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Cultivation Techniques of *Secamone afzelii* and Effect of its Ashes on Rats Prolactin Contains

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Abstract **Objectives:** This study was carried out to assess effect of *Secamone afzelii* on lactation and perpetuate availability. **Methodology:** Sections of *Secamone afzelii* plants were cultured under natural conditions in the immediate environment and then observed for 7 days. The ethanol extract with or without ash of plant was administered orally to female rats at a dose of 225 mg/mL/kg bw, twice a day for 4 days. Then, the level of prolactin was measured in these animals. **Results:** The results of the cultivation technique of *Secamone afz*elii indicate a significant difference between stems with armpits and those without. The first mentioned produce buds unlike those without armpits. In addition, *Secamone afzelii* extract significantly increases the prolactin content in treated rats. The effect of the extract is potentiated by the ash of the same plant: the prolactin content in the rats treated with the extract + ash mixture is significantly higher than that of the rats treated only with the extract. **Conclusion:** It appears that the extract of *Secamone afzelii* (enriched or not in ash), would stimulate milk secretion in female rats. In addition, the cultivation techniques applied to this plant could promote its durability, availability and easy access.

Keywords: *Secamone afzelii, lactogen, durability, cultivation techniques, ash*

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1. Introduction

Exclusive breastfeeding is the act of feeding the newborn only with breast milk without the addition of water, decoctions, fruit juices, milk, herbal tea or other foods except prescribed drugs [1]. The beneficial effect of colostrum is widely recognized for the health of young children. Colostrum, which is yellowish in color, is primary breast milk, very rich in antibodies essential for the development of infant immunity and for the effective fight against infections and/or conditions. Breast milk plays a key role in the protection of newborns against allergies [2], as well as in growth and physiology. Also, breast milk is a complete food and satisfies the nutritional needs of the newborn during its first months [3]. Breast milk also increases psycho-emotional development in children [4].

However, according to the World Health Organization (WHO), around 2.289 million children die in the first year of life worldwide. This is due to malnutrition, linked to improper feeding practices to which children are subjected [5]. Faced the decisive role of appropriate nutrition, especially in infants, the WHO General Assembly recommends exclusive breastfeeding for the first six months of the child's life, followed by a weaning period that results in the addition of appropriate complementary foods, until around two years of age [2]. The United Nations of International Children's Emergency Fund (UNICEF) estimated in 2010 that the lives of 1.2 million children around the world could be saved each year if the practice were to become widespread [6].

In West and Central Africa, only 20% of young children under six months of age are exclusively breastfed [7]. In rural areas, in most developing countries, malnutrition remains a major nutritional problem in infants and young children [8]. In Côte d'Ivoire, the overall rate of exclusive breastfeeding regardless of the child's age is 33.51% [9]. This rate generally decreases gradually, going from 46.67% in the first month to 16.67% at the end of the first six months. The decrease in the prevalence of exclusive breastfeeding is linked to the decline in interest in this nutritional practice or may be related to socio-professional factors [9]. Added to this is the increasingly growing inability to produce milk in particular in nursing women. This incapacity can be caused by insomnia, stress [10]. Durable solutions for the stimulation of milk secretion remain very unlikely to this day.

Secamone afzelii (Asclepiadaceae) is a plant traditionally used to stimulate breast milk production in lactating women [11]. This plant is widespread in West Africa including Côte d'Ivoire and has a wide ecological adaptation [12]. However, its availability is currently difficult in dry seasons [13]. This study is part of the vast program of mother-child health and nutrition-child health, initiated jointly by UNICEF and the Ministry of Public Health of Côte d'Ivoire. It aims to sustain the production of *Secamone afzelii* through cultivation techniques and optimize milk secretion by determining the effect of the ash of this plant on lactation in the spleen.

2. Material and Methods

2.1. Plant Material

The plant material, identified at the National Floristic Center of the Félix Houphouët-Boigny University in Abidjan, consists of the plant *Secamone afzelii* (Schult) K. Schum (Asclepiadaceae) with specimen number 120801. Samples of this plant were collected in Bingerville in the District of Abidjan.

2.2. Animal Material

Animal are adult female Wistar rats (*Rattus norvegicus*), 14 weeks aged and weighing on average 255 ± 5 g. These rats were pre-screened at week 4 to avoid mating. The upkeep and feeding conditions were carried out according to standards. Animals were housed in groups of 6 in metabolic and metal cages. They were placed in conditions to isolate them from stress. In addition, solid and liquid metabolic wastes were removed daily to avoid microbial contamination.

2.3. Cultivation of *Secamone afzelii* Plant

Nine (9) plots, each containing five (5) *Secamone afzelii* plants were used. On each plot, all the plants were sectioned at the level of the stems. The severed part attached to the floor contained armpits. Another plot also containing seedlings cut at the stem level with the part fixed to the ground devoid of armpits served as a control. All the plots were assessed under the natural conditions of the immediate environment.

2.4. Preparation of *Secamone afzelii* Extract

Samples of *Secamone afzelii* (aerial part) were collected, washed, dried and then powdered. One hundred (100) grams of this powder was dissolved in 1.5 L of distilled water. The whole was heated at 80 to 90°C for 45 min. The resulting homogenate was cooled for 5 min and then filtered through a small mesh poplin cloth. To the residue obtained, one (1) L of distilled water was added. The whole was heated at 80 ° C for 30 min then filtered again. The collected filtrate was subjected to partial purification by adding an equal volume of ethanol to it. After stirring for 3 min, the mixture was stored in a cold room for 24 h and centrifuged at 3000 rpm for 15 min. The resulting pellet was dissolved in a small volume of water and then lyophilized. The powder obtained was the ethanol extract.

2.5. Ash Preparation

The ash content of *Secamone afzeliia* determined according to the A.O.A.C method [14]. The ash was obtained by total incineration of the organic matter. To do this, one (1) g of sample was weighed and placed in porcelain crucibles previously weighed under vacuum. The whole was then placed in a muffle furnace (J.P SELECTA / Spain) at 520°C for 48 hours. The crucibles containing the ash were subsequently removed from the furnace and placed in a desiccator (GLASWERKWERTNEIM, 2 bars / Germany) for 5 min before being weighed.

2.6. Animal Experimentation

Four (4) groups of female rats were orally given different solutions (255 mg/mL/Kg bw) twice a day for 4 days: in the morning at 8 a.m. and in the evening at 4 p.m [15]. Group 1 (control) received distilled water. Group 2 (reference) received galactogil. Group 3 received the ethanol extract of *Secamone afzelii*. Group 4 received the ethanol extract supplemented with the ash of *Secamone afzelii*. On the 5th day, blood was collected from all the rats and centrifuged at 3000 rpm for 10 min using a JOUAN centrifuge. The serum obtained was used for the determination of prolactin.

2.7. Determination of Prolactin in Serum

Prolactin was determined according to the method of Djiane *et al.* [16]. The assay principle combines the Sandwich enzyme immunoassay method with a final fluorescence detection. Prolactin concentrations were calculated automatically using a VIDAS-type immunoanalyzer.

2.8. Statistical Analysis

Results were expressed as mean ± standard deviation. Statistical analysis was performed by analysis of variance and differences between the means were determined by Neuwman-Keuls test using Statisticat 7.1 software. The difference between the values was significant when $P < 0.05$.

3. Results

3.1. Domestication of *Secamone afzelii*

The results were obtained 7 days after sectioning the stems (Table 1), and indicate that the onset of growth of the stem sections results in the appearance of buds in the armpits. The growth by budding is observed in the plots containing the stems of *Secamone afzelii* with armpits. On the control plot, the stems without armpits present an absence of budding.

Table 1. Buds influenced by the nature of the experimental stems

Plots	Numbers of trials	Appreciation of buds
1	5	Presence
2	5	Presence
3	5	Presence
4	5	Presence
5	5	Presence
6	5	Presence
7	5	Presence
8	5	Presence
9	5	Presence
Control	5	Absence

3.2. Ash Content

The ash content of *Secamone afzelii* is 2.44 ± 0.45%.

3.3. Effect of *Secamone afzelii* Extract on Prolactin Contents

For each substance administered, the plasma prolactin contents observed on all the groups of rats are presented in Table 2. The results show that the prolactin contents in the rats treated with the ethanol extract of *S. afzelii* and galactogil are significantly different than that of control rats (10.41 ± 0.11 ng/mL). The results also show that the ethanol extract of *S. afzelii* supplemented with ash significantly increased the prolactin content in the rats compared to that of the rats treated only with the ethanol extract.

Table 2. Plasma prolactin contents in female rats

Animal groups	Plasma prolactin contents (ng/mL)
Group 1 Distilled water	10.41± 0.11[a]
Group 2 Galactogil (255 mg/mL/Kg bw)	15.26± 0.84 [d]
Group 3 Ethanol extract *of S. afzelii* (255 mg/mL/Kg bw)	11.35± 0.50 [b]
Group 4 Ethanol extract + ash of *S. afzelii* (255 mg/mL/Kg bw)	12.51 ± 0.90 [c]

a, b, c and d* Values followed by the same letter are not significantly different at the 5% level.

4. Discussion

The cultivation technique applied to *Secamone afzelii* indicates that the growth results in the appearance of buds in the armpits of the stem sections in contact with the soil. The absence of armpits in control stems where any bud did not appear, shows that growth begins at the armpits level. The appearance of buds in the stem sections with armpits is a new growth or vegetative development observed. The application of this cultivation technique results in new plants of *S. afzelii*. Regeneration in *S. afzelii* by cultivation techniques that involve harvesting procedures was possible in view of the results by the application of a prior harvesting system. They could promote the sustainability of *S. afzelii*. Control of production for a significant yield would ensure that the plant can be recycled.

Concerning the ash of *S. afzelii*, the content (2.44 ± 0.45%) is comparable to those found in other plants (2.5%), reported by Heller [17]. However, this value is low when compared to the total ash content reported by Kandalkar *et al*. [18] which is 5.5 ± 0.3%. Ash contents of the order of 5.5 ± 0.3% could be explained by the collection of plant samples, in arid and dry seasons.

The serum prolactin contents in the female rats treated with *S. afzelii* extract is significantly higher to that of the control rats (10.41 mg / mL). However, this value (11.35 ± 0.5 ng / mL) is significantly lower than that of rats treated with galactogil (15.26 ± 0.84 ng / mL). This result suggests the presence of an active lactogenic principle in the plant *S. afzelii* which would be pectins, acid polysaccharides [19]. In fact, studies showed that pectins induce the production of prolactin [20], a hormone that stimulates breast development [21] and the secretion of β-casein [22]. Normal values of this hormone in rat *Rattus norvegicus* are in the range 8.31-10.44 ng/mL [23]. Beyond these values, prolactin induces milk secretion [23].

Regarding the prolactin content obtained in female rats treated with ethanol extract of *S. afzelii* supplemented with ash, it is significantly higher than that of the rats treated only with the ethanol extract. This could be explained by an additional supply of the extract of *S. afzelii* in minerals which would stimulate the production of prolactin. In fact, minerals constitute metal cofactors which would activate the enzymes involved in the induction of the production of prolactin. It should be noted that a mineral fraction composed of phosphorus and calcium is used in the manufacture of galactogil [24]. This could justify the role of ashes sought in the milk secretion. The ash contained in the nutritional intake enhances the effectiveness of the active fractions on lactation [15].

5. Conclusion

This experimental study initiated a reproductive pathway for *Secamone afzelii* which can guarantee its sustainability throughout the year. It also shows that this plant contains substances with lactogenic power. The lactogenic potential would be enhanced by the presence of minerals in concerted action with the active principle. Easy access to the plant, in addition to its lactogenic potential, could constitute a lasting solution relating to milk secretion in lactating women.

Authors' Contributions

KKE and KCS: designed and coordinated the study.

AYP: carried out the laboratory experiments.

KCO: provided analytical help and chemicals for the assays.

BGH: helped in analysis and interpretation of data

KKAM and AV: participated in the drafting the work or the revising.

All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare that there is no conflict of interest.

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