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PURIFICATION COLUMN ON SILICA AND CHEMICAL CHARACTERIZATION OF A COUMARIN ISOLATED FROM METHANOL EXCERPT OF THE STEMS OF PLANT *SECAMONE AFZELII (ACLEPIEDACEAE)* FROM ABIDJAN - IVORY COAST

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SUMMARY

The purpose of our study is to isolate the first molecule of *Secamone afzelii* and caracterize the family molecular of pure product. The research component is distinguished by a TLC, a luminescence to 366 nm with a front, Rf = 0.6. This molecule is not visible to the naked eye, nor to 254 nm, on the TLC.

The column chromatography on silica helped isolate the product search with a yield of purification equal to 18,67 ± 0.72%. The various tests carried out on the phytochemical extracted. The molecule isolated could be considered to coumarin.

KEY WORDS: *Secamone afzelii*, silica column, purification, coumarin, TLC.

RESUMEN:

La finalidad de nuestro estudio es aislar una molécula de *Secamone afzelii* y la caracterización de la familia molecular del producto puro. El componente estudiado es identificado por TLC, con luminiscencia a 366nm y con un Rf= 0,6. Esta molécula no es visible a simple vista, ni a 254 nm en el TLC.

La columna de cromatografía de silica permitió aislar el producto, con un rendimiento del 18,67 ± 0.72%. Se realizaron distintas pruebas sobre el extracto fitoquímico La molécula aislada puede ser la cumarina.

PALABRAS CLAVE: *Secamone afzelii,* columna de sílice, purificación, cumarina,

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INTRODUCTION

In the world, 80% of people use medicinal plants for medicine, lack of access to medicines prescribed by modern medicine but also because these plants often have a real impact. Today, traditional knowledge is less and less transmitted and tends to disappear. That is why ethnobotany and Ethnopharmacology working to identify anywhere in the world, plants deemed active and it belongs to modern research to clarify the properties and validate the use[1-4].

The search for new molecules should be undertaken within the plant and animal biodiversity using data Ethnopharmacology. This approach allows you to select plants potentially active and increase significantly the number of discovery of new products assets. Interest chemists relate to natural molecules extracted from plants and animals, is increasingly growing. Several authors have studied compounds isolated from plants with multiple interests [5-7]. In recent years we are particularly interested in plants recognized by users as having antioxidant properties. Among these plants are *Secamone afzelii* the family *ASCLEPIEDACEAE*. The methanol extract was tested and found to have antioxidant properties. The molecules responsible for this important quality would be bioactive flavonoids[8-9]. So far no author has isolated molecules of this plant.

The originality of this study is to isolate and characterize the chemical family of the first molecule of *Secamone afzelii* from a methanol extract

MATERIAL AND METHODS

1. Vegetal material:

The vines of *Secamone afzelii* we studied were harvested in Abidjan in small bush of the University of Abobo-Adjame, which is an extension of the Banco forest. The bodies were washed under running water continuously for fifteen (15) minutes. Then the leaves were separated from the stems. These were dried in an oven at 70 ° C for one week. The body was dry pulverized by a grinder (type RETSCH 811,100) for a powder which were conducted all studies

2. Method:

Extraction by maceration

The extraction method by macerating material in a solvent is to leave a certain amount of plant material in a suitable solvent for a sufficiently long period so that the solvent reaches out molecules based on their polarity. Figure 1 summarizes the extraction method that we used.

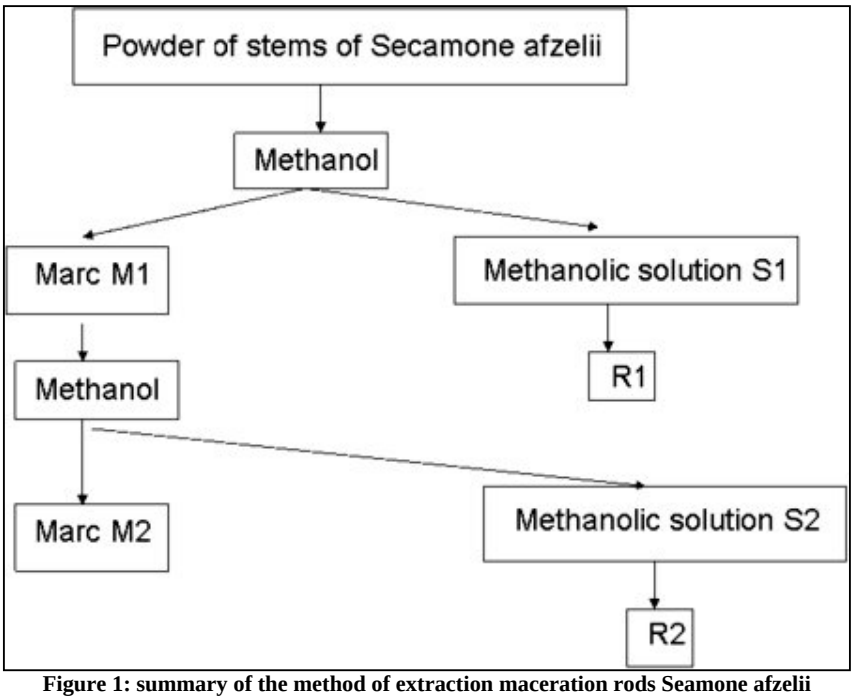

Figure 1: summary of the method of extraction maceration rods Seamone afzelii

100 g powder rod is macerated in distilled methanol (500 ml) for a week, then filtered by Büchner. The solution raw methanol S1 is vacuum distilled through an evaporator (Buchi R110 type MKE 6540 / 2) until a solid gross dry R1. The maceration is resumed with M1 to have a solid gross dry R2. Finally mass of R1 and mass R2 gives 9 g.

Purification column of silica.

The thin-layer chromatography (TLC) with developing the mixture chloroform / methanol (9.5 / 0.5) was conducted on the gross extract methanol. This reveals CCM to 366 nm and Rf = 0.6 luminescent a product that is not visible to the naked eye, nor visible to 254 nm. The methanol extract was purified on a column chromatography with silica, in order to isolate the compound luminescent at UV 366 nm. The column is mounted with hexane. The height of silica is 15 cm and the inner diameter of the column is 4 cm.

Phytochemical tests.

Several tests described in the literature[6] have been made to characterize the chemical family to which it would be possible to include this molecule.

RESULTS:

1. Purification

Purification column silica is first elected to the well-hexane mixture and then with hexane / chloroform (50 / 50).

The desired product is obtained with pure 100% chloroform. Purification yields are co-signed in Table 1.

Experiment	1	2	3
Mass of crude (g)	2	1,5	3,8
Pure Product (g)	0,38	0,30	0,71
Performance of individual experience (%)	19	20	17
Yield (%)		18,67 ± 0,72	

Table 1: Values returns to purify the desired product

The yield purification 18.67 ± 0.72% shows that in the methanol extract, this compound appears with a remarkable rate.

2. Characterization

The various tests have been made[6, 10] to find the chemical family to which the isolated molecules are summarized in Table 2.

Classe of compound	Quinones	Alcaloids	Terpenoids	Coumarins	Flavonoids	Tanin
Reaction observed	No reaction	No reaction	No reaction	Experience N° 1 : Fluorescent stain to 366 nm with or without NH3 (Positive). Experience N°2 : The cycle lactone reaction test (positive)	No reaction	No reaction

Table 2: Phytohemical Screening realized into pure product

The first test to see whether the isolated molecule is a coumarin was positive. We conducted another experience on the molecule belonging to the family of coumarin.

DISCUSSION

This test is that the cycle of coumarin lactone[6, 10]. This test was positive. In view of the two experiences, we can say that the isolated molecule may belong to the family of coumarin.

It is worth noting that spectroscopic analysis in 1H and 13C NMR, then SDM, we can confirm that the first approach and infer the structure of this molecule. But already these initial results will help the interpretation of spectra.

In conclusion, a study of thin-layer chromatography of a methanol extract of stem *Secamone afzelii*, led us to purify the extract methanol. The yield 18.67 ± 0.78% of the product sought to characterize the fact that this compound is revealing that UV visible to 366 nm, was purified.

The phytochemical screening done on the pure compound permits conclude that the isolated molecule is a coumarin.

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***Secamone afzelii* is a plant used in traditional medicine against various pains. The antioxidant capacity of methanol extracts obtained from this plant was described previously by Mensah et al (2004) and Houghton et al (2005). In this work, the authors show a simple and rapid method for purify and characterized partially one of the components with antioxidant capacity present in the metanolic extract of *S. afzelii*. This component was identified as belonging to the family of coumarins. The importance of this study is the potential use of the pure extract by their antioxidant capacity. It is known that compounds of the family of coumarins to act as antioxidants in biological systems. _____**

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The coumarins are compounds very abundant in nature. Chemically derived from the cyclization of O-hydroxycinnamic acid and it have different radicals in the 6- and 7-position. The coumarins are synthesized in the roots and it accumulates in young tissues. They are abundant in dicotyledonous (Rutaceae, Umbelliferae, legumes, solanaceae, ...). One of their functions in plants is antigerminative.

From a chemical point of view the coumarins can be simple (umbelliferone, esculetol, fraxetol,...) or compounds (furocoumarins [psolareno, imperatorine, bergapten ...], 7.8-furocoumarins [pimpinellin, angelicin, ...], 3-pyranocoumarins [samidine, visnadine, ...]). Plants with simple coumarins: tonka beans, meliloto, horse-chestnuts. Plants with compound coumarins bergamot, angelica,

Keller ... Its pathway is complex, starting from shikimic acid through of cinnamic acid and resulting in various coumarins.

The coumarins has diverse pharmacological properties (which is not synonymous with therapeutic application): sedatives (tonka bean), antispasmodic (Kella), phlebotonic (Indian horse chestnut).

The furocoumarins are photosensitives (bergamot, angelica, Ammi majus). At present, some plant species are used in therapeutic in form of the dry extract (capsules) or as oral solution.

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