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Major Elements, Trace Elements and Effect of Landolphia Myrtifolia on Cardiac Activity in the S.A.V.A Region, Madagascar

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ABSTRACT: The majority of the world's population uses medicinal plants for various reasons. Landolphia myrtifolia, which is a cultivated plant, has often naturalized and is widely consumed by populations in the S.A.V.A region thanks to its cardiogenic activities. This study is to determine the mineral elements contained in Landolphia myrtifolia during heart attack in northern Madagascar. Our study took place in Madagascar-INSTN in the department of X-ray fluorescence and the environment. The method used is Energy Dispersive X-Ray Fluorescence. Thus, three chemical elements were found: heavy metals in trace, undesirable elements, nutrients which are: potassium (K), calcium (Ca), vanadium (V), titanium (Ti), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), lead (Pb), aluminum (Al), phosphorus (P). The plant used during heart attacks contains more nutrients than toxic elements. It is advisable for people to seek professional advice to receive the necessary care during a heart attack.

KEYWORDS: Landolphia myrtifolia, ED-XRF, mineral elements, heart, SAVA Region.

I. INTRODUCTION

Landolphia myrtifolia is a climbing vine that can reach up to the forest canopy and can be used in various areas of daily life. In Madagascar, although we are in the process of developing, plants are among the treatments that people use during heart attacks. They use the plant for cardiogenic activity. The plants are taken either as a decoction or infusion. They consume it, according to these people, to treat people with lower limb edema and chronic fatigue followed by shortness of breath with the slightest exertion. But why consume it? According to the people, tradition and the Tangalamena prescribe the plant for its antihypertensive, anti-edematous, and cardiogenic effects. People consume it in doses according to their own desires, without exact dosage, but they take it whenever they need it. The interest of this work is to determine the mineral elements contained in the leaves and stem of Landolphia myrtifolia, used by populations during heart attack in the North of Madagascar as well as to improve health. X-ray Fluorescence Spectrometry is an analysis technique that allows to identify and determine the elements that make up a sample. This technique allows to measure very varied samples: minerals, metals, oils, water, plants, cements, etc. [17]. We hope that this article succeeds in convincing decision-makers, especially populations in a state of heart attack to take very serious and respectful measures to prohibit the consumption of Landolphia myrtifolia during heart attack in order to preserve health.

II. METHODOLOGY

1. Sample Preparation

Several samples were collected from two (02) different districts in the S.A.V.A region of northern Madagascar, namely the ANTALAHA district and the SAMBAVA district. Sample preparation followed a standardized protocol aimed at obtaining homogeneous and representative specimens for ED-XRF analysis: drying, grinding, sieving, and packaging.

2. X-ray Fluorescence Analysis Protocol

The X-ray Fluorescence and Environment Technology Department (TXRF) within INSTN-Madagascar has a SPECTRO XEPOS X-ray fluorescence spectrometer used for qualitative and quantitative analyses of the elements present in medicinal plant samples. The figure below illustrates an overview of this spectrometer.



Fig 1: Overview of the SPECTRO XEPOS X-ray Fluorescence Spectrometer

The Spectro XEPOS is an X-ray fluorescence spectrometer that uses the ED-XRF method for sample analysis. The excitation method is secondary targets. When the targets are exposed to the primary X-rays delivered by the tube, they emit rays to excite the atoms in the sample. Once these atoms are excited, they in turn emit fluorescence lines, which are then measured by a semiconductor detector. The technical data for proper use of the spectrometer during sample analysis are provided in the table opposite:

Table 1: Technical Data of the SPECTRO XEPOS

Operating voltage range	95-120 V/200 - 240 V 50/60 Hz
Power consumed	150 W

III. EXPERIMENTAL RESULTS

1. Results from the Pharmacological Test

1.1 Effect of Landolphia myrtifolia extract on cardiac contraction

Injection of the extract into the aorta increases the amplitude of cardiac contraction. This increases with the concentration of the extract injected into the infusion set. Compared to the initial contraction amplitude, the extract causes an increase of 26 ± 0.03 % to 75 ± 0.04 % at concentrations ranging from 0.4 to 0.8 mg/ml. These values demonstrate that the extract improves cardiac contraction.

Table 2: Landolphia myrtifolia on cardiac contraction

Concentration	Witness (mg/ml)	0,4 mg /ml	0,6 mg /ml	0,8 mg /ml
Amplitude of contraction	$12,6 \pm 0,02$ %	$26 \pm 0,03$ %	$52,8 \pm 0,36$ %	$75 \pm 0,04$ %

1.2 Effect of Landolphia myrtifolia extract on cardiac output

Before infusion, this flow rate was 0.58 ± 0.06 ml/sec, compared to 0.76 ± 0.01 , 0.85 ± 0.06 , and 0.92 ± 0.03 ml/sec in the presence of the extract at concentrations of 0.4, 0.6, and 0.8 mg/ml. These results demonstrate that the extract improves cardiac output.

Table 3: Landolphia myrtifolia on cardiac output

Concentration	Witness (mg /ml)	0,4 mg /ml	0,6 mg /ml	0,8 mg /ml
Cardiac output	$0,58 \pm 0,06$ ml /sec	$0,76 \pm 0,01$ ml /sec	$0,85 \pm 0,06$ ml /sec	$0,92 \pm 0,03$ ml /sec

2. Results following XRF analysis

Table 4 shows the average contents expressed in % of the mineral elements in the Landolphia myrtifolia samples according to the parts analyzed.

Table 4: Average content in Landolphia myrtifolia from ANTALAHA and from SAMBAVA

	ANTALAHA		SAMBAVA	
	Leaves (%)	Stems (%)	Leaves (%)	Stems (%)
Phosphorus (P)	$9,89 \pm 0,17$	$12,39 \pm 0,15$	$14,12 \pm 0,09$	$16,61 \pm 0,43$
Potassium (K)	$9,41 \pm 0,27$	$12,08 \pm 0,18$	$11,06 \pm 0,20$	$14,69 \pm 0,15$
Calcium (Ca)	$13,04 \pm 0,25$	$15,54 \pm 0,27$	$15,70 \pm 0,20$	$15,80 \pm 0,31$
Titanium (Ti)	$0,10 \pm 0,01$	$0,13 \pm 0,02$	$0,08 \pm 0,001$	$0,14 \pm 0,01$
Vanadium (V)	$0,17 \pm 0,02$	$0,13 \pm 0,01$	$0,14 \pm 0,01$	$0,14 \pm 0,02$
Manganese (Mn)	$0,30 \pm 0,04$	$0,69 \pm 0,01$	$0,41 \pm 0,03$	$0,71 \pm 0,01$
Iron (Fe)	$0,68 \pm 0,02$	$1,35 \pm 0,05$	$0,63 \pm 0,01$	$1,40 \pm 0,12$
Nickel (Ni)	$0,20 \pm 0,03$	$0,29 \pm 0,03$	$0,27 \pm 0,01$	$0,25 \pm 0,03$
Copper (Cu)	$0,33 \pm 0,03$	$0,43 \pm 0,02$	$0,33 \pm 0,03$	$0,40 \pm 0,02$
Zinc (Zn)	$3,77 \pm 0,08$	$4,80 \pm 0,10$	$3,50 \pm 0,12$	$4,83 \pm 0,03$
Bromine (Br)	$0,04 \pm 0,001$	$0,07 \pm 0,01$	$0,02 \pm 0,001$	$0,09 \pm 0,03$
Lead (Pb)	$0,04 \pm 0,001$	$0,22 \pm 0,02$	$0,18 \pm 0,02$	$0,43 \pm 0,01$
Aluminum (Al)	< LD	< LD	$0,23 \pm 0,02$	$1,05 \pm 0,03$

IV. DISCUSSIONS

1. Discussion following XRF analysis

- Phosphorus Case

According to the analysis results, Landolphia myrtifolia contains phosphorus in every part of the plant. On average, the phosphorus concentration on the analyzed stem is highest in the SAMBAVA district, at $16.61 \pm 0.43\%$, and in the ANTALAHA district, at $12.39 \pm 0.15\%$. On the leaf, the highest concentration is found in the SAMBAVA district, at $14.12 \pm 0.09\%$, and in the ANTALAHA district, at $9.89 \pm 0.17\%$. Phosphorus is very important for the health of the body because it is very useful for the proper functioning of the nervous system. The United Nations in 2015 established that phosphorus consumption should be 150-220 mg.kg-1 per day [22] while the European Food Safety Authority (EFSA) recommended the value of 550 mg per day [4].

- Potassium Case

The results of Landolphia myrtifolia analyses showed that they contain potassium, some even representing a quarter of the total amount. The highest concentration of potassium analyzed in the stems was found in the SAMBAVA district, at

$14.69 \pm 0.15\%$, and the lowest was $12.08 \pm 0.18\%$ in the ANTALAHA district. In the leaves, the SAMBAVA district had the highest concentration, with an average of $11.06 \pm 0.20\%$, and the lowest average was found in ANTALAHA, at $9.41 \pm 0.27\%$. Potassium is a chemical element that exists in the body as an ion, i.e. a constituent that carries an electrical charge [14]. EFSA has recommended the daily value of 1599 mg per day [4].

- Calcium Case

Calcium also plays an essential role in blood clotting, maintaining blood pressure, and muscle contraction [13]. Landolphia myrtifolia from the S.A.V.A region also contains a fairly significant amount of calcium. The highest concentration of calcium analyzed in the leaves is found in the SAMBAVA district, which is $15.70 \pm 0.20\%$, and the lowest is $13.04 \pm 0.25\%$ in the ANTALAHA district. In the stems, the SAMBAVA district has the highest concentration with an average of $15.80 \pm 0.31\%$, and the lowest average is found in ANTALAHA, which is $15.54 \pm 0.27\%$. The World Health Organization recommends a consumption of 1.5 to 2.00 g/kg per day [20], so it would be better to control the consumption of Landolphia myrtifolia to avoid excess calcium in the body to prevent threats to their health.

- Titanium Case

The results of Landolphia myrtifolia analyses show the presence of titanium. This is an undesirable element. The highest concentration in the leaves is found in the ANTALAHA district, at $0.10 \pm 0.01\%$, and the lowest average is in SAMBAVA, with an average of $0.08 \pm 0.001\%$. In the stems, the concentration in the SAMBAVA and ANTALAHA districts has an almost identical average of $0.014 \pm 0.001\%$. Even in very small quantities, caution is necessary.

- Vanadium Case

The analysis results show that Landolphia myrtifolia contains vanadium in very low amounts compared to other elements present. Even in trace amounts, consumption of Landolphia myrtifolia must be controlled to avoid excessive vanadium intake. On average, the highest concentration in the leaves is found in the ANTALAHA district ($0.17 \pm 0.02\%$), and the lowest average is in SAMBAVA ($0.14 \pm 0.01\%$). In the stems, the concentration in SAMBAVA district is the highest ($0.14 \pm 0.02\%$), and the lowest average is $0.13 \pm 0.01\%$ in ANTALAHA district.

- Manganese Cases

The results of analyses of Landolphia myrtifolia from the SAVA region contain very low amounts of manganese. Manganese is represented at a rate not exceeding 2%. Regarding the average, the highest concentration in the leaves is found in the ANTALAHA district ($0.41 \pm 0.03\%$), and the lowest average is in SAMBAVA ($0.30 \pm 0.04\%$). In the stems, the concentration in the ANTALAHA district is the highest ($0.71 \pm 0.01\%$), and the lowest average is in SAMBAVA ($0.69 \pm 0.01\%$). Poisoning cases are rare, but excess manganese has an asphyxiating effect on the central nervous system. No benefit of manganese supplementation has been proven in pregnant women [11]. The A.F.S.S. recommended a consumption limit of 0.6 to 4.3 mg per day [1].

- Iron Case

According to the results of Landolphia myrtifolia analyses, the presence of iron was found in a lower concentration. Iron levels vary from one district to another. The highest concentration of iron analyzed in the leaves is found in the ANTALAHA district, which is $0.68 \pm 0.02\%$, and the lowest is $0.63 \pm 0.01\%$ in the SAMBAVA district. In the stems, the SAMBAVA district has the highest concentration with an average of $1.40 \pm 0.12\%$, and the lowest average is found in ANTALAHA, which is $1.35 \pm 0.05\%$. Iron is a trace element essential to living organisms. They are also high during growth [2]. In 2017, the World Health Organization recommended a daily intake of 120 mg [20].

- Nickel

Nickel is also present in Landolphia myrtifolia in the SAVA region, but in very small quantities, reaching less than 1%. Nickel is present at a very low level, reaching less than 1%. On average, the highest concentration in leaves is found in the SAMBAVA district, at $0.27 \pm 0.01\%$, and the lowest average is in ANTALAHA, with an average of $0.20 \pm 0.03\%$. In stems, the concentration is highest in ANTALAHA, with an average of $0.29 \pm 0.03\%$, and the lowest average is $0.25 \pm 0.03\%$ in ANTALAHA. The effects of high nickel consumption are the threat of developing lung, larynx and prostate cancer, nausea, vomiting and dizziness, pulmonary failure, birth failure, asthma and chronic bronchitis, heart problems [6]. The World Health Organization recommended 5 $\mu\text{g/kg}$ per day [20] while in 2001, the A.F.S.S. A recommended a consumption of 60 μg per day [1].

- Copper Case

According to the analysis results, copper is contained in *Landolphia myrtifolia* at a very low level, that is, in trace amounts, but in trace amounts. It is an undesirable element. Copper is also present in very low quantities. Throughout the district of the SAVA region, these values do not exceed 1%. The concentration in the leaves is similar in the districts of ANTALAHA and SAMBAVA, at $0.33 \pm 0.03\%$. In the stems, ANTALAHA has the highest concentration, with an average of $0.43 \pm 0.02\%$, while the lowest is in SAMBAVA, with an average of $0.40 \pm 0.020\%$. These results indicate that there is no difference in copper concentration in the five districts of the SAVA region in the leaf parts. On the other hand, in the stems, there is a difference in concentration of at least copper. The risk of a deficiency is extremely rare. Excess copper is more common and can be caused by a hereditary metabolic disease [19]. In 2015, the United Nations recommended the consumption of 11 to 37 mg/kg per day [22].

- Zinc Case

The presence of zinc in *Landolphia myrtifolia* has also been noted, but in very low quantities. Zinc is an element with an average that varies from one district to another. On average, the highest concentration in the leaves is found in the ANTALAHA district, at $3.77 \pm 0.08\%$, and the lowest average is in SAMBAVA, with an average of $3.50 \pm 0.12\%$. In the stems, the concentration in SAMBAVA district is the highest, with an average of $4.83 \pm 0.03\%$, and the lowest average in ANTALAHA district is $4.08 \pm 0.10\%$.

Pregnant women, in particular, are at risk of catching a cold, flu, or any other infection that can reduce zinc levels in the body, which can be dangerous for the fetus [5]. According to the World Health Organization, zinc supplementation in pregnant women is only recommended in the context of rigorously conducted research [20] while in 2015, the EFSA recommended a daily consumption of 1.6 mg [4].

- Bromine Case

In *Landolphia myrtifolia* in the SAVA region, the analysis revealed the presence of bromine in a very low quantity. Bromine is present at a very low average, not reaching 1%. On average, the highest concentration in the leaves is found in the ANTALAHA district, which is $0.04 \pm 0.001\%$, and the lowest average is in SAMBAVA, with an average of $0.02 \pm 0.001\%$. In the stems, the concentration in SAMBAVA district is the highest, with an average of $0.09 \pm 0.03\%$, and the lowest average is $0.07 \pm 0.03\%$ in ANTALAHA district. Bromine is one of the elements dangerous to living organisms. It causes nervous system dysfunction and can disrupt genetic material [16].

- Lead Case

Lead is also an element with a very low average. These averages vary depending on the district. On average, the highest concentration in leaves is found in the SAMBAVA district, which is $0.18 \pm 0.02\%$, and the lowest average is in ANTALAHA, with an average of $0.04 \pm 0.001\%$. In stems, the concentration in the SAMBAVA district is the highest, with an average of $0.43 \pm 0.01\%$, and the ANTALAHA district has the lowest average, which is $0.22 \pm 0.02\%$. Lead is a toxic element [18]. The major toxic effect of lead during fetal development would result in lasting neurobehavioral deficits during childhood [12]. According to the World Health Organization, a daily intake of 3.6 $\mu\text{g/kg}$ is recommended [21].

- Aluminum Case

When aluminum is ingested, 95% of it is eliminated in the feces without it having passed the intestinal barrier. Of the fraction that manages to cross this barrier, 83% is eliminated in the urine. Even if practically all absorbed aluminum is eliminated, our body contains approximately 30 to 50 mg of aluminum, stored mainly in the bones, liver, and lungs [12]. Only the sample from SAMBAVA found traces of aluminum, which are 1.05 ± 0.03 in the leaf and 0.23 ± 0.02 in the stem.

4.2 Discussion following the pharmacological test

The isolated heart of a fatigued frog was used as an experimental model. The extract was infused through the aorta to study its effect on contraction and heart rate. Its effect on cardiac output was studied by perfusing the heart through the venous sinus. Our results show that the extract increases the contraction amplitude of the isolated heart of a fatigued frog. It decreases the heart rate and increases the filling time. The extract increases the amplitude of cardiac contraction, reflecting an increase in contraction force. In addition, this increase is accompanied by an acceleration of cardiac

contraction speed. These effects reflect the positive inotropic effect of the extract. This effect could be attributed to the cardiac glycosides revealed during the phytochemical screening performed on this extract.

V. CONCLUSION

In our research, samples were collected from two (02) different districts in the S.A.V.A region in the north of the island, namely the SAMBAVA district and the ANTALAHA district. This plant is used by populations in northern Madagascar during heart attacks. *Landolphia myrtifolia* extract improves cardiac output. These results show that the extract is a cardiostimulant. The analyses of this plant were carried out using an energy dispersive X-ray fluorescence method at INSTN-Madagascar. The parts of the plant were analyzed and the elements found were the same: Potassium (K), Calcium (Ca), Bromine (Br), Manganese (Mn), Iron (Fe), Nickel (Ni), Copper (Cu), Zinc (Zn), Titanium (Ti), Vanadium (V), Lead (Pb), and Phosphorus (P). Some of these elements are nutrients such as phosphorus, potassium and calcium and they are in the majority in quantity. It can be said that *Landolphia myrtifolia* can replace some foods containing these elements. But despite the presence of these nutrients, there are also toxic elements such as Bromine (Br), Nickel (Ni) and Lead (Pb) which are very harmful to human health. These effects are very dangerous that it is necessary to take precautions to avoid certain complications for users of *Landolphia myrtifolia*. It is preferable for people to make good consultations with specialists to have the care they need during a heart attack.

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