



TLC Profiling of Leaves Extracts of Some Aloe Threatened Species Endemic to Madagascar for Their Antioxidant Activity

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Abstract:

The World Health Organization reported that at least 80% of populations rely on traditional medicine and medicinal plants for their primary health care. Due to their phytochemical compounds, the plants of the Aloe genus are reported to have high potential antiCovid-19 (and antioxidant properties). The aim of this study is to evaluate the in vitro antioxidant activity of some Malagasy endangered species of Aloe genus. The ethanolic extract of few Aloe of Madagascar leaf extracts was fractionated by liquid-liquid partition using hexane. In total 18 different fractions from 9 species have been used to determine their antioxidant activity through in vitro model by using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay. Both hexanic extract and aqueous extract displayed antioxidant activities in four species. The most evident antioxidant activity was expressed by A. helenae.

Keywords:

antioxidant properties; aloe spp.; conservation; Madagascar

I. Introduction

The World Health Organization (WHO) reported that at least 80% of populations rely on traditional medicine and medicinal plants for their primary health care (Ngbolua et al., 2011a, b; Ngbolua et al., 2016a). The use of medicinal plants for various health problems is not only a choice but is also linked to poverty due to and the high costs of modern medicines (Ngbolua et al., 2016b; Inkoto et al., 2018; Ngbolua et al., 2019a). In fact, the excess production of Oxygen Reactive Species (ORS) can become toxic to the major components of the cell and gives rise to oxidative stress, which will lead to numerous pathologies such as neurodegenerative diseases (Alzheimer, Parkinson), diabetes, cancer, inflammatory diseases, aging, etc. Cells use many antioxidant strategies to eliminate or minimize the oxidative damage (Iteku et al., 2019). These plants contain an arsenal of bioactive compounds like coumarins, flavonoids, terpenoids, alkaloids, tannins, etc. which are endowed with interesting and relevant pharmacological properties including antioxidant activity (Bongo et al., 2017). Aloe L. genus is a largely African genus having a major center in terms of diversity in South Africa and Madagascar, and it contains at least 624 species (Lindsey et al., 2003).

Aloe colonized all major habitats in Madagascar from rainforests to high peaks of the central taxa highlands and the dry forests of the South (Orlando, 2013). It is a typical element of the succulent flora of the eastern Indian Ocean islands of Madagascar, the Mascarene islands of Mauritius, Reunion and the Seychelles (Dee et al., 2018). The same author reported 132 native Aloe spp., all of which are endemic to Madagascar and consider the island as a major center of diversity of Aloe. Aloes come in a variety of growth forms, from small miniatures to tall trees, and anything in between (Klopper, 2013). Apart from its morphological and geographical distribution heterogeneities, phylogenetic evidence showed (excluding Lomatophyllum morpho-group) a non-monophyletic unit in Malagasy Aloe spp (Dee et al. 2018) which leads to the difficulty of identifying them at the species level.

It has been known also for years pharmacological properties of Aloes in the world. Due to its phytochemical compounds, a non-native and extensively cultivated *A. vera* (L.) Burm.f. (syn.: *Aloe barbadensis* Miller) is reported to have high potential antiCovid-19 (Mpiana et al., 2020a, b) and antioxidant properties (Miladi et al., 2008). The aim of this study is to fill the gap in phytochemical studies of Malagasy endangered species of Aloe genus by testing in vitro its antioxidant activity.

II. Research Method

2.1 Plant Material

Different species used in this research and its conservation status are presented in table 1 below.

Table 1. List of Malagasy Endangered Species of *Aloe* Genus

| Plant species | Used material | Conservation Status |
|--|---------------------|--|
| <i>Aloe capitata</i> var <i>angavoana</i> J.-P.Castillon | Leaf powder extract | Endangered (Rakotoarisoa et al., 2014) |
| <i>Aloe capitata</i> var <i>quartziticola</i> H. Perrier | Leaf powder extract | Endangered (www.geocat.kew.org , 2019 modified) |
| <i>Aloe helenae</i> Danguy | Leaf powder extract | Endangered (IUCN 3.1) |
| <i>Aloe cipolinicola</i> (H.Perrier) J.-B.Castillon & J.-Castillon | Leaf powder extract | Endangered (Rakotoarisoa et al., 2014) |
| <i>Aloe analavelonensis</i> Letsara, Rakotoar. & Almeda | Fresh leaf extract | Vulnerable (Letsara et al., 2012) |
| <i>Aloe ivakoanyensis</i> Letsara, Rakotoar. & Almeda | Fresh leaf extract | Critically Endangered (Letsara et al., 2012) |
| <i>Aloe suzannae</i> Decary | Fresh leaf extract | Endangered (IUCN 3.1) |
| <i>Aloe suarezensis</i> H. Perrier | Fresh leaf extract | Endangered (Rakotoarisoa et al., 2014) |
| <i>Aloe guillaumetii</i> Cremers | Fresh leaf extract | Critically Endangered (Rakotoarisoa et al., 2014) |

Biological materials (mainly fresh leaves) were provided by Tsimbazaza Zoological and Botanical Park between January and May 2019. They were added along with the author personal living collections. To make the powder of collected leaves, about 100 g of different species (*A. capitata* var *angavoana*, *A. capitata* var *quartziticola*, *A. helenae* and *A. cipolinicola*) and (*A. analavelonensis*, *A. ivakoanyensis*, *A. suzannae*, *A. suarezensis* and *A. guillaumetii*) (Figure 1) collected in January and May respectively was preserved in a freezer at -20 °C. Afterwards, these leaves were dried by lyophilization (Temperature -40 °C under pressure 0,850 mBar during 24 hours), then, grounded in order to make a fine powder. For each species 10 g and 15 g of powder was used for extraction respectively.



(a) *Aloe analavelonensis* Letsara, Rakotoar. & Almeda



(b) *Aloe helenae* Danguy



(c) *Aloe ivakoanyensis* Letsara, Rakotoar. & Almeda



(d) *Aloe suzannae* Decary



(e) *Aloe capitata* var *quartziticola* H. Perrier



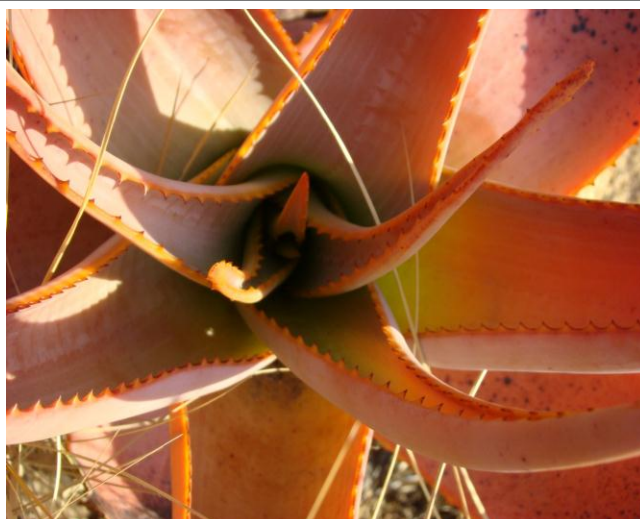
(f) *Aloe capitata* var *angavoana* J.-P. Castillon



(g) *Aloe guillaumetii* Cremers



(h) *Aloe cipolinicola* (H.Perrier) J.-B.Castillon & J.-Castillon



(i) *Aloe suarezensis* H. Perrier

Figure 1. Picture of Malagasy endangered species of *Aloe* genus

2.2 Extract Preparation

Each fresh material and powder was extracted with 100 mL of ethanol 80° at a temperature 70 °C for 1 hour. After filtration, the ethanol was evaporated under pressure at 45 °C for about 20 minutes. The obtained extract was then de-pigmented by Hexane and the quantity of the solvent used depended on samples. Afterwards, two phases were obtained namely: n-hexane soluble fraction and aqueous extract separately. The n-hexane soluble fraction was evaporated to have more concentrated solution and at last 10 mL of each extract were collected (figure 2a, b).



Figure 2a. Concentrated n-hexane extract

Figure 2b. Aqueous extract

2.3. Determination of Antioxidant Capacity

This method is based on the degradation of DPPH radical (2, 2 DiPhenyl-1-PicrylHydrazyl). The DPPH radical is a violet-colored radical; the addition of antioxidant reduces this radical and causes the mixture to discolor. This radical decolorization measured by spectrophotometer at 517 nm is proportional to the concentration of antioxidants (Bongo et al. 2017). The antioxidant activity of the separated extracts was estimated in terms of hydrogen-donating or radical scavenging ability, using the 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) method in the TLC plate (Brand-Williams, 1995). A coloration change will show the antioxidant activity in the soaked plate by the DPPH. The presence of antioxidant capacity of the extract was estimated visually after TLC at room temperature. The following solutions were combined to make the eluent: Hexane 1, Methane 9, ED 0.2 for separation of the extracts. The efficiency of the deposit, the migrations was controlled under UV 254 nm light.

III. Results and Discussion



Figure 3a. Photo of TLC Spotted with 18 Sports after Migrations

Unaided eye observation shows three different spots movement, yellow, purple and dark green mainly in the n-hexane extracts. This explains the separation of molecules that contains the extracts. On the other hand the observation under the UV lights showed more detailed images of the molecules migrations.



Figure 3b. TLC profiling under UV 254 nm

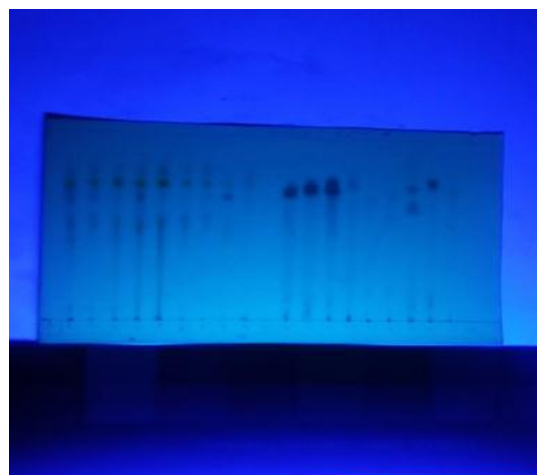


Figure 3c. TLC profiling under UV 365 nm

Among those migrated molecules, some reacted with the DPPH radical and produced a yellowish spot, which could be explained by an antioxidant activity. Its reaction was expressed by the following figure.

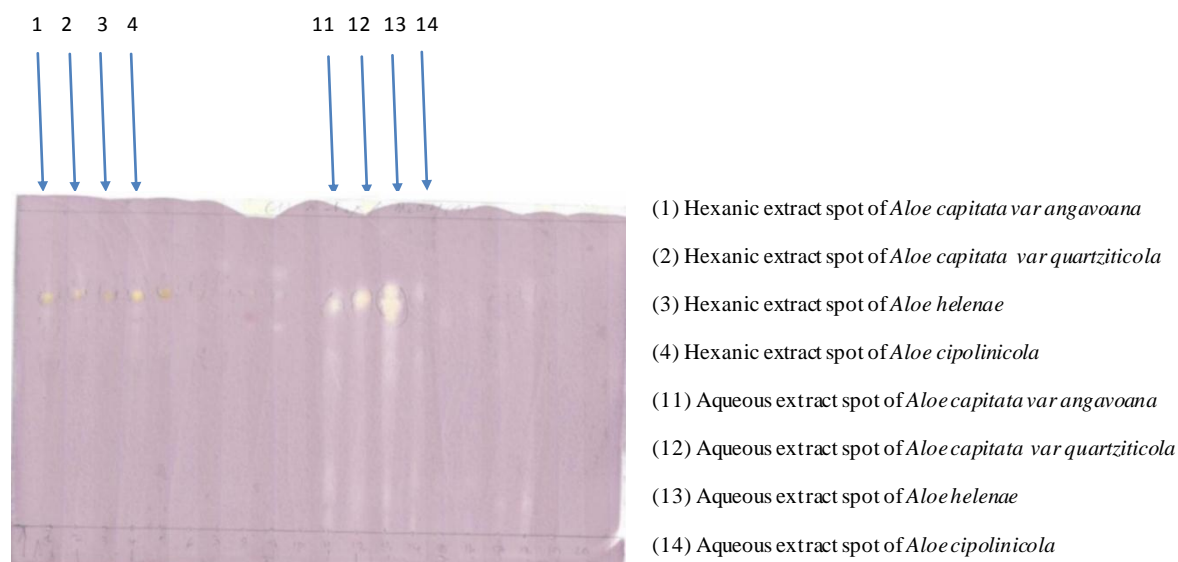


Figure 3d. TLC Spotted Plate Sprayed with DPPH Radical

The figure 2d showed the TLC plate with migrated spots, which expressed antioxidant activity. They belong to the following species extracts: *A. capitata* var *angavoana*, *A. capitata* var *quartziticola*, *A. helenae* and *A. cipolinicola*. The most evident reaction is in the aqueous extract of leaf powder of *A. helenae* (spot 13).

SARS-CoV-2 is the pathogen agent of the new corona virus disease that appeared at the end of 2019 in China. There is, currently, no effective treatment against COVID-19. Recent findings based on molecular docking study revealed that some Aloe derived compounds are potential inhibitors of the main protease (3CLpro) responsible for the replication of coronaviruses (Mpiana et al., 2020a). The present research revealed that the plant of the Aloe genus could serve as anti-oxidative therapy for ameliorating injuries of Covid-19-infected patients. It also was reported that some plants belonging to Aloe genus has a large abroad of antiviral activity on several types of virus (Haemorrhagic Viral Rhobdavirus Septicaemia, Herpes simplex virus type 1, Herpes simplex virus type 2, Varicella-Zoster virus, human immunodeficiency virus, Influenza virus, poliovirus, Cytomegalovirus, Human papillomavirus) including coronavirus SARS-CoV-1 and are consumed orally in several forms and are safe (Mpiana et al., 2020b).

Given the ethno-medical importance the plant species of Aloe genus for the population and their scientifically validated pharmacological properties, it is therefore desirable to develop sustainable strategies for the conservation of these species. One option to be explored is their domestication with a view to their use as material for drugs manufacture. Thus, the cultivation of Aloe congensis in agro-ecosystems in forest and savannah environments of Democratic Republic of the Congo (DRC), particularly in the Nord Ubangi Province, would allow a better comparative study with species from Madagascar, as is the case for some ongoing studies (Randrantoarimbola et al., 2020). This part of DRC belongs to the Ubangi eco-region, a subgroup of Northeastern Congolian lowland forests. This eco-region is one of the 200 globally priority terrestrial eco-regions known as the "G200" (Ngbolua et al., 2020a, b, Ngbolua et al., 2019b, c, d; Gbolo et al., 2019; Ngunde-te-Ngunde et al., 2019).

IV. Conclusion

The findings of this research showed that there are nine endangered species of Aloes in Madagascar. The TLC profiling revealed that four species showed traces of antioxidant activity. The anti-oxidative reaction was different depending on the nature of the extract (from powder or fresh) and plant species. A very characteristic reaction was shown in the aqueous extract of leaf powder of *A. helenae*. Research is in progress to isolate and identify the antioxidant compounds in those fractions.

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