Preliminary phytochemical analysis of the ethanolic extract of *Xerophyta stenophylla* Baker

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**Abstract**

The species *Xerophyta stenophylla* Baker of the family Velloziaceae is known in Angola as “Chá do Iona” by the people of the Muxima region, Luanda. This species is alternatively used for alleviating schistosomiasis symptoms. The information available in the literature on “Chá do Iona” is scarce. Thus, we investigated the leaf and stem extracts' preliminary phytochemical and biological screening to assess their antioxidant potential. The phytochemical profile was determined in hydroethanolic extract (5.95%). The extract yield for this plant species was 0.26%, and there was a positive presence of flavonoids, coumarins, anthraquinones, tannins, saponins, and alkaloids and a negative presence of anthocyanins. Fifteen substances were identified by gas chromatography coupled with mass spectrometry, among which cineole <1.8> and mentha-1 (7), 8-dien-2-ol <trans-p-> showed areas of 19, 31% and 17.59%, respectively. The tests for antioxidant activity confirmed the antioxidant potential of this plant. These preliminary results indicate that *Xerophyta stenophylla* Baker has promising pharmacological potential and can be officially included in the Angolan pharmacopeia.

**Keywords:** Schistosomiasis; *Xerophyta stenophylla* Baker; Muxima; Pharmacological.

**Resumo**

A espécie *Xerophyta stenophylla* Baker da família Velloziaceae é conhecida em Angola como “Chá do Iona” pelos habitantes da região de Muxima, Luanda. Usado para tratar a esquistossomose. Há pouca informação disponível na literatura sobre “Chá do Iona”, portanto neste trabalho propôs-se realizar a triagem fitoquímica e biológica preliminar das folhas e caules da planta para avaliar seu potencial antioxidante. A determinação do perfil fitoquímico foi feita em extrato hidroetanólico (5.95). O rendimento do extrato para esta espécie vegetal foi de 0.26% e houve presença...
posiblemente de flavonóides, cumarinas, antraquinonas, taninos, saponinas e alcaloides e negativos para antocianinas. 15 substâncias foram identificadas por cromatografia gasosa acoplada à espectrometria de massa, entre as quais cineol <1,8> e mentha-1 (7), 8-dien-2-ol <trans-p-> apresentaram áreas de 19, 31% e 17,59% respectivamente. Os testes de atividade antioxidante confirmaram uma atividade antioxidantes desta planta. Estes resultados preliminares indicam que X. stenophylla Baker tem um potencial farmacológico promissor e pode ser incluído oficialmente na farmacopeia angolana.

Palavras-chave: Esquistossomose; Xerophyta stenophylla; Muxima; Farmacológica.

1. Introduction clave: Esquistosomiasis; Xerophyta stenophylla Baker; Muxima; Farmacológica.

Plants constitute a current alternative for the search for new therapeutic agents. In fact, they have been used since ancient times for healing purposes, as one of human beings’ desires has always been to try to fight their diseases. For this, they made use of organisms and products that nature offers. Particular uses have been passed down orally or in writing and from generation to generation throughout history, right up to the present day.

Many of the applications of medicinal plants are related to magico-religious practices. The result has often been an overevaluation of certain medicinal plants; that is, by attributing to them an infinity of properties which they really do not possess.

Currently, interest is increasing in the study and use of medicinal plants, both in developed and developing countries. However, the main causes are different in each of them. In developed countries it is, a priori, a fashion, which tries to combat the excessive consumption of synthetic drugs or to avoid the side effects that derive from them. On the other hand, in developing countries it is more of a socioeconomic problem, as the majority of the population does not have the necessary financial resources for pharmacological therapy. However, in most cases there is no scientific proof of the usefulness of plant products. This situation, existing in most countries of the African continent, revealing the importance of scientifically validating medicinal plants of traditional and popular use, when it really corresponds (Bragagnollo et al., 2018).

In this context, as already mentioned, it can be said that the emergence of parasitic diseases globally is often associated with low economic development and poor living conditions of a population, especially the lack of basic sanitation and inadequate housing conditions.

According to the World Health Organization (WHO), schistosomiasis affects approximately 270 million people worldwide, and an estimated 700 million more people are at risk of contamination in endemic areas. The main species of Schistosoma that cause disease in humans are Schistosoma guineensis, S. hematobium, S. intercalatum, S. japonicum, S. mansoni, and S. mekongi. The three species involved with the most reported cases are S. hematobium, S. japonicum, and S. mansoni (Grimes et al., 2015).

On the African continent, all species are found except S. japonicum and S. mekongi. (Siza et al., 2015). The infection is caused by repetitive contact with water, whether for professional, recreational, or domestic reasons. In the initial stage, the
disease is asymptomatic and can evolve to extremely severe clinical forms and lead to death (Ministry of Health-Angola, 2010).

Urinary tract lesions caused by *S. hematobium* are manifested by symptoms and signs such as hematuria, dysuria, and suprapubic pain. These symptoms can progress to renal failure in the advanced stages due to obstructive uropathy (UPO) and cancerous forms, such as bladder squamous cell carcinoma (Chan, 2011).

### The disease situation in Sub-Saharan Africa and Angola

In Sub-Saharan Africa (SSA) approximately 76%, and particularly in Angola about 68% (see Figure 1) of the population lives close to rivers, lakes, lagoons, and other sources of water, habitat(s) of intermediate host mollusks (Carvalho et al., 2018). The existence of mollusks that act as intermediate hosts in favorable ecological conditions, such as temperature, light, pH, salinity, and the contact of the population with these water sources, are determining factors for maintaining the high prevalence and high morbidity rates (Tibiriça et al., 2011).

![Figure 1 - Map of the African continent (Sub-Saharan Africa) and the map of Angola.](image)

On the other hand, factors such as the frequency, duration, and time of day when contact occurs and the interaction between species and parasitic individuals are essential in the epidemiological pattern in a given region (Cimerman et al., 2011).

Among the countries on the African continent (54), Angola is one of the 16 countries with the highest rate of affected by the disease (Becker et al., 2013). The tropical climate, the existence of many watercourses (rivers and lagoons), and the consumption of untreated water by the populations living by the rivers contribute to the disease being endemic in the country (Bahia, 2019).

According to a report by the Ministry of Health (MINSA, 2001), studies on schistosomiasis were first developed in Angola in 1896. Noting that the first provinces selected for the research were: Huíla, Bengo (Ambriz) and Cabinda, that is, the first located in the southern part and the following two in the northern region of Angola. However, in the 1980s, studies related to urinary schistosomiasis in Angola were already considered important, proof of this, Grácio (1982) at the same time
presented a field research work, wherein terms results he reports a prevalence of 93% of cases in the municipality of Cubal and 85% in Cateque (province of Benguela); both municipalities located in southern Angola.

Rey (1991), a few years later, reported the presence of hematologic schistosomiasis in practically all the provinces of the country, except the provinces of Zaire and Cabinda, this for reasons of lack of plausible information from a scientific perspective. Thus, the author in his research reported a prevalence of 35% of schistosomiasis

in inhabitants of the Bom Jesus (located in Luanda), 30% in the inhabitants of the Comuna da Funda (Luanda), 45.3% in the Commune of Cassoneca located in Bengo (Angola). And according to Sousa (1996), in his research, he reports that Cassoneca commune (located on the country's Atlantic coast) had a prevalence of 69% among inhabitants who bathed in rivers, 64% among those who bathed in lakes, 39% among those who bathed in springs and 28% among those who bathed in households.

In this context, according to the author's De Sousa et al., (2021), of the main transmitters of the disease is the catfish species. In the schistosomiasis distribution map prepared by the Angolan Health and Medical Assistance Services, in the 1970s the disease in question was present in almost all the provinces of the country.

Currently, in the community of Muxima (area located along the Kwanza River), that is, in the municipality of Quissama (Luanda), the disease scenario is quite problematic, as the disease is notorious in main inhabitants aged between 5 and 30 years (Maghema, 2005).

The locality of Muxima

The town of Muxima belongs to the Municipalities of Quiçama (Kissama in the Ambundu script), located in the province of Bengo (Angola), founded in 1599 by Portuguese settlers. The term Muxima comes from the national language kimbundu, which, translated into English, means “heart”. The locality is inhabited by approximately 45.262 inhabitants and comprises an area of approximately 12.046 km². This region is currently the most extensive center of religious tourism in Angola, as it is located on the banks or close to the Kwanza River (see Figure 2).

Figure 2 - The Muxima village: is located in the Municipality of Quiçama (Kissama), Bengo province - Angola.
As for the geographic coordinates, it is located in the southeast, having the following references: 9° 33' 0" South and 13° 55' 0" East. The same village is inhabited or inhabited mainly by Bantu populations of the Ambundo ethnolinguistic group, who speak the kimbundu language. The region's climate is tropical (as illustrated in Figure 1), prevails with a dry season from May to August and a rainy season from September to April. During the dry season, the average temperature is close to 21°C, and in the rainy season, the temperature can reach 39°C. Muxima vegetation varies widely from Kwanza Riverbanks to the interior regions, from dense forest, mosaic forest-savanna, open forest, and dry tropical forest with cacti and *imbondeiros* and large areas of the grove.

**Description of Xerophyta stenophylla Baker**

*X. stenophylla* is a plant that belongs to the Vellziaceae family and was first described by the British botanist John Gilbert in 1878 (Thomas Sheppard, 1907). A flowering plant belonging to the Velloziaceae class of tropical monocots. Approximately 270 species are distributed in seven genera: Acanthochlamys, Aylethonia, Barbacenia, Barbaceniopsis, Burlemarxia, Vellozia, and Xerophyta. All are perennial trees, shrubs, or herbaceous plants distributed in South America and Africa (Ohashi, 2005; Lima et al., 2014). This family has immense biodiversity concentrated in Brazil and a similar representative in southern Africa, mainly due to the genus *Xerophyta*.

**Uses of Xerophyta stenophylla Baker**

Data from the World Health Organization (WHO) indicate approximately 80% of unconventional drugs for the global population. Natural products are employed to address the lack of primary medical care (Da Silva et al., 2021). The use of plant and phytochemical extracts for therapeutic purposes is one of the oldest ancient forms of human medicinal application (Araújo, 2014). In Africa, due to the difficulty of accessing synthetic drugs (high cost), approximately 80% of the population in developing countries makes use of medicines based on natural products (alternative therapies) (Isaac, 2014).

According to Araújo (2014), phytotherapy has grown remarkably in recent years, making sure that 25% of all world prescriptions are for herbal medicines today. Preventively, natural products exhibit a sizeable therapeutic index with reduced adverse effects when used by the Muxima region population. The ancestral knowledge of populations in the area of plants is used to elaborate formulations based on the *X. stenophylla* Baker plant used alternatively in therapy against schistosomiasis. This plant is used for infusions (Iona tea), treating lung infections, gonorrhoea, abdominal pain, diarrhoea, constipation, male infertility, headache, vision problems, adult epilepsy, asthenia, toothache, cough, chest pain, and tuberculosis (Eric Bossard, 1996). Thus, an investigation of the phytochemistry of the specimens obtained only in Angola was carried out, to evaluate their intervention in terms of treatments.

**2. Methodology**

Initially, a compilation of literature located in the libraries of the Universidade Federal Fluminense (UFF) was carried out, then an effort was made to locate other bibliographies generated by other study centres, especially the Angolan centres, such as; the Botany Center of Angola, Center for Science and Technology of the Ministry of Health, libraries of Universidade Agostinho Neto (UAN) and the Center for Research and Higher Education in Tropical Agriculture of Angola (CPESATA). Thus, these literature-level searches were carried out to identify relevant scientific data on the phytochemistry and pharmacology of *X. stenophylla* Baker.

We first searched for the Velloziaceae family, combining the keywords "Velloziaceae and Schistosoma" and selected articles and dissertations in which schistosomes were related to *X. stenophylla* Baker.

Based on the knowledge acquired through different works in the literature, in terms of research strategy, it can be said
that the present study was built strongly inspired by the aforementioned investigations aiming to evaluate plants for the treatment of possible diseases, among which Hematobic schistosomiasis in the inhabitants of the province of Luanda.

As already mentioned, for the present study, the village of Muxima, in the Municipality of Quissama, which is part of Luanda, was selected. However, a study was carried out in the community residing in the village of Muxima. Interviews were carried out with the objective of delimiting the semantic field associated with the plant and, above all, with schistosomiasis. It is worth mentioning that the term most frequently used by the community in the region in question is “urinating blood”.

The field work itself was carried out, taking into account the authorization of the authorities of the region. Along this path, it was initially investigated how the disease is known and the treatment plant and what are the prevailing ideas about the cause of the illness. For this purpose, 45 people were interviewed. As for the questions selected and asked to the community of the region in question, there are some, which are: have you ever heard of schistosomiasis? Ever heard of urinating blood? How do people get this disease? 87% of respondents said they had already heard about the disease of “urinating blood” and only 13% said they also knew the term “schistosomiasis”.

During her stay in that location, the researcher talked to some doctors about patients diagnosed with schistosomiasis. Some of the doctors said that it was a very common disease in the population in the region, but that the population did not consider it a lethal disease.

These preliminary investigations led to the conclusion that a disease called “Urinating blood” is associated with parasites, bathing in rivers, and pond water (still water). To carry out the preliminary study, the researcher remained at the site for 15 days. During this period, systematic observation of the study area was carried out and interviews to identify key informants and also collect the plants used for treatment, to evaluate their properties in the laboratory.

**Collection of plant material**

The plant material of the leaves and stems of *X. stenophylla* Baker (Iona) was collected in July and August 2018 in the region of the province of Kwanza Sul, municipality of Quibala, Angola, located in the southeast at latitude 10° 44′ 01″ South; 14° 58′ 47″ East, with an average annual temperature of 30°C. The species were identified at the Botany Department of Luanda - Angola, duly authorized by the Ministry of Higher Education, Science, Technology and Innovation in coordination with the National Center for Scientific Research of Angola (CNIC), later transferred to the herbarium of the Faculty of Pharmacy of the Fluminense Federal University (UFF), in Niterói - Brazil.

**Preparation of extracts**

The leaves and stems were dried in an oven with forced ventilation at a temperature of approximately 30°C for 48 h and sprayed (302 g) in a knife mill (Micro mill type WILLIE TE-648 brand TECHNAL). Then, the sample was subjected to extraction by cold maceration in 2 L of ethanol (ethanol 95% and H2O 5%). The reaction flask was covered with aluminum foil to prevent ultraviolet rays. Three extractions were performed at 48 h between each extraction until complete material exhaustion, obtaining the hydroalcoholic extract. After extraction, the extracts were filtered, evaporated, and concentrated at 40°C using a rotary vacuum evaporator (85-01 LABTEC LB, São Paulo, Brazil).

**Phytochemical screening**

A preliminary phytochemical screening of crude extracts was also performed in the present study to identify the presence of secondary metabolites such as flavonoids, coumarins, tannins, anthraquinones, saponins, alkaloids using the methodology described by (Matos, 1997; Seca et al., 2018).
**Extraction of essential oils**

To extract essential oils, 292 g of previously dried leaves and stems of the X. stenophyla Baker plant were used and submitted to a steam distillation procedure to isolate the essential oil. The sample recovered in water was extracted with ethanol (95%). Essential oil from leaves and stems: evaluation of antimicrobial activity and antioxidant activity. Noting that the antioxidant activity of the extracts and phases were analyzed by bleaching the solution, monitored by wavelength spectrometry, as shown in Figure 4.

**Chromatographic analysis of essential oils and identification of compounds**

The essential oils were analyzed by gas chromatography coupled to mass spectrometry (GC-MS-QP5000, Shimadzu) with electronic ionization under the conditions described by (Iara et al., 2015). Compound identification was performed by comparing the retention times and mass spectra obtained with those reported in the literature (Adams, 2007). In addition, the
compound fragmentation pattern was compared with the NIST mass spectrum library.

3. Results and Discussion

Chromatographic analysis

The results of the gas chromatography analysis show *X. stenophylla* ethanolic extracts with the largest area for compounds cineole<1.8-> with 19.31% and mentha-(7),8-dien-2-ol<trans-p-> with 17.59% (Table 1). The compound with the smallest area was caryophylla-4 (12), 8 (13)-dien-5β-ol, with 0.93%. In the literature, mono- and sesquiterpenes are related to several biological activities, such as monoterpenic cineol 1-8 antiviral activity (Astani et al., 2011). Other monoterpenes also have different biological activities described in the literature, such as larvacide (Kweka et al., 2016), antiproliferative (Gautam et al., 2016), and antifungal activities (Zhang et al., 2016).

### Table 1. Results of gas chromatography coupled with masses of essential oils.

| TR | Substance | %     |
|----|-----------|-------|
| 7.568 | Cimeno | 1.45 |
| 7.824 | Cineole<1,8> | 19.31 |
| 10.895 | Mentha-2,8-dien-1-ol<trans-> | 1.78 |
| 11.463 | Mentha-2,8-dien-1-ol<cis-> | 1.37 |
| 11.62 | Pinocarveol<trans-> | 2.72 |
| 12.53 | Pinocarvone | 1.52 |
| 13.653 | Mentha-1(7),8-dien-2-ol<trans-> | 16.05 |
| 15.316 | Mentha-1(7),8-dien-2-ol<cis-> | 17.59 |
| 15.849 | carvonacetona | 2.34 |
| 28.112 | Occidentalol | 1.59 |
| 31.211 | Eudesmol<Y-> | 1.65 |
| 31.367 | Caryophylla-4(12),8(13)-dien-5β-ol | 0.93 |
| 31.872 | Eudesmol<β-> | 1.41 |
| 31.986 | Eudesmol<α> | 2.57 |
| 32.818 | Guia-3,10(14)-dien-11-ol | 11.00 |

% Quantified Substances 83.28

Source: Authors.

Preliminary phytochemical survey

Preliminary phytochemical exploration was performed to identify the main secondary metabolite classes present in *X. stenophylla* stems and dry leaves. This analysis revealed the presence of flavonoids, coumarins, anthraquinones, tannins, saponins, and alkaloids. Phytochemicals have broad biological activity because of their antioxidant properties (Simões et al., 2010). The yield for the *X. stenophylla* Baker ethanolic extract was 0.26%. Regarding the concentrations in response to the colorimetric analyses, the extracts of *X. stenophylla* Baker always revealed much more intense colors.

The results of the phytochemical screening for ethanol extracts from *X. stenophylla* were considered positive due to the formation of precipitates and the appearance of color and negative due to their absence. The most significant results expressed in several precipitates revealed that the classes of major compounds are tannins, coumarins, and flavonoids (two crosses) (Table 2). Regarding the presence of anthocyanins, the ethanolic extract revealed a negative result (-).
Table 2. Results of the phytochemical survey of *Xerophyta stenophylla* Baker.

| Bioactive substances | *Xerophyta Stenophylla* (Ethanol extract) |
|----------------------|------------------------------------------|
| Alkaloids            | +                                        |
| Saponins             | +                                        |
| Anthraquinones       | +                                        |
| Tannins              | ++                                       |
| Coumarins            | ++                                       |
| Flavonoids           | ++                                       |
| Anthocyanins         | -                                        |

Source: Authors.

Antioxidant activity

The preliminary analysis results shown in Table 3 reveal a bioactivity potential and the *X. stenophylla* antioxidant activity. Additionally, the antioxidant activity was determined using the free radical 2,2-diphenyl-1-picryl-hydrazyl (DPPH) method. The total phenol content (TPC) and total flavonoids (TF) from *X. stenophylla* Baker exhibited antioxidant activity compared with DPPH and FRAP.

Table 3. Preliminary metabolic and antioxidant analyses of *X. stenophylla*.

| Types of tests | Average + S.D |
|----------------|---------------|
| TPC            | 1388.0 + 69.60 |
| TF             | 553.74 + 91.46 |
| DPPH           | 1262.65 + 136.90 |
| FRAP           | 1453.11 + 151.20 |

TPC - Total phenol content (mg GAE/100 g of plant material); TF - Total flavonoids (mg CAE/100 g of plant material); DPPH (μmol Trolox equivalents/100 g of plant dry material); FRAP - (mg FSE/100 g of plant material); S.D. Standard deviation. Source: Authors.

Discussion

Velloziaceae is a native plant family endemic in Africa and the American continent, specifically in South America, comprising five genera (Acanthochlamys, Barbacenia, Barbaceniopsis, Vellozia, and *Xerophyta*) and approximately 274 species (Mello-Silva, 2018). Most species are distributed in Neotropical America (Barbacenia, Barbaceniopsis, and Vellozia). Other occur in Africa, Madagascar, and the Arabian Peninsula (*Xerophyta* and Vellozia) and one in China (Acanthochlamys) (Mello-Silva et al. 2011).

The phytochemical potential of the Velloziaceae family was obtained directly from bibliographic reviews. Unfortunately, plant ethnobotanical references are scarce. The few data available were obtained from manuscripts already published by Angolan authors, such as dissertations, old newspapers such as the voice of Angola, and digital platforms from outside the African continent. However, the active compounds present in the Velloziaceae family allow the supposition of these substances in the species *X. stenophylla* Baker once this specimen is traditionally used for schistosomiasis treatment in the region.

Most precisely, this family possesses natural habitat at cerrado, a region called rupestrian fields, which comprises
mountainous, sandy, rocky soils, high solar irradiation conditions, low viability of water, and nutrients. Although Velloziaceae lives in these conditions, the family has surprising longevity. These plants with high resistance and longevity suggest the presence of special protective metabolites, which may have promising biological activities, such as antioxidant, anti-inflammatory, and antitumor activities. The Velloziaceae family is ubiquitous in rocky fields and can dissolve quartzite rocks, releasing strongly bound phosphorus (P) through carboxylate release and contributing to the growth of rocks. This element accelerates the formation of impoverished soil by releasing nutrients derived from rocks into the ecosystem and allowing the long-term establishment of other species for the biological weathering of rocks (Porder, 2019).

Nevertheless, the results were obtained concerning the Velloziaceae family. According to the literature, numerous terpenes and flavonoids were identified and isolated, the latter class being known for its antioxidant activity. In addition, some data certified the presence of bioactive compounds such as terpenes and biological activities (Nora et al., 2014; Vasconcelos et al., 2017; Silva et al., 2019) such as fungicidal and bactericidal properties of resins, water repellents, anti-inflammatory, anticytolytic and pro-anabolic activities in human chondrocytes, and healing capacity. Furthermore, other pharmacological properties, such as analgesics, antibiotics, and anticancer properties (Silva et al., 2015), were also mentioned.

References were found on the phytochemical potential of the Velloziaceae family obtained directly from bibliographic reviews. Unfortunately, the ethnobotanical references of the plants are scarce, and the few available were obtained from works already published in theses by Angolan authors, dissertations, old newspapers such as the voice of Angola, and digital platforms from outside the African continent. However, knowledge of the active compounds present in the plants of this family allows one to suppose its existence in the species X. stenophylla Baker, as it is a type of traditional use for the treatment schistosomiasis in the region.

4. Conclusion

According to the results obtained concerning the Velloziaceae family, the preliminary phytochemical analysis of the ethanolic extract obtained from the stem and dry leaves of X. stenophylla Baker proved to be positive for the presence of secondary metabolites such as flavonoids, coumarins, anthraquinones, tannins, saponins, and alkaloids. In contrast, for anthocyanins, the results were negative.

The secondary metabolites identified in the ethanolic extract have functional properties, namely, antioxidants, and contain bioactive substances. Therefore, we can redefine the species X. stenophylla Baker, which has a relevant potential to be made official as belonging to the phytotherapeutic arsenal of Angola.

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