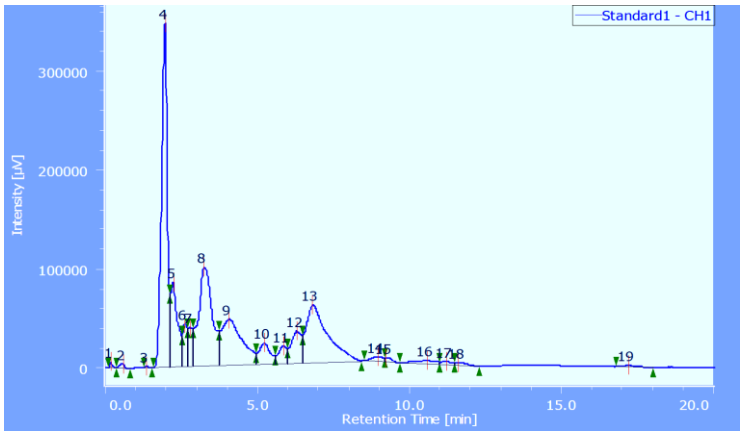

Fig 3: Chromatogram of chloroformic of *Tapinanthus bangwensis*

Figure 4 give the results of the analysis by HPLC of the methanolic extract of *Tapinanthus bangwensis*. The chromatogram contains 11 peaks with relatively important surfaces of integration. The analysis shows that the extract contains polar molecules (peak 3 and peak 4), molecules of average polarity (peak 6, peak 7, peak 8 and peak 9) and molecules non-polar (peak 10 and peak 11).

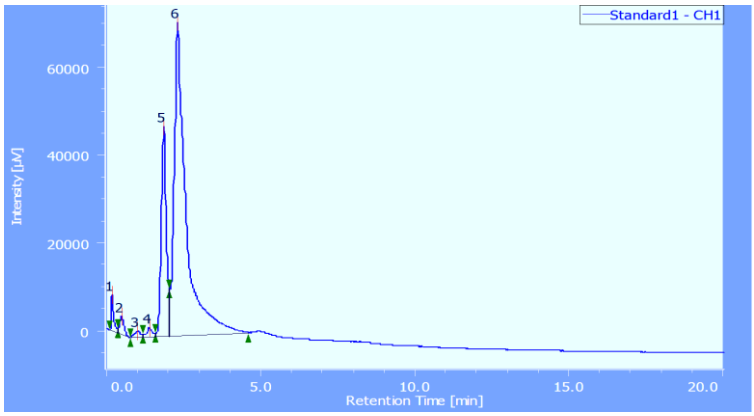

Fig 4: Chromatogram of methanolic extract of *Tapinanthus bangwensis*

Figure 5 give the results of the analysis by HPLC of the hexanic extract of *Tapinanthus bangwensis*. The chromatogram contains 11 peaks with relatively important surfaces of integration. The analysis shows that the extract contains polar molecules (peak 3 and peak 4), molecules of average polarity (peak 6, peak 7, peak 8 and peak 9) and molecules non-polar (peak 10 and peak 11).

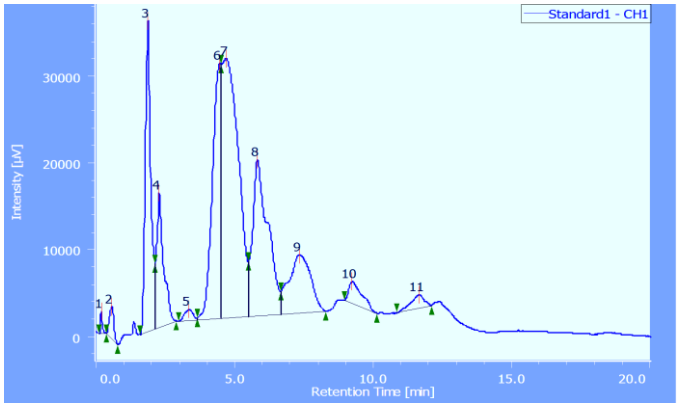

Fig 5: Chromatogram of cyclohexanic extract of *Tapinanthus bangwensis*

DISCUSSION

The effect of the methanol extract of *Tapinanthus bangwensis* is more significant on *Sitophilus zeamais* and *Callosobruchus maculatus* (maize weevil). Moreover, the results of phytochemical screening showed that the methanol extract contains tannin, polyphenols and flavonoids. The ubiquitous polyphenols in nature provocate a disturbance of the natural traction of insect. It can be fast the first day for quercetin or later on the fourth day for narangine, syringaldehydeorvanillic acid. It is accompanied in some cases(caffeic and ferulic acid,vanillin,luteolin-7-glucoside) a knock down effect. After eight days the insects are in a coma or dead state. The toxicity of polyphenolsis positively correlated with the attractive power of compound [7].The tannins have a direct toxic effect on some insect species [8]. Tannins influence on growth, development and fecundity of several insect pests [9]. The reduction in growth caused by tannins has major disadvantages for the insect with a lower number of eggs and smaller eggs. This would affect the survival and health of individuals in the subsequent generation [10]. Thus, aromatic plants and their allelochemicals molecules exert a dual activity:

- On adults by rapid toxic inhalation (monoterpenes) on the one hand and action which contributes to the insecticidal activity of the aromatic plant of a lower intensity but is exercised in the period (polyphenols);
- On the different phases of the reproductive cycle: inhibition of fertility and larvicidal and ovocide activity at neonatal and later stages.

The insecticidal activity observed with the extracts with the cyclohexane and the Chloroform of *Tapinanthus bangwensis* can be due to the presence of active ingredients similar to Viscotoxine. Kerharo and Adam [11] show that *Tapinanthus bangwensis* contains of Viscotoxine which is a toxic active ingredient.

The Angiosperms contain alkaloids which are secondary metabolites made up by the secondary nitrogen atoms, tertiary or quaternary in their structures [12]. They are metabolically active and play a significant role in the physiology of the plants or the organizations. The alkaloids have repulsive properties or anti appetizing in the connection of the devastating insects [13].

Several studies showed that species of the family of Capparidaceae showed the insecticidal effect of the organic extracts on the devastating insects of stock of harvest. Among this work, it can quote those of Gueye *et al* [14] which showed the insecticidal activity of *Boscia senegalensis* on *Caryedon serratus* (groundnut beetle). Many work also showed that the organic extracts of plant give insecticidal effects on the devastating insects of stored food products. The toxicity of the extracts with organic solvents of *Afrostyrax lepidophilus*, *Trichilia gilgiana*, *Drypetes gossweileri* and *Zanha golungensis* with regard to *Sitophilus zeamaïs*, *Tribolium castaneum* and *Rhyzopertha Dominica* is shown by work of Aba Toumnou [15].

The whole of the chromatograms, obtained with the organic extracts (cyclohexanic, chloroformic and methanolic) of *Tapinanthus bangwensis*, shows the presence of polar and fairly polar compounds. These results confirm those obtained during the phytochimic tests carried out on these same extracts.

Indeed, it was shown at the time of screening that *Tapinanthus bangwensis* contains polyphenols, alkaloids and saponins which are polar compounds [16].

In addition, the chromatogram of the cyclohexanic extract of *Tapinanthus bangwensis* presents the same profile as that obtained by Tokusoglu *et al.;* [17] under the same chromatographic conditions (elution by the same binary system water acetonitrile 60:30, V/V (phase reverses) and by using a detector UV after having fixed the wavelength at 254nm) and the

presence of three flavonoids marks (Kaempherol, Quercetin and Myricetin).

The chromatogram, obtained with the methanolic extract of *Tapinanthus bangwensis*, presents the same profile with that obtained with the butanolic extract of the species *Génista ferox* by Mekkiou [18] under the same conditions of analysis with two major peaks. The purification of these extracts led to a diversity of phenolic secondary metabolites with twenty-four isolated products [18].

In the same way the chromatogram of the methanolic extract shows the same characteristics of several classes of flavonoids in methanol with two absorption bands : flavons (250-280; 310-350), substituted flavonols 3-OH (250-280; 330-360nm), the Penduletin (250nm and 340nm), the isoflavons (240-280 and 245-275nm), the Apigenin 7-O glucosid (268nm and 363nm), the Isovitexin and the Saponarin [19], Penduletin (250nm and 340nm), the isoflavons (240-280 and 245-275nm), Apigenin-7-O-glucoside (268nm and 363nm), Isovitexin and Saponarin [19]. These extracts thus contain flavons and/or flavonols. These results corroborate the fact that the extracts of *Tapinanthus bangwensis* contain phenolic compounds which are likely to be responsible for the biological activities noted in the results of the various studies carried out.

CONCLUSION

The biological tests of the three extracts of *Tapinanthus* bangwensis *on Sitophiluszeamais* and *Callosobruchus maculatus* shows that methanol offers better insecticidal activity extract. This result is also corroborated by the chemical analyses by the liquid chromatography high efficiency HPLC-UV and the phytochemical study of the different extracts. Thus, this extract will be subject to bio-guided fractionation to isolate the active principle (s).

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