

Ethnobotanical, Antimicrobial and Phytochemical Screening of *Euphorbia Thymoflia L.* (Kinononono madiniky) to Cure the Menstrual Hemorrhage of a Woman Sakalava Bemazava in the Northern Region of Madagascar

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Abstract The antibacterial and antifungal effect *Euphorbia Thymoflia L.* (Kinononono madiniky) was evaluated in the panel of bacteria (*Enterobacter cloacae*, *Klebsiella oxytoca*, *Escherichia coli*, *Salmonella enteridis*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Staphylococcus aureus*) and fungi (*Candida albicans*) and protozoa (*Trichomonas vaginalis*). The extract of *Euphorbia Thymoflia L.* (Kinononono madiniky) is sensitive against bacteria, fungi, protozoa tested. *Enterobacter cloacae* (11.5 mm), *Klebsiella oxytoca* (9mm), *Salmonella enteridis* (11mm), *Streptococcus pneumoniae* (11.5mm), *Pseudomonas aeruginosa* (12mm), *Bacillus cereus* (10mm) and *Candida albicans* (11mm) and protozoa *Trichomonas vaginalis* (12 mm). *Euphorbia Thymoflia L.* contains elements of Flavonoids, Sterols and Triterpenes, Tannins and Polyphenols, but the Alkaloids, Anthraquinones, Saponosides. Sakalava Bemazava women use this plant as a medicine against menstrual haemorrhage. In the 12 Fokontany of the rural commune of Antranokarany; the women investigated practical heat bath, decoction and washed with boiling water cool. 5 antranokarany, 12 Ambalamahogo, 12 Ankotika, 10 Ankoala, 9 Ampamakia, 7 Marosely, 11 Antrema, 12 Befitina, 15 Androhiba, 11 Mangabe, 8 Ampodrabe, 10 Antanimena. Traditional healers play an intermediary role between population and modern medicine. The plant can be used to discover new bioactive natural compounds that can serve as leads in the development of new pharmaceuticals with fewer side effects and risks of resistance.

Keywords *Euphorbia Thymoflia L.* (Kinononono madiniky), Ethnobotany, Phytochemical screening, Traditherapists, Menstrual haemorrhage

1. Introduction

In the north of Madagascar, where the Sakalava breed is found, plants remain the main medical means of care for the populations. The district of Ambanja, DIANA region where lives the Sakalava breed Bemazava the "Kinononono madiniky" is used to stop the bleeding of a woman. It is very abundant in the district of Ambanja. At present, medicinal

plants play a key role as a pillar of traditional health systems in many developing countries such as Madagascar. Currently, the bioactive compounds present in these medicinal plants are used as drug sources [1]. According to the World Health Organization (WHO), more than 80% of the world's population depends on medicines derived from these medicinal plants for primary health care needs. The use of medicinal plants as a source of relief from the disease can be traced back to before the recorded history. These phytomedicines are safe and respectful of the environment [2]. Phytomedicines have become increasingly popular and their use is widespread. Plants produce a diverse range of bioactive molecules, called phytochemicals. Phytochemicals

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are bioactive chemical substances of plant origin. They are considered secondary metabolites because the plants that produce them may not need them [3, 4]. These secondary metabolites are synthesized naturally in all parts of the body of the plant; bark, leaves, stem, root, flower, fruit, seeds, etc., that is, any part of the body of the plant may contain active components, making them rich sources of different types phytochemicals. Generally, these phytochemicals are secondary metabolites such as flavonoids, steroids, alkaloids, resins, fatty acids, tannins and phenolic compounds, etc. [5].

It is classified in the family Euphorbiaceae.

Scientific classification [1, 2]

Kingdom: Plantae

Clade: Angiosperms

Clade: Eudicots

Clade: Rosids

Order: Malpighiales

Family: Euphorbiaceae

Tribe: Euphorbieae

Subtribe: Euphorbiinae

Genus: *Euphorbia*

Species: *E. Thymifolia(L)*

Venacular names: Kinononono madinika, Tsikatsakatsanjaza, Rononontandraka

This ruderal annual herb, prostrate, with a stem long of 20 cm at most is often purple. Its small, narrow leaves, 4-8 mm long and 2-4 mm wide, obtuse at the apex, are slightly stringed at the base. Its solitary inflorescence with a 1-2 mm long peduncle produces trigonal, smooth, yellowish green fruit and tiny seeds [1-4].

2. Materials and Methods

2.1. Method of Work for the Ethnobotanical Study

2.1.1. Presentation of the Study Area

The Rural Municipality of Antranokarany is part of the District of Ambanja, Region DI.A.N.A, located in the North of Madagascar. It is composed of 11 Fokontany (Villages): Atranokarany, Befitina, Ankotika, Ambalamahogo, Ampamakia, Marosely, Antrema, Mangabe, Androhibe, Ampodrobe, Ankoala, Antanimena and counts 5000 habitats on 150000 of the district of Ambanja, that is to say 3.33% of this population. It is composed of Sakalava breed Bemazava. This area is characterized by a warm tropical climate and plover with dense and diverse forest vegetation.

2.1.2. Method of Work for the Ethnobotanical Study

The working method consists of ethnobotanical surveys. They took place in three stages in the month of April 2017; direct interview with populations aged between 15 and 35 and 10 renowned traditional healers including six (6) men and four (4) women. The survey consists of recipes recommended for the treatment of disorder and menstrual hemorrhage.

2.2. Phytochemical Screening Material

2.2.1. Preparation of the Powder of the Samples

Our samples were dried at room temperature. Afterwards, we proceeded separately to the grinding in a wooden mortar then to sieving to obtain the powder of "Kinonono madinika". The fine powders obtained were well kept, in well labeled bags and in the desiccator [6-9].

2.2.2. Chemical Screening of Different Organs

a) Detection of alkaloids

It takes 1g powder leaves and macerated in 10 ml of 1% HCl solution for 24 hours. The macerate is filtered and tested with a few drops of MEYER and DRAGENDORFF reagent. The alkaloids form with a white precipitate with the MEYER reagent, while they form a red precipitate with the DRAGENDORFF reagent [7-10].

b) Detection of flavonoids

In the analysis, it takes 5 to 10g of powder are brought to the boil for 5 minutes in 100ml of water. After cooling and filtration, take the 5 ml of the filtrate and then add 5 ml of hydrochloric alcohol (5 ml of 95% ethyl alcohol, 2 ml of distilled water, 2 ml of 32% hydrochloric acid HCl), 0.5 g about magnesium chips and a few drops of iso amyl alcohol.

The appearance of the pink, orange or red coloring in the iso amyl alcohol supernatant layer indicates the presence of flavonoids. The same reaction carried out without adding magnesium and heating for 2 minutes in a water bath allows the characterization of leuco anthocyanin. It is positive if there is appearance of a red color [7-11].

c) Detection of anthraquinones

To detect anthraquinones, it is necessary to 5g of plant material powder soaked with a few drops of HCl 1/5, are macerated in 30ml of the chloroform-ether mixture (1/1) in a capped vial for 24 hours. After filtration, 2 ml of filtrate are stirred with 2 ml of the 1/10 sodium hydroxide solution. The presence of quinone is translated by the transfer of the color from red to violet of the aqueous phase [12-14].

d) Detection of sterols and terpenes

To analyze the sterols and terpenes, it takes 1g of plant material powder is macerated for 24 hours in a clogged vial containing 20ml of diethyl ether. 5 drops of the solution are evaporated on the watch glass. The residues are taken up in 2 drops of acetic anhydride. Addition of the drop of concentrated sulfuric acid gives sterol or terpene compounds, a purple color turning green. A negative result in these two tests indicates the absence of sterol and terpene products [10-13].

e) Saponin detection

The saponins require 5 g of crushed plant material, a decoction is made in 50 ml of water for 15 minutes, 10 ml of filtrate are taken in another test tube and shaken vigorously, the tube is allowed to stand for 10 minutes, the persistence of foam after 10 minutes indicates the presence of saponins in

the sample [10-13].

f) Detection of tannins and polyphenolics

He takes the 5 ml of the decoction of the Kinonono powder and drops a few drops of 1% ferric chloride. The appearance of a particular coloration or a precipitate indicates the presence of tannins in the "Kinonono madinika" powder [11-14].

g) Detection of sterols and polyterpenes

Sterols and polyterpenes were detected in residues R1 and R5 by the Liebermann reaction. An aliquot of residue is dissolved hot in 1 ml of acetic anhydride in a capsule, then taken up in a test tube in which are poured 0.5 ml of concentrated H₂SO₄. The appearance of a purple coloration that turns blue then green indicates a positive reaction [11-14].

2.2.3. Antimicrobial Susceptibility Testing

a) Pure cultures of bacteria (*Enterobacter cloacae*, *Klebsiella oxytoca*, *Escherichia coli*, *Salmonella enteridis*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Staphylococcus aureus*) and fungi (*Candida albicans*) and protozoa (*Trichomonas vaginalis*) were used for 'study. Bacterial strains were maintained on nutrient agar slopes at 4°C. One loop of each bacterial strain was added to a 50 ml sterile nutrient broth in a 100 ml conical solution. The requests were then incubated for 24 hours to activate the test strain. Purified fungi cultures were maintained on Sabouraud Dextrose Agar Agar (SDA) slopes at 4°C. It was transplanted onto SDA plates and incubated at room temperature for 5-8 days. Developed spores were harvested and a spore suspension was used for antimicrobial testing [16-18].

b) Agar diffusion method

The antimicrobial activity of plant extracts was evaluated by the agar diffusion method. The surface of the agar plate is inoculated by uniformly spreading the bacterial inoculum over the entire surface of the agar. For fungi, SDA was inoculated with fungal spore suspension at a tolerable

temperature and transferred to sterile Petri dishes. Then, a 6 mm diameter hole is aseptically punched with a sterile corn borer and a 20 µl volume of the extract is introduced into the well. Then the bacterial plaques are incubated overnight. Similarly, the fungal plates were incubated at room temperature for 5 to 10 days. The antimicrobial agent diffuses into the agar medium and inhibits the growth of the microbial strain tested. The antimicrobial activity was determined by measuring the zone of inhibition and expressed in millimeters (mm). Five sets of plates are used for antimicrobial studies as well as control plates [17, 18, 21-24].

3. Results and Discussion

3.1. Ethnobotanical Results

There are women in 12 Fokontany of the rural commune of Antranokarany use the "Kinonono madiniky" as medicine. In our survey, it takes age group of women between 15 to 35 years old. As the Antranokarany Fokontany the 5 women investigated used the "Kinonono madiniky" to cure menstrual hemorrhage or disorder. 11 for women in Mangabe, 12 in Ambalamahogo, 12 in Ankotika, 10 in Ankoala, 9 in Ampamakia, 7 in Marosely, 11 in Antrema, 10 in Befitina, 15 in Androhibe, 8 in Ampodrabé and 10 in Antanimena. The "Kinonono madiniky" is among the most used medicine plant in the rural municipality of Antranokarany. All the traditherapeutes surveyed are advised women and give the "Kinonono madiniky" as effective medicine to cure the illness very rependu in the race Sakalava. Where does this disease come from? According to our survey Sakalava women are the most hardworking and bear the burden like bucket, dead woods, culture on brulis. The traditherapeutes mention that it is the hardness that is to say the too hectic Sakalava woman causes the menstrual hemorrhage. The women surveyed confirm that neither the pharmaceutical drug capable of curing diseases nor the medicinal plant called "kinonono madiniky".

Table 1. The results of the ethnobotany survey

Local names	Number of POPs. Female	Organs used	Preparation method	Mode of administration	Diseases
Antranokarany	5	Leaves set and fresh root	- decoction - Steam bath	- Oral voice - places above the container the sex of the woman	- menstrual blurred - menstrual bleeding
Ambalamahogo	12	Leaves set and fresh root	- decoction - Steam bath	- Oral voice - places above the container the sex of the woman	- menstrual blurred - menstrual bleeding
Ankotika	12	Leaves set and fresh root	- decoction - Steam bath	- Oral voice - places above the container the sex of the woman	- menstrual blurred - menstrual bleeding
Ankoala	10	Leaves set and fresh root	- decoction - Steam bath	- Oral voice - places above the container the sex of the woman	- menstrual blurred - menstrual bleeding

Local names	Number of POPs, Female	Organs used	Preparation method	Mode of administration	Diseases
Ampamakia	9	Leaves set and fresh root	- decoction -Steam bath	- Oral voice - places above the container the sex of the woman	- menstrual blurred -menstrual bleeding
Marosely	7	Leaves set and fresh root	- decoction -Steam bath	- Oral voice - places above the container the sex of the woman	- menstrual blurred - menstrual bleeding
Antrema	11	Leaves set and fresh root	- decoction -Steam bath	- Oral voice - places above the container the sex of the woman	- menstrual blurred -menstrual bleeding
Befitina	10	Leaves set and fresh root	- decoction -Steam bath	- Oral voice - places above the container the sex of the woman	- menstrual blurred - menstrual bleeding
Androhibe	15	Leaves set and fresh root	- decoction -Steam bath	- Oral voice - places above the container the sex of the woman	- menstrual blurred - menstrual bleeding
Magnabe	11	Leaves set and fresh root	- decoction -Steam bath	- Oral voice - places above the container the sex of the woman	- menstrual blurred - menstrual bleeding
Ampodrabe	8	Leaves set and fresh root	- decoction -Steam bath	- Oral voice - places above the container the sex of the woman	- menstrual blurred - menstrual bleeding
Antanimena	10	Leaves set and fresh root	- decoction -Steam bath	- Oral voice - places above the container the sex of the woman	- menstrual blurred - menstrual bleeding

3.2. Phytochemical Screening

Table 2. Summary of phytochemical screening

Antinutritional Factors	Results	Conclusion
Alkaloids	-	Absence of alkaloid
Flavonoids	+	Presence of Flavones
Sterols and Triterpenes	+++	Presence of Sterols
Anthraquinones	-	Absence of Anthraquinones
Saponosides	-	Absences of Saponosides
Tannins and Polyphenols	+++	Presence of other types of phenolic compounds. other than pyrogallal and catechistic

The "Kinonono madiniky" called *Euphorbia Thymoflia* Linn are widely distributed in the rural district of Antranokarany. Phytochemicals are natural substances that are modified by chemical or enzymatic reactions to develop drugs, cosmetics, pesticides or biodegradable plastics.

a) Alkaloids

Alkaloids are organic nitrogen-containing chemical substances having a pharmacodynamic action. This name derives from the word alkaline; originally, the term was used to describe any base containing nitrogen (or amine). He is absent in the "Kinonono madiniky".

b) Flavonoids

Flavonoids are phenolic compounds, many of which are pigments responsible for the coloring of many flowers and some fruits. Plant dyes and other natural compounds derived

from the chromone have almost all skeletons of flavin more or less modified by addition or subtraction of the oxygen group and are the family of flavonoids. Flavonoids attract and guide pollinators and the reproduction of the flowering plant. They are widely distributed in the vegetable kingdom where it exists most often in soluble form of glycosides. The main activities attributed to flavonoids are a vitamin P property. Experience shows that all its compounds are capable of decreasing capsular permeability and strengthening their resistance. Flavones have activity in the bleeding vein of a woman's vagina.

c) Tannins

Tannins are complex mixtures of esters and carbohydrate ethers. They are also classified as water-soluble polyphenolic compounds derived from shikimic acid. There are classically two groups of tannins:

- the condensed tannins or proanthocymidols
- the hydrolysable tannins.

The tannins have pharmacological properties either internally, their application exerts an anti-diarrheal and antiseptic effect; or externally, the tannins waterproof the outermost layers of the skin and mucous membranes thus protecting the underlying layers.

d) Saponins

Saponins are substances abundantly prevalent in the vegetal reign and owe their name to the fact that their aqueous solution foam abundantly. But in the "Kinonono madiniky" he is absent.

e) Anthraquinones

The anthraquinones are in the form of quinones whose compounds corresponding to the oxidation of aromatic compounds and characterized by a -1,2 cyclohexadienone-3,5 (ortho-quinone) unit. Natural quinones belong to three main groups: Benzoquinones, Naphta quinones and Anthraquinones.

Quinones are of great technical importance (dyes) and biochemical (redox catalysts in cells). They synthesize the quinones using different methods, according to their structures.

Benzoquinone is obtained by oxidation of aromatic amines or phenols. The oxidation of naphthalene and larger aromatic polycycles gives directly quinones.

f) Steroids and Terpenoids

- steroids

Steroids can be considered as triterpenoid having lost up to 3 methyls.

They constitute an important class of biological compounds such as: sterols per se, adrenocortical and sexual hormones, aglycones of cardiac glycosides, saponins and some alkaloids.

Steroids are a family of compounds that contain the per-1,2-cyclopentano phenanthrene backbone and are part of lipids. These compounds are frequently found in plants and animals and are among the most important natural products. The sterols have a hydroxyl group on the carbon atom 3, a double bond between the carbon atoms 5 and 6 and a side chain attached to the top 17 of the perhydrocyclopentano phenanthrene ring.

Cholesterol is the most representative of sterols; it gives birth to the majority of steroids. Steroids have a very high rate in Kinonono. Our note mentions that they stop the hemorrhage in the uterus.

- Triterpènes

It is called terpene, a series of constituents of fragrant vegetable essences generally obtained by steaming. These are relatively volatile essential oils (C₁₀ or C₁₅) (essence of mint, pine, eucalyptus, rose, lemongrass).

Some terpenes used as medicine were already known in antiquity. Nowadays, camphor and α -pinene are of commercial importance. Terpenoids play a protective role of vagina to different bacteria, the action of terpenoid very remarkable in vagina as a microbial barrier and stop all bad microbial actions.

3.3. Antimicrobial Results

The antibacterial and antifungal effect of Euphorbia Thymoflia Linn was evaluated against a panel of bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiellaoxytoca*, *Streptococcus pneumoniae* and *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella enteridis*) and fungi (*Candida albicans*). The sensitivity profile was given in Table 3. The extract of Euphorbia Thymoflia Lin was sensitive against the bacteria and fungi tested. The

extract of Kinonono madiniky powder is active or gram-sensitive like *Streptococcus pneumoniae*, *Bacillus cereus*, because the inhibition halo (x) is between 9mm and 14mm.

Table 3. Antimicrobial Test

Test Germs	Kinonono Madiniky Powder	Reference Antibiotics	
	Inhibition Halo Diameter (mm)	Nalidixic Acid (NA 30)	Fusidic Acid (FA 10)
<i>Streptococcus pneumoniae</i>	11.5	-	25
<i>Staphylococcus aureus</i>	6	-	23
<i>Salmonella enteridis</i>	11	18	-
<i>Enterobacter cloacae</i>	11.5	20	-
<i>Escherichia coli</i>	6	18	
<i>Klebsiellaoxytoca</i>	9	22	-
<i>Pseudomonas aeruginosa</i>	12	20	
<i>Bacillus cereus</i>	10		12
<i>Candida albicans</i>	11	-	14
<i>Trichomonas vaginalis</i>	12	-	14

Diameter of the discs: 6mm

Cell concentration: 106 cells / ml

Concentration of the extract / disc: 200 μ g

Extract volume: 20 μ l

Standards: x (inhibition halo diameter) <8mm: resistant; 9mm <x <14mm: sensitive; 15mm <x <19mm very sensitive; 20mm: extremely sensitive

It is less active compared to the reference antibiotic fusidic acid 10 μ g. Regarding gram + bacteria such as *Salmonella enteridis*, *Enterobacter cloacae*, *Klebsiellaoxytoca*, *Pseudomonas aeruginosa*, the extract is active or sensitive especially on *Pseudomonas aeruginosa*. The inhibition halo (x) is between 9mm and 14mm, but it is less active than the reference antibiotic Nalidixique.

The antifungal effect of extract is marked; it is active with *Candida albicans*. The Kinonono madiniky has an effect with *Trichomonas vaginalis* of an inhibitor halo (x) is equal to 12mm compared to the reference antibiotic Fusidic acid.

Kinonono madiniky is a very popular medicine for Sakalava woman; it can cure menstrual haemorrhage and trouble. According to medical studies, the different Sakalava Bemazava women's diseases are due to the daily lifestyle. There are abnormal external or internal factors (unnatural). Sakalava women too excess of hygiene, they shower vaginal that is to say they wash the vagina too much with water with each pee. The second cause, from the age of 15, women is separated with their parents in case the women did not go to school. It is sexual intercourse too early can be involved, not only by transmission of germs but by action in contact with highly alkaline sperm. The vaginal environment consists of a liquid phase (water + substances from the blood + cervical mucus) and solid elements (vaginal mucous cells, protective

white blood cells against infections and bacteria (good or bad for health).

The normal vaginal flora is characterized by a large bacterial diversity of lactobacillus type (called Döderlein Flora) and a great diversity of colonizing species of intestinal or oropharyngeal origin. So, the customs of Sakalava Bemazava cause menstrual hemorrhage and haze. The results confirm that the gift comes from God for the traditherapeutes is true to cure the menstrual hemorrhage and disorder. The presence of elements such as flavonoids, the abundance of Sterols and Triterpenes, Tannins and Polyphenols kill the bacteria and ensure the mucous membrane to protect the vagina of a Sakalava woman.

4. Conclusions

Euphorbia Thymoflia L. (Kinononono madiniky) is a highly recognized plant in the rural district of Antranokarany. Traditherapeutes (traditional practitioners) use for the treatment of various vaginal diseases. The study can be used as a springboard to fight infectious diseases. The presence of Sterols and Triterpenes, Tannins and Polyphenols and also their activities on bacteria like (*Streptococcus pneumoniae*, *Salmonella enteridis*, *Psuedomonas aeruginosa*, *Bacillus cereus*) and fungi such as (*Candida albicans*) and protozoa like (*Trichomonas vaginalis*) are very important in the pharmacoresistance is further research.

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