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ANALYSIS BY HPLC OF THE CHLOROFORMIC EXTRACTRESULTING FROM SEEDS OF *ALYSICARPUS OVALIFOLIUS* (SCH. AND TH.) TESTED ON LARVAE OF MOSQUITO.

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ABSTARCT

Introduction: *Alysicarpus* is a kind of plants with flowers belonging to the family of Fabaceae, made up of 106 species.[1] The *Alysicarpus* kind is resistant to the drought, tolerating with salinity and resistant to the diseases and the ravagers. These properties are associated with their secondary wealth of metabolites such as the flavonoids, the alkaloids tannins etc. These secondary metabolites currently constitute tracks of made up of origin vegetable used like repulsive or toxic products against insects and ravagers. One can set the example of rotenone, rotenoïdes and pyrethrins.[2] Indeed, In the fight anti-mosquito, the active matters of insecticides of synthesis used present several disadvantages. To ensure a better intervention, while preserving to the maximum the natural environment, of new preventive methods as of new products are constantly required.[3] The family of the leguminous plants whose been part the *Alysicarpus* kind offers a panoply of capable compounds to help to fight against certain insects like the mosquitos. The latter are responsible for diseases like paludism. This disease remains public health problems in Senegal because the burden of the disease is always heavy in particular in certain areas of the country and the deaths which areallotted to him persist.[4] The important place that the mosquitos in the terrestrial environment as in the aquatic environment occupy on the one hand, and the fight against the diseases transmitted by their punctures on the other hand, make these Arthropods an important equipment of study for the biologists.[5] Then, the natural substances with bioactives molecules resulting from the plants currently arouse a very particular interest by their multiple biological activities (antibacterial, antioxydant and insecticidal).[5] It is in this context that this study on *Alysicarpus ovalifolius* (Sch is registered. and Th.) which, to our knowledge, was not yet the object of studies pushed in this direction. With an aim of filling this gap, this study presents the bioactivity and HPLC of the Chloroformic fraction of *Alysicarpus ovalifolius* analyzes tested on larvae of mosquito.

MATERIAL AND METHODS

1. Material

1.1. Plant material

Vegetable equipment is composed of seeds of *Alysicarpus ovalifolius*. It was collected in the botanical garden of the Technology and Faculty of Science (UCAD, Senegal) in the month of July 2015.

1.2. Biological material

Animal equipment consists of larvae of mosquitos collected in channel IV[6] opposite the University Amadou Hampaté Ba which is next to the High school Blaise Diagne of Dakar.

2. METHODS

2.1. Preparation and preservation of vegetable equipment

After having collected the plant, the various parts of the plants are separate. Thus, the seeds are dried in the shade with the room temperature of the laboratory. Following three weeks of drying, they are crushed using an electric crusher but also using a traditional mortar. The broyat consists of seed clear green powder of *Alysicarpus ovalifolius*. The water contents were sought and it appeared that the seeds have a water content of 3%, therefore lower than 10%. What indicates according to **Bassène E. (1994)** that the seeds are well preserved.

2.2. Extraction of the secondary metabolites and fractionation by chromatography oncolumn.

2.2.1 Method of extraction

The process of extraction is the maceration. The sample is impregnated in solvent (1g/10mL) during 72 hours. That is to say 30 grams of seed powder of *Alysicarpus ovalifolius* macerated in 300 ml ofchloroform during 72h. After filtration and evaporation of solvent, the recovered extract is subjected to has chromatography one silica column with gradient of elution.

2.2.2. Fractionation by chromatography on column

The chromatography on column constitutes a general method of separation and purification of compounds. The chromatography known as of adsorption is based on the differences in intensity ofthe forces of adsorption of the compounds on the surface of certain divided or adsorbent solid bodies: alumina, lime, silica, etc… The mixture to be separated, dissolved in a generally organic solvent crosses the adsorbent (called stationary phase) placed in a column out of glass. The components differently selected are divided into rings along the column, the ring low corresponding to the compound less adsorbed.[7]

The method is the following one: the chromatography is started with hexane; it is then developed by increasing the quantity of the ethyl acetate of 20%. There are on the whole twelve fractions, the twelfth fraction being éluée with methanol 100%. The figure hereafter represents the diagram of fractionation of the chloroformic extract.

2.3. Statistical analysis

For the data of the biological tests with the powders of sheets, the variables measured are: time, the number of died insects, the amount and the nature of the extracts like their interaction. 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 are subjected to a variance analysis, model fixed with four factors (plants, extracts, 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 curves and the tables obtained are used to have the result of the analyses.[8]

2.4. Methods of analysis by HPLC-UV

The fractions which proved more effective are analyzed by liquid chromatography high efficiency (HPLC-UV).
A liquid chromatograph Thermo high efficiency was used. It is provided with an automatic pump and is coupled with a system of detection with absorption UV with bar of diode. The unit is controlled by a computer provided with and the acquisition operating software of the data (Chromnav).

A column in opposite phase (silica is grafted by carbonaceous chains linaires and éluant it used is polar) of type Hypersil-keyston C18 (size of the particles: 5µ; length 250mm; diameter interns 4,6mm) 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 1mL/min. Under these conditions, the duration of the analysis is of 20min. The wavelength of detection and 230nm.

The witnesses (Quinine, Rutin, Gallic acid, Emodine, Cinchonin) are solubilized in ethyl Acetate. The analysis by HPLC was carried out on the basis of comparison of times of retention of the extracts with those obtained with the witnesses.

3. RESULTS

3.1. Results of the extractions and fractionation

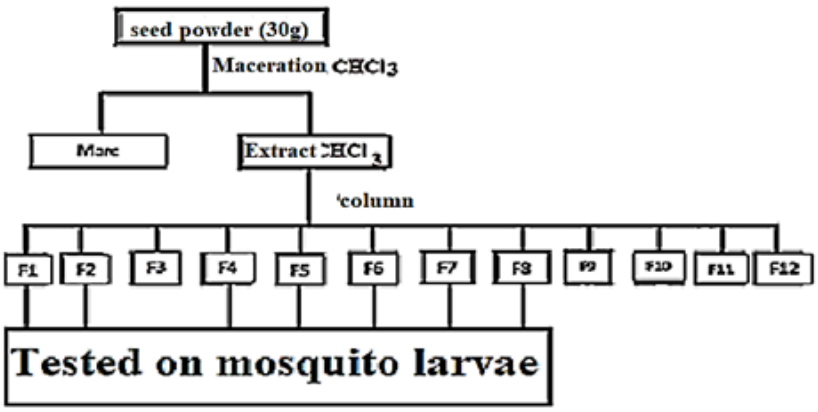

Figure 1: Diagram of fractionation of the chloroformic extract of seeds of *Alysicarpusovalifolius.*

Twelve fractions were obtained, among which seven were tested on the larvae of mosquitos after analysis by thin layer chromatography (CCM). Figure 2 gives the result of the tests of contact of thefractions on the larvae of mosquito according to time.

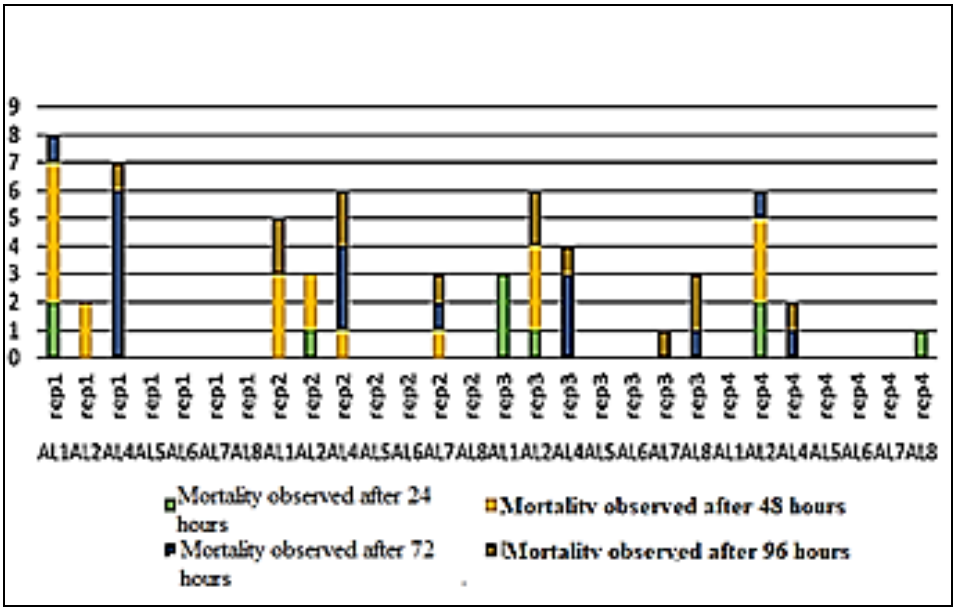

Figure 2: Chloroformic mortality of the larvae ofmosquitos according to the fractions of seeds of *Alysicarpus ovalifolius* and time.

The analysis of the diagram above shows that the fractions AL1et AL4 are most active with the firstamount (5 and 6 larvae out of 10 died) respectively with 48 hours and 72heures. Then, the effectiveness of these fractions on the larvae, a less way, is still noticed on the level of amounts 2 and 3. In addition AL2 also showed an activity on the larvae with an average of two larvae out of ten for the first two amounts and three larvae out of ten with the two last.

3.2. Results of analysis of the fractions by HPLC

3.2.1.Chromatographic profiles of the witnesses

The witnesses used, for the identification of the active ingredients responsible for the larvicide activity of the fractions, are five: quinine (alkaloid), rutin (bioflavonoïde), gallic acid (tannin), the emodin (anthraquinone), and cinchonin (alkaloid). These witnesses can be divided into three families: alkaloid, flavonoid and tannin. The identification is bioguidée owing to the fact that several biopesticides is part of these last families.

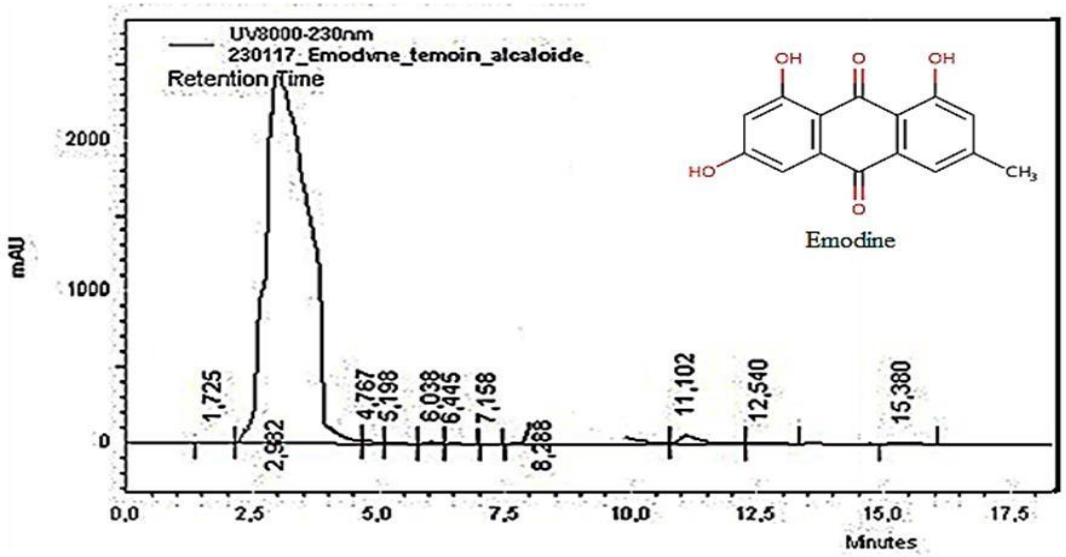

Figure 3: Profile chromatographic of emodin.

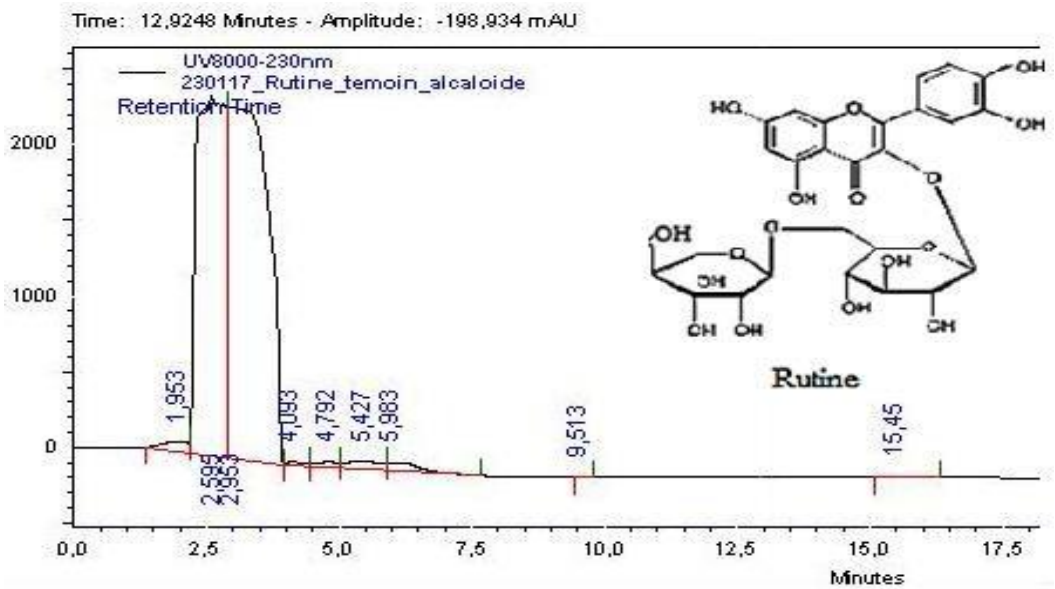

Figure 4: profile chromatographic rutin.

3.2.2. Results of the analysis of the fractionsresulting from seeds of *Alysicarpusovalifolius*

Results of analysis of the chloroformic fraction Al1

Figure 5 represents the chromatogram of the Al_1. Fraction presents a peak at the time of retention 2.983 minutes very close to that of the emodine (Tr=2,982 minutes). This fraction would contain emodine.

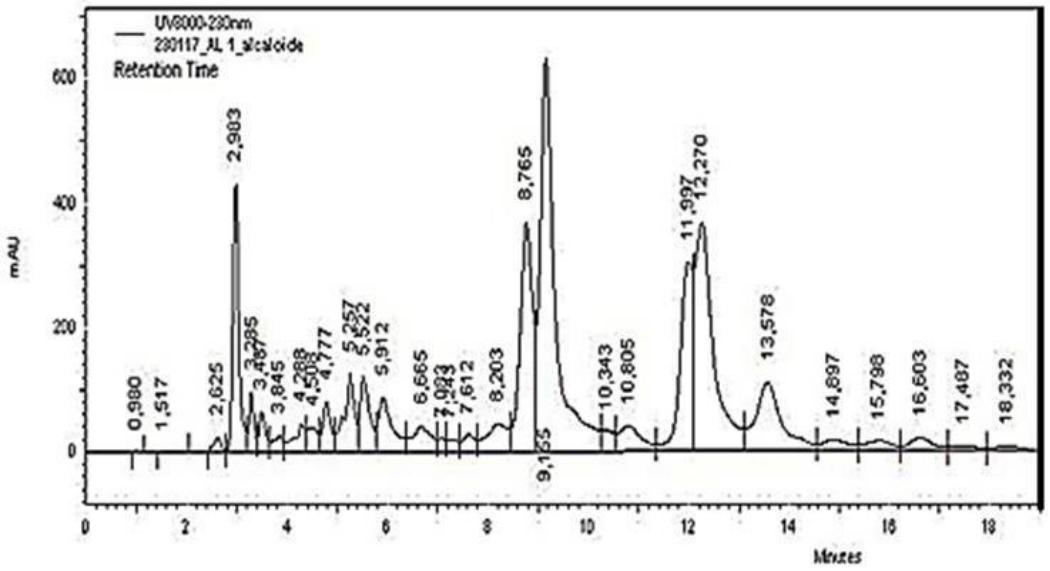

Figure 5: Chromatogram of the Al1 fraction.

Results of analysis of chloroformic fraction AL2

Figure 6 below represents the chromatogram of the chloroformic fraction AL2. Chromatogram shows majority peak with a time of 2.593minutes retention. This peak corresponds to that of the pilot rutin whose time of retention is 2.595minutes.

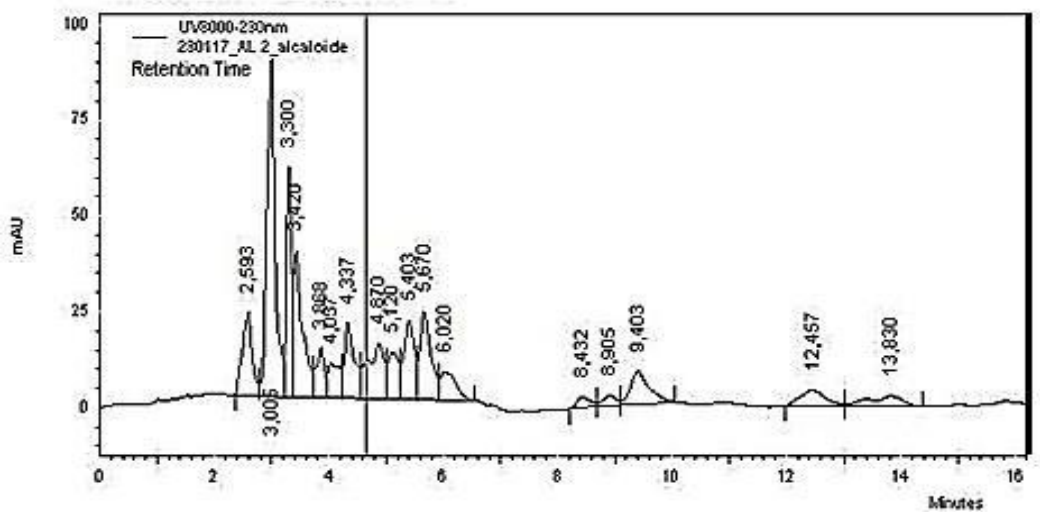

Figure 6: Chromatogram of fraction AL2.

Results of analysis of chloroformic fraction AL4

Figure 7 here after represents the chromatogram of fraction AL4. It presents 17 peaks among which, a peak with a time of retention of 2.993min comparable to that of the émodine (time of retention: 2.982 min). The fraction would contain emodin then.

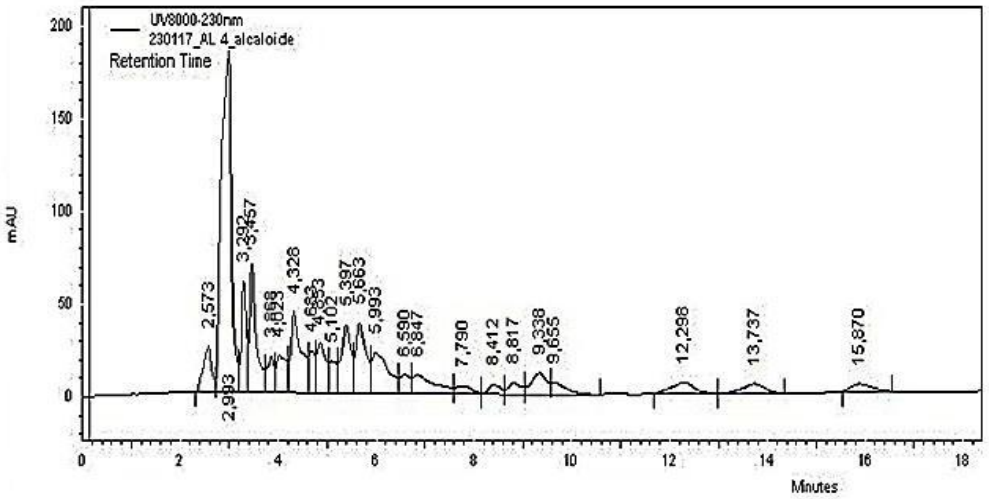

Figure 7: Chromatogram of chloroformic fraction AL4.

4. DISCUSSION

The choice to use the chloroformic extracts for fractionation is justified by their wealth of alkaloids and their major insecticidal activity on the larvae of mosquitos. Following fractionation, chloroformic extracts of seeds of *Alysicarpus ovalifolius*, we noted that fractions AL1 and AL4 (elutes with hexane/acetate of ethyl following a gradient of elution…) are more active on the followed larvae of mosquitos by fraction AL2 (eluant: hexane/acetate of ethyl), their effectiveness extending enters 24 and 72 hours. Indeed, in these fractions, there are émodine and rutin. Rutin is a natural bioflavonoïde. It seems derived from quercetin. However, three of the derivatives glycolyses of this last, are mortals for the larvae of Heliothis zea and Pectinofora gossypol, with a concentration of 0.2%.[9] The émodine is an anthraquinone. It is a powerful pesticide which would directly inhibit the development of the hyphas of the mildew.[10]

5. CONCLUSION

At the end of this work having studied the larvicides activity of the chloroformic fractions of *Alysicarpus ovalifolius*; some of them proved to be effective on the larvae. The potentially active fractions were analyzed by CLHP, in order to compare their chromatographic profiles with those of the standards and to obtain information on the chemical nature of the components. The whole of the chromatograms showed, the existence, in the chloroformic extracts of the most effective fractions, the presence of flavonoïde (rutin), and anthracene drive (emodine). One also notes others not identified more or less polar compounds.

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