



## CHEMICAL CHARACTERIZATION OF ANGOLAN VEGETABLE SPECIES USED TRADITIONALLY IN THE TREATMENT OF SCHISTOSOMIASIS

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### Abstract

Schistosomiasis is an endemic disease in 13 of the 18 Angolan provinces, representing 72.2% of the national territory. As this is a serious public health problem, this study aimed at the preliminary chemical evaluation of two Angolan species traditionally used in Muxima Community (Luanda, Angola) for the treatment of signs and symptoms of schistosomiasis. In this community, mixtures containing leaves and stems of Tchambanda (*Crotalaria ononoides*, Benth) and Iona (*Xerophyta stenophylla*, Beker) are used as decoctions. The scientific literature presents few studies with these species and the contributions related to their chemical, pharmacological and functional properties may validate their ancient use by the Angolan populations. Georeferencing, harvesting of plant organs, identification and herborization of the species were the first steps of the investigation. Afterwards, identification and quantification of major chemical constituents' classes in the extracts (ethanolic and/or aqueous) were performed, as well as the evaluation of possible functional properties. The results showed that both species are very promising regarding the bioactive content, showing the presence of essential oils, flavonoids, coumarins, tannins, anthraquinones, saponins, alkaloids, as well as antioxidant properties. The present work is the first chemical study carried out with these species and further chemical and pharmacological evaluations will be carried out to contribute to the knowledge of their functional and pharmacological properties.

**Keywords:** *Crotalaria ononoides*, *Xerophyta stenophylla*, Functional properties

## CARACTERIZAÇÃO QUÍMICA DE ESPÉCIES VEGETAIS ANGOLANAS USADAS TRADICIONALMENTE NO TRATAMENTO DA ESQUISTOSSOMOSE

## Resumo

A esquistossomose é uma doença endêmica em 13 das 18 províncias angolanas, representando 72,2% do território nacional. Por se tratar de um grave problema de saúde pública, este estudo objetivou a avaliação química preliminar de duas espécies angolanas tradicionalmente associadas à Comunidade Muxima (Luanda, Angola) para o tratamento de sinais e sintomas de esquistossomose. Nesta comunidade, misturas contendo folhas e caules de Tchambanda (*Crotalaria ononoides*, Benth) e Iona (*Xerophyta stenophylla*, Beker) são utilizadas como decocções. A literatura científica apresenta poucos estudos com esta espécie e as contribuições relacionadas às suas propriedades químicas, farmacológicas e funcionais poderão validar o seu uso milenar pelas populações angolanas. O georreferenciamento, a colheita dos órgãos das plantas, a identificação e a herborização das espécies foram os primeiros passos da investigação. Posteriormente foram realizadas a identificação e a quantificação das principais classes de constituintes químicos nos extratos (etanólicos e / ou aquosos), bem como a avaliação de possíveis propriedades funcionais. Os resultados obtidos mostraram que ambas as espécies são bastante promissoras quanto ao conteúdo de substâncias bioativas, mostrando a presença de óleos essenciais, flavonoides, cumarinas, taninos, antraquinonas, saponinas, alcaloides, bem como propriedades antioxidantes. O presente trabalho é o primeiro estudo químico realizado com essas espécies e outras avaliações químicas e farmacológicas serão realizadas para contribuir com o conhecimento de suas propriedades funcionais e farmacológicas.

**Palavras-chave:** *Crotalaria ononoides*, *Xerophyta stenophylla*, Propriedades funcionais

## INTRODUCTION

The use of medicinal plants as medicines is probably as old as the existence of man himself. Data from the World Health Organization (WHO) show that about 80% of the world's population use some kind of herb to relieve some painful or unpleasant symptoms.

The use of natural products in Angola is documented since the arrival of the Portuguese. The use of plants by indigenous inhabitants is described for diverse curative or palliative purposes (MUHMMAD et al. 2018). Due to the richness of plant complexes used to treat various diseases throughout the Angolan territory, the country has been the target of scientific interest, since there is a shortage of publications involving research on plant species considered national (SILVIA et al. 2019).

In Angola, schistosomiasis is an endemic disease in 13 of the 18 provinces, corresponding to 72.2% of the national territory (MONICA et al. 2015). Therefore, it is a very important public health problem, requiring appropriate interventions and possible eradication measures.

*Xerophyta stenophylla* (Iona, Velloziaceae) and *Crotalaria ononoides* (Tchambanda, Leguminosae) are medicinal plants from Muxima region (Luanda, Angola) widely used by the local population in preparations (decoctions) for the treatment of various diseases, mainly for symptom relief and treatment of schistosomiasis. In Angola they grow abundantly in the municipality of Quibala, province of Kwanza Sul and the rest of the southern region of the country (Namibe) (MAGHEMA, 2005). The scientific literature describes few studies with these species and the contributions related to their chemical, pharmacological and functional properties may validate their millenary use by Angolan populations. In this sense and meeting a demand from the scientific bases on the traditional use of Angolan plants, the present study aimed at a preliminary chemical study of these two species seeking in identifying their possible functional properties and potential valorization as sources of bioactive compounds.

## MATERIALS AND METHODS

**Extraction of essential oils:** Hydrodistillation was performed in a modified Clevenger apparatus using dry and ground leaves and stems of the two plant species separately (292g of Tchambanda and 302g of Iona) according to (KAPPES, 2011).

**Chromatographic Analysis of Essential Oils:** The essential oils were analyzed by a GCMS-QP5000 (Shimadzu) using electron ionization according to the conditions described by (IARA et al 2015). Retention Indices (RI) were calculated by comparing retention times of an aliphatic hydrocarbon mixture (C9-C30) analyzed under the same conditions (VAN DEN DOOL E KRATZ, 1963). Compounds identification was performed in comparison with their retention times and mass spectra with those reported in the literature (ADAMS, 2007). The MS fragmentation pattern of the compounds was compared to the NIST mass spectra library.

**Plant materials and preparation of extracts:** Leaves and stems powders (292g of Tchambanda (9°31'S;13°58'E) and 302g of Iona (10°44'01"S;14°58'47"E) were macerated with 2L of aqueous ethanol (90%) under shade. This procedure was repeated until exhaustion of plant materials. The extracts were filtered and concentrated using vacuum rotatory evaporation (85-01 LABTEC LB, São Paulo, Brazil) at 40°C.

**Identification tests of secondary metabolite classes in plant extracts:** The presence of flavonoids, coumarins, tannins, anthraquinones, saponins and alkaloids were analyzed in the ethanolic extracts of the two species according to Matos (1997).

**Total soluble phenolics, total flavonoids and antioxidant analyses:** Samples of each plant material (7g, leaves and stems) were subjected to decoction with 300 mL of distilled water in triplicate. Aqueous filtrates were freeze-dried and stored at 4°C until analysis. For determination of the chemical parameters, the extracts were reconstituted with distilled water. Total soluble phenolics of extracts were determined with Folin–Ciocalteu reagent according to the method of Vinha et al. (2015) with minor modifications. Total flavonoids were determined according to Costa et al. (2018) with some modifications. The anti-radical ability (DPPH radical scavenging assay) of the extracts was evaluated according to Costa et al. (2018) with minor modifications. An analytical curve was prepared with Trolox and the antioxidant capacity expressed as  $\mu\text{mol}$  Trolox equivalents per 100g of plant material on dw. The FRAP assay was carried out according to Costa et al. (2018). The standard curve was constructed using ferrous sulphate and the results were expressed as ferrous sulphate (FSE) equivalents per gram of dw.

## RESULTS AND DISCUSSION

The essential oils presented yields of 0.26% and 0.041% for the species *Xerophyta stenophylla* and *Crotalaria ononoides*, respectively. The species *X. stenophylla* presented as major constituents the terpenes Guaia-3,10(14)-dien-11-ol, 13.67%; trans- $\rho$ -Mentha-1(7),8-dien-2-ol, 12.77%; 1,8-Cineol, 12.58%; cis- $\rho$ -Mentha-1(7),8-dien-2-ol, 11.18%; trans-Carveol, 3.68%;  $\alpha$ -Eudesmol, 2.57%, while the species *C. ononoides* presented the terpenes E-Caryophyllene, 18.04%; Bisabolol, 12.28%; Bicyclogemacrene, 6.4%; Spathulenol, 6.13%;  $\delta$ -Cadinene, 3.72%;  $\alpha$ -Copaene, 2.89%. As the plant materials used for the extraction of essential oils were obtained from the local herbalists of Muxima (where the local population purchase these medicinal plants for use), the low yields obtained may be related to the drying process and storage of these plant materials. Further evaluations of materials processed under controlled conditions will be conducted in the future for comparative purposes.

The ethanolic extracts yields for *X. stenophylla* and *C. ononoides* were of 0,26% and 0,041%, respectively. The analysis of the main classes of secondary metabolites showed the positive presence of flavonoids, coumarins, anthraquinones, tannins, saponins and alkaloids for *X. stenophylla*, while *C. ononoides* showed positive results for flavonoids, coumarins, tannins and alkaloids. Regarding concentrations in response to colorimetric analyzes, *X. stenophylla*

extracts always showed much more intense colors than *C. ononoides*, except for the presence of alkaloids, where both species presented similar intensities.

In comparison to *C. ononoides* decoction extract, *X. stenophylla* presented very promising results regarding its bioactive content as well as its antioxidant properties (Table 1).

Table 1. Metabolic and antioxidant preliminary analyses of *Xerophyta stenophylla* and *Crotalaria ononoides*

	<i>Xerophyta stenophylla</i>	<i>Crotalaria ononoides</i>
	Mean ± S.D.	Mean ± S.D.
TPC	1388.0±69.6	505.0±98.8
TF	553.74±91.46	351.82±52.39
DPPH	1262.65±136.90	289.10±50.27
FRAP	1453.11±151.20	572.37±89.16

TPC - Total Phenolics Content (mg GAE/100g of plant material); TP - Total flavonoids (mg CAE/100g of plant material); DPPH ( $\mu$ mol Trolox equivalents/ 100g of plant material); FRAP - (mg FSE/100g of plant material); S.D. - Standard Deviation.

## CONCLUSIONS

This is the first work showing the preliminary chemical study carried out with *Xerophyta stenophylla* and *Crotalaria ononoides*. The results obtained in the chemical evaluations of the extracts and their possible functional properties, namely antioxidant properties, show that these species have an important potential to be officialised as species belonging to the Angolan phytotherapeutic arsenal. Further chemical and pharmacological evaluations will be carried out to contribute to the knowledge of the functional and pharmacological properties of these species.

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