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Purification and characterization of a coumarin in methanolic leaf extracts of *Secamone afzelii* (Asclepiedacea) from Côte d'Ivoire

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Secamone afzelii, coumarin, TLC, column chromatography

1 SUMMARY

Leaves of *Secamone afzelii,* plants which is recognized as having several biological properties including being an antioxidant, were macerated in methanol. When separated by Thin layer Chromatography (TLC), the crude methanolic extract showed a compound that is visible at UV 366 nm. This product is luminescent and has Retention Factor (R_f) = 0.6 on TLC. The molecule was identified and purified by column chromatography on silica. This purification was performed with a yield of 16.08 ± 0.97. This demonstrates presence of this molecule in the methanol extract of leaves of *S. afzelii.* The phytochemical tests carried out on the purified molecule showed it may belong to the family of coumarins.

2 INTRODUCTION

Plants are presently a source of medicines for many people, especially in developing countries where many illnesses are treated primarily with traditional medicines obtained from plants. The modern pharmaceutical industry itself still relies largely on the diversity of secondary metabolites in plants and animals for the discovery of new molecules with novel biological properties. Plants seem to be an inexhaustible source, since only a small proportion of the 400,000 known plant species have been investigated for phytochemical and pharmacological properties, and considering that each species may contain up to several thousands of different constituents (Hostettmann *et al*, 1998).

As part of the search for new molecules or biologically active compounds of plant origin, it is preferable to base the choice of plants on some criteria. One of the used criteria is that of plant use in traditional medicine or folk medicine. This criteria usually relies heavily on the experience and knowledge of indigenous people in the area where the plant grows. Another possibility is to consider the ecosystem in which the plant species grow.

In this study we are particularly interested in a plant *Secamone afzelii* (Asclepiedacea), which has been identified to have anti-oxidant properties. The work of Mensah *et al.* (2004) and that of Zabri *et al.* (2008) has shown that the antioxidant property in the methanol extract of *Secamone afzelii* could

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be due to the presence of flavonoids. This plant is also known by traditional healers as having anti-inflammatory and, anti-bacterial effects, as well as activity against the hemorrhoids, pneumonia, and bloating in children (Edjanohoun E. *et al.*, 1979 and ACCT, 1989).

The main objective of this work was to isolate by chromatography on an open silica column, a molecule that was detected by Thin Layer Chromatography (TLC) and visible at 366 nm, but not at 254 nm or to the naked eye.. The purified phytochemical was further analysed to characterise and classify it.

3 MATERIALS AND METHODS

3.1 Plant material: *Secamone afzelii* plants were collected in Abidjan in a bush existing at the University of Abobo-Adjame. This bush is an extension of the forest of Banco Abidjan. The plants were washed under running water continuously for ten (10) minutes. The leaves were then separated from the stems and dried separately in an oven at 70 °C for one week. The dried leaves were pulverized by a grinder (type RETSCH 811,100) and a powder was obtained and used in the experiments.

3.2 Extraction by maceration: The method of extraction by maceration was used in which a given amount of plant material is left in a suitable solvent so that plant molecules move into the solvent according to their polarity. Figure 1 summarizes the extraction method used.

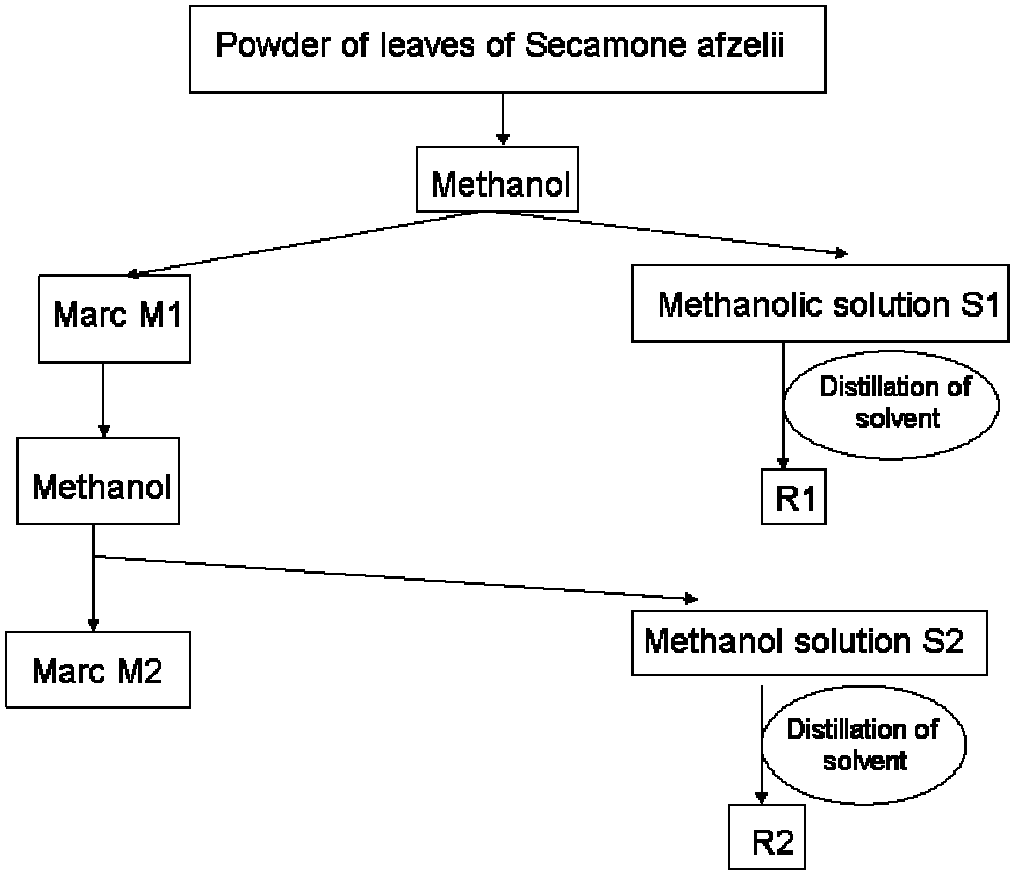

Figure 1: Method of extraction by maceration of *Secamone afzelii* leaves with methanol.

A sample of 100g of the powder of the leaf was macerated and placed in distilled methanol (500 mL) for one week, then filtered on Buchner. The crude methanol solution (S1) was distilled under

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vacuum using a Rotavapor evaporator (Buchi R110 MKE type 6540 / 2) until dry matter was obtained (R1). Maceration was done with M1 of 100gm to get R2. Finally, the mass of R1 and R2 made a gross total of 12 g.

Purification by thin layer chromatography (TLC) on silica column using a mixture of chloroform / methanol (9.5 / 0.5) was performed on the raw methanolic extract. By TLC observation under UV light at 366 nm a compound with, R_f = 0.6 was detected. The product is luminescent at 366 nm under UV light and not visible to the naked eye. It appeared black at 254 nm.

The methanol extract was purified by column chromatography using silica, in order to isolate the luminescent compound observed at UV 366 nm. The separation column used has an internal diameter of 4 cm, and was packed with silica to a height of 15 cm, and used hexane. Tests were conducted on the purified phytochemical compound. The results were compared to those of several tests described in literature (Dohou *et al*., 2003 and Békro *et al*., 2007) and this enabled characterization of the chemical family of the purified molecule.

4 RESULTS AND DISCUSSION

Purification on silica column was eluted first with hexane and then with a mixture of hexane / chloroform (50/50). The desired pure product was obtained with 100% chloroform. This product has been described by Zabri *et al* (2009) and was isolated from the methanolic extract of *S. afzelii* stems. Yields of purification are presented in Table 1.

Table 1: Purification levels of the methanol extract of leaves of *Secamone afzelii*.

Parameter	**Experiment**		
	1	**2**	**3**
Mass of crude (g)	3,5	4,0	3,5
Pure Product (g)	0,55	0,59	0,61
Performance of individual compound (%)	15,71	15,11	17,43
Yield (%)		**16,08 ± 0,97**	

The yield of purification was 16.08 ± 0.97%, which shows that the methanol extract of leaves of this plant contains a substantial amount of the product. This result is close to that reported by Zabri *et al*. (2009) on the stems of *S. afzelii*.

Various tests were carried out as described previously (Dohou *et al*., 2003; Békro *et al*., 2007) to determine the chemical family to which the isolated molecule belongs (Table 2). The two tests for the identification of coumarins were positive. These tests are characteristic and support the conclusion that the molecule isolated from the leaves of *S. afzelii* could be a coumarin. It is worth noting that analysis by NMR spectroscopy then SDM would enable us to confirm the findings of these preliminary analysis methods and to deduce the structure of this molecule. The coumarins are recognized to have different biological properties, especially to be anti-coagulants (Alan, 1988; Abemethy, 1999; Jean, 1999; Felter *et al*., 2006). Further research on the activity of the pure molecule would confirm some of the properties of this plant.

CONCLUSION

Thin-layer chromatography (TLC) of a methanolic extract of leaves of *Secamone afzelii* detected a compound that is visible under UV light at 366 nm and appears as a luminescent spot. This product was further purified with a yield of 16.08 ± 0.97%, which shows a remarkable presence of the molecule in he plant. The phytochemical tests carried out on the pure compound were positive for the identification of coumarins. The molecule could therefore be considered to belong to the family of coumarins.

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Table 2: Phytochemical tests to identify the family of molecular product in methanolic extract of the leaves *Secamone afzelii.*

	Chemical class of compound					
	Quinones	Alcaloïdes	Terpénoïdes	Coumarines	Flavonoïdes	Tanin
Reaction observed	No reaction	No reaction	No reaction	**1st test**: Spot Glow to 366 nm with or without NH3 **(positive).** **2nd test**: reaction test on the cycle lactone **(positive).**	No reaction	No reaction

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