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Phytochemical Screening Of Two Medicinal Native Plants Of Madagascar: Smilax Anceps, Dianella Ensifolia

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Abstract – Traditional medicine is widely used in several countries. Madagascar has a great advantage due to its rich biodiversity characterized by several endemic species. The local population uses plants to treat many diseases, as infusion or cataplasm obtained from the seeds and the leaves. This study concerns the valorization of two endemic plants known by their analgesic property. Qualitative phytochemical analysis done to these plants confirm the presence of some phytochemicals like polyphenols.

Keywords – Phytochemical screening, qualitative analysis, polyphenol, endemic plants

I. INTRODUCTION

Madagascar is located in an area which is characterized by a rich biodiversity. The endemic rate of plants is very high, with 12,000 species listed on 2011 [1]. As in many countries rich in plant biodiversity, traditional practice is widely used to cure many diseases.

This study is focused on the test of chemical components presence in two native Malagasy plants: Dianella ensifolia and Smilax anceps. These two plants were collected in the eastern part of the Island, Andasibe, located on 18° 28′ S, 48° 28′ E.

We specially chose these two native plants because of the medicinal characteristics shown on the plants of the same family [2] [3] [4].

Different methods were used in this study to highlight the presence of chemical compounds by phytochemical screening which is an excellent method to detect chemical components for qualitative analysis.

II. MATERIALS AND METHODS

The material used in this study is fresh plants collected in the eastern part of Madagascar. These plants usually grow everywhere over the island, but we specially chose this part because of its favorable climate allowing to obtain good quality of plants.

2.1. Collection of plant materials

Fresh plants were collected within Andasibe forest on August 24th, 2021. This place is a rural commune located in the East of Madagascar, in the region of Alaotra Mangoro, 18° 28′ S, 48° 28′ E.

Table 1: Brief presentation of the two plants

	SMILAX ANCEPS	DIANELLA ENSIFOLIA
Photos of the plants	 ***Fig 1: Smilax anceps (fresh plants)***	 ***Fig 2 : Dianella ensifolia (fresh plants)***
Family	Smilacaceae	Liliaceae
Other scientific names	Smilax cynodon cordem, Smilax herbacea thumb, Smilax kraussiana meisn, Smilax semiamplexicaulis bojer, Smilax mossambicensis	Dianella nemorosa
Vernacular denomination	Roidambo, Avaotra, Vahanievotro, Fandrikibodisy, Roipatana	Kivazavazaha, Kivondrombohitra, Masonomby, Ombilahidiny, Tsivazavazaha, Voamasonomby
Chemical components contained in the plants issued from the same genus	Flavonoid, steroid	Flavonoid, quinone, chromon

To carry out the different tests, we used the leaves and the stems of the plants which were cut into small pieces, then dried in an airy room for three weeks, at room temperature. The mixture was regularly stirred to accelerate the drying-processor. When plants were completely dried, we reduced them into fine powder by using mechanical blender. The powder was then transferred in containers and labelled for future use.

2.2. Preparation of organic extracts

The plant powder was macerated in ethanol solution 95% for ten days, at room temperature. We used about 400 grams of the powder that we macerated in about two liters of ethanolic solution. After those ten days, we filtered the solution, so that we obtained the raw solution. Then, we did a liquid-liquid extraction by using solvents with increasing polarities: n-hexane, ethyl acetate and methanol. After each operation, the solvent was evaporated by using rotavapor at the evaporation temperature of the solvent concerned.

The preparation process is shown on figure 3.

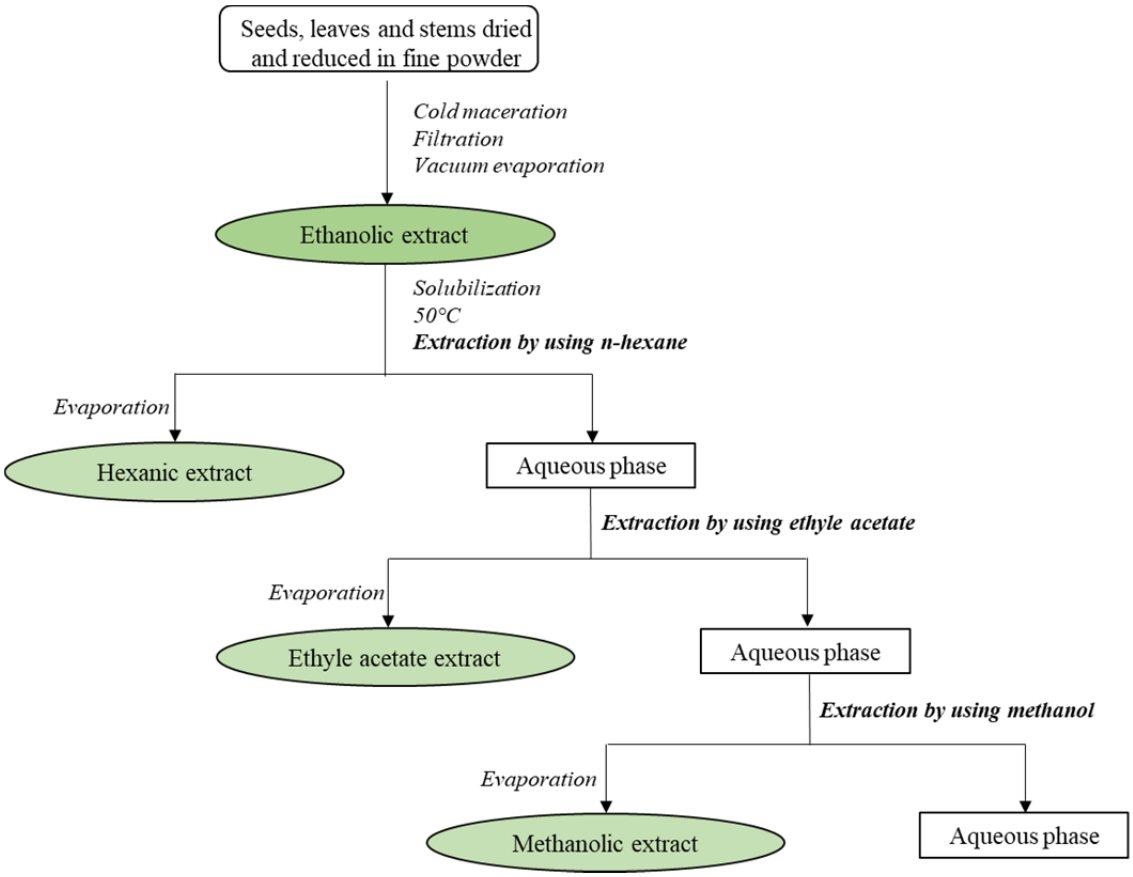

Fig.3: Process preparation used to obtain organic extracts

2.3. Qualitative phytochemical analysis

This part describes the methods used for phytochemical screening. All the tests were done on the raw extract and on each type of organic extract.

2.3.1. Test for coumarin

The extract is dissolved in hot water and then cooled at room temperature. The solution was divided in two tubes: a tube used for a control and a tube for the test. Then we added ammonium hydroxide 10 grams per liter. The presence of coumarins is indicated by a fluorescence color.

2.3.2. Test for leucoanthocyan and flavonoid

To detect the presence of flavonoid, we did the Wilstater-test, the Wildtater-test modified and the BatSmith-test. We depigmented the extract by using hexane and then we took the extract with ethanol. The solution was poured in five different tubes.

- Tube 1: Control tube
- Tube 2: 0,5 mL of concentrated HCl and few fragments of magnesium ribbon were added to the extract. The presence of flavonoids is indicated by apparition of a change of coloration
- Tube 3: We use the same protocol as used for the Tube 2 and then we added 1 mL of water and 1 mL of isoamyl alcohol. The apparition of pink or magenta red color on the upper phase indicates that the extract contains flavonoid. Red color indicates the presence of flavone, purple-red color indicates the presence of flavonols.

- Tube 4: We added a few quantity of hydrochloric acid and we have heated in water-bath for half an hour. If it appears red purplish coloration, it will indicate the presence of leucoanthocyan.
- Tube 5: we added a few quantity of hydrochloric acid. The presence of anthocyan is indicated by apparition of red purplish color.

2.3.3. Test for polyphenol and tannin

The extract was mixed with a solution of sodium chloride 10% and some water. The solution was filtered and distributed into four tubes.

- Tube 1: control tube
- Tube 2: we added a few drops of gelatin 1%. The apparition of precipitation indicates a presence of polyphenols.
- Tube 3: we did the same experience as for the Tube 2 and added a few drops of chloride sodium. If the solution precipitates, the extract contains tannins.
- Tube 4: we added a few drops of FeCl3 with methanolic alcohol. If blue greenish coloration appears, the extract contains condensed tannins. And if it appears black blueish color, the extract contains hydrolysable tannin.

2.3.4. Test for alkaloid

To look for the presence of alkaloids, we used Mayer's and Wagner's reagents which are added to the chlorohydric acid extract of the extract. The presence of alkaloid is indicated by appearance of precipitate which causes turbidity.

2.3.5. Test for steroid and triterpene

To look for the presence of steroid and triterpene, we put the extract in ethanolic solution and added chloroform then anhydrous sodium sulphate. The solution is then poured into five tubes:

- Tube 1: Control tube
- Tube 2: we used a saturated solution of antimony. A fluorescent yellow color indicates the presence of steroid.
- Tube 3: the Libermann Burchard test requires $(CH_3CO)_2O$ and few drops of sulfuric acid. A purple reddish color indicates the presence of triterpenoid and a purple color means that the extract contains steroid.
- Tube 4: the Salkowski test requires to use 1 ml of sulfuric acid. If a red ring appears, the extract contains insaturated sterols
- Tube 5: we added picric acid to the solution. A red purplish ring indicates the presence of lactonic steroid

2.3.6. Test for triterpene

The extract is dissolved in chloroform (2 ml), is evaporated to dryness. Then 2 ml of concentrated sulfuric acid was added. The presence of terpenoids is indicated by apparition of reddish-brown coloration.

2.3.7. Test for saponin

The extract is dissolved in water. The solution is then agitated vigorously for about half a minute. If there is a persistent foam (about 1 cm) after half an hour, it means that the extract contains saponins.

III. Results And Discussion

Table 2: Result of phytochemical screening of Dianella ensifolia

	DIANELLA ENSIFOLIA			
Chemical families	**Raw extract**	**Hexanic extract**	**Ethyl acetate extract**	**Methanolic extract**
Coumarin	-	-	-	-
Leucoanthocyan	+++	+	++	++

Flavone	+++	++		
Flavonol	-	-	-	-
Polyphenol	+++	+++	+++	+++
Tannin	+	-	-	-
Condensed tannin	-	-	-	-
Alkaloid	-	-	-	-
Iridoid	-	-	-	-
Steroid	+	-	+	-
Unsatured steroid	++	-	+	++
Triterpene	-	-	-	-
Saponin	-	-	-	-

Table 3: Result of phytochemical screening of Smilax anceps

	SMILAX ANCEPS			
Chemical families	**Raw extract**	**Hexanic extract**	**Ethyl acetate extract**	**Methanolic extract**
Coumarin	+++	-	+	+++
Leucoanthocyan	-	-	-	-
Flavone	-	-	-	-
Flavonol	+++	+	+++	++
Polyphenol	+++	+++	+++	+++
Tannin	+	+	+	+
Condensed tannin	+++	+	++	++
Alkaloid	-	-	-	-
Iridoid	-	-	-	-
Steroid	+	+	+	+
Unsatured steroid	+++	-	+	++
Triterpene	-	-	-	-
Saponin	-	-	-	-

Note: '+' indicates tracks of the product; '++' indicates presence in low concentration; '+++' indicates presence in high concentration; '-' indicates absence

The result of phytochemical screening of two endemical plants of Madagascar is tabulated in Table 3 and Table 4. The analysis revelated the presence of various phytochemicals in the different extracts of the two plants.

Dianella ensifolia is a plant rich in leucoanthocyan, flavone, polyphenol and unsatured steroid. Tests show that leucoanthocyan is essentially present in the raw extract. We identified tracks in the hexanic extract and low concentration in ethyl actetate extract and in methanolic extract. Flavone is in high concentration in the raw extract but in low concentration in hexanic extract. We could identify that Dianella ensifolia contains a high concentration of polyphenol. Steroid is in tracks and unsatured steroid, in low concentration, is especially present in methanolic extract.

Smilax anceps contains coumarin in raw extract, and the test showed that this chemical component is essentially in the methanolic extract. Flavonol is showed to be in high quantity in this plant: in the raw extract and then in the ethyl acetate extract. Hexanic extract and methanolic extract contain also flavonol but in smaller quantities. The test done in the different extracts showed that the plant contains polyphenol in high quantity. Smilax anceps contains tracks of tannin in all the types of extract. This plant also contains high concentration of condensed tannin in raw extract and this component is in low concentration in ethyl acetate and methanolic extracts. This plant does not contain neither alkaloid nor iridoid. Steroid is present in the plant in tracks. Saponin and triterpene do not exist in this plant.

Analysis of the two plant extracts showed that they contained different chemical components such as coumarin, leucoanthocyan, flavone, flavonol, polyphenol, steroid and unsatured steroid which are known to have physiological activities [5]. Phenolic components have properties such as anti-aging, anti-carcinogen, apoptosis, anti-atherosclerosis cardiovascular protection [6]. These compounds improve endothelial function and inhibit the proliferation of cancer cells. Scientific studies reported that steroid and unsatured steroid have antibacterial properties [7]. Steroid and unsatured steroid are very important because they have relationship with different compounds, for example with sex hormones. Tannins are known to inhibit fungi, viruses and bacteria, yeasts [8].

The presence of chemical components in one type of extract and its absence in other type can be explained by the possibility of biosynthetic and physiological reactions which may exist inside the plant or by the environment effect that always modifies the things.

IV. Conclusion

The results of the analysis done revealed that the plants contain medicinal important components and constitute a good source for useful drugs. Therefore, phytochemical screening is very important and helpful to find chemical constituents, to quantify their content and to locate the source of pharmacological active compounds. In this study, the results obtained that suggesting the identified phytochemical components may be the origin for the identification of bioactive constituents. So, these two plants may be an important reservoir of bioactive compounds. These two plants are widely used by local population and further work will be done to characterize the active components.

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