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Insights into an endemic medicinal plant species of Madagascar and Comoros: The case of Famelona (*Chrysophyllum boivinianum* (Pierre) Baehni, Sapotaceae family)

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ABSTRACT

Chrysophyllum boivinianum (Pierre) Baehni is an endemic plant of Madagascar and Comoros. In Madagascar, it is known by the local name "famelona". The wood of *C. boivinianum* is exploited for carpentry as material for building houses and ships. Its leaves are used in traditional medicine to treat fever, muscle pain and scorpion bites as well as to heal wounds. In Madagascar, it is widely used by the local population to treat many diseases. Thus, a deeper assessment of its valorisation strategy is becoming ever more crucial. The objective of this study was to determine the phenolic and organic compounds in the leaves and stems of *C. boivinianum* in relation to their biological activities and local uses. Solvents composed of methanol, water and chloridric acid were used for leaf and stem extractions. The two extracts were then compared with leaf infusions and stem decoctions by spectrophotometric and chromatographic analysis in order to determine phytochemical composition, and antioxidant and antimicrobial activities. The results showed that the leaf extracts contained the highest amount of total polyphenolic compounds (TPC) (805.16 ± 1.08 $mg_{GAE}/100\ g_{DW}$), followed by leaf infusions at 477.87 ± 38.49 $mg_{GAE}/100\ g_{DW}$. The stem extracts and stem decoctions had lower TPC than did the leaf extracts, with 249.12 ± 7.11 $mg_{GAE}/100\ g_{DW}$ and 191.66 ± 14.88 $mg_{GAE}/100\ g_{DW}$, respectively. The leaf infusions showed much higher antioxidant activity (49.67 ± 0.45 mmol Fe^{2+}/kg_{DW}) than did leaf extracts (27.60 ± 0.32 mmol Fe^{2+}/kg_{DW}). This activity was influenced by high amounts of chlorogenic acid and caffeic acid, at 217.08 ± 2.89 $mg_{GAE}/100\ g_{DW}$ and 13.02 ± 0.15 $mg_{GAE}/100\ g_{DW}$, respectively. The leaf infusions were also rich in gallic acid (15.19 ± 1.63 $mg_{GAE}/100\ g_{DW}$). The stem extracts and stem decoctions also had lower antioxidant activity, at 19.86 ± 7.35 mmol Fe^{2+}/kg_{DW} and 12.53 ± 0.15 mmol Fe^{2+}/kg_{DW}, respectively. The leaves of *C. boivinianum* showed a higher content of bioactive compounds than the stems, and the infusions represented the best method for extracting biomolecules with high amounts of healthy properties and antioxidant activity. Regarding antimicrobial activities, leaf extracts had higher antimicrobial activity against *Salmonella typhii*, `Escherichia coli` and `Candida albicans` than did stem extracts. This activity was influenced by the high rates of polyphenols in the leaves.

1. Introduction

Madagascar is a country rich in biodiversity. Its flora is very diversified and comprises 13,000 plants species, 80% of which are endemic, that are commonly exploited by the local population (ANGAP, 1998).

Famelona is a plant in the Sapotaceae family. Famelona is known by the scientific name *Chrysophyllum boivinianum*, which is synonymous with *Gambeya boiviniana* Pierre. Famelona is an endemic plant of Madagascar and Comoros (Louppe et al., 2008). This species is found in the Madagascar eastern humid forest between 0 and 1500 m above sea level (e.g. Tsiazompaniry forest) and it grows on a variety of substrates such as sandy or lateritic soils (Louppe et al., 2008).

The *C. boivinianum* plant has multiple uses. Its stems are exploited for various purposes, including carpentry, furniture and the construction of

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houses and ships (Ramamonjisoa et al., 2012). Its wood is often used for furniture, as it is light and uniform in structure. The heartwood colour is cream to yellowish brown or pinkish brown. The density of famelona wood is 630 to 710 kg/m^3, which corresponds to a heavy essence that is preferred by consumers. The machinability of famelona wood is easy, and the wood presents excellent finishing after painting (Andrianantenaina, 2014). Wood exploitation is an important human pressure on this species, but the Tavy, or traditional rice farming, also poses a permanent threat for any species occurring in this eastern ecosystem. Wood exploitation and permanent deforestation are equally the main anthropogenic pressures on this species: indeed famelona is the fifth-most used wood in the wood industry in the capital of Madagascar (Ramananantoandro et al., 2013).

Chrysophyllum boivinianum trees are large: the famelona tree can reach 25 m in height and 70 cm in diameter. Its bark is clear and smooth and has a white latex (Fig. 1), which is a characteristic of the Sapotaceae family (Randrianaivo, 2013). The leaves are simple, whole on smooth margin, pointed and arranged in a spiral (Figs. 2, 3). The leaves are elliptic, obovate and approximately 12 cm in length and 2 to 3.5 cm wide. The leaves of *C. boivinianum* have prominent lateral veins on abaxial surfaces (more than 10 straight lateral nervures). The flowers are axillary, bisexual, regular, sessile or with short pedicel, grouped in fascicles. The sepals are free, 2.5 to 3 mm in length and pubescent. The corollas are tubiliform, approximately 2.5 cm long. The stamens are inserted in the tube of the corolla. The superior ovary is locular, tapered, glabrous, and short styled. The fruit is a globular berry that is approximately 2.5 to 4 cm in diameter, rounded and dark green in colour (Fig. 3). Each fruit contains 3 to 5 ellipsoid seeds that are flattened and in brown in colour (Fig. 4) (Louppe et al., 2008). At maturity, the seeds are very sensitive to caterpillars, which severely reduce the longevity of seeds. The fruiting period of *C. boivinianum* in Madagascar is from June to September.

The fruit of *C. boivinianum* is edible and is eaten by the local population in the eastern forest; the fruit is also a food that is appreciated by lemurs (SNGF, 2012), which are responsible for the dispersal of this plant. Leaves and bark have often antiseptic and antibacterial properties: local populations use the leaves for healing and coagulation. Infusion of Famelona leaves are used in traditional medicine to treat fever and muscle pain, while bark decoctions are used to treat syphilis

Fig. 1. Stem of *C. boivinianum*.

Fig. 2. Herbarium specimen of *C. boivinianum*.

(Rabearivony et al., 2015). Randrianarivelojosia et al. (2003) reported that Famelona leaves are used to counteract scorpion stings and relieve fatigue and, according to Rindraniaina (2012), they are also used to treat digestive and genital diseases. Traditionally, *C. boivinianum* has been considered a sacred plant. Some Malagasy believe it can give wealth and ensure security and that it can avoid the wrong things in life and lead to the best things in life (Rindraniaina, 2012). It is a revitalising plant, as indicated by its vernacular name.

A recent study of the *C. boivinianum* bark showed anti-inflammatory activity against ethyl acetate extracts. Analysis of the chemical composition was carried out by various chromatographic techniques, and the molecular structures were elucidated using spectrometric methods including nuclear magnetic and mass spectrometry as well as both IR spectrometry and GC/MS. Nine molecules were identified, including lupeol acetate; β-amyrin acetate; α-amyrin acetate; taraxasterol acetate; lupeol fatty acid ester and β-amyrin fatty acid ester; chondrillasterol; β-sitosterol; and β-sitosterol-3-O-glucoside (Rasoanaivo et al., 2014).

Strong pressure due to its intensive over-exploitation, poor knowledge of its genetic resources and climate change are threatening *C. boivinianum* as well as the forest ecosystems on which it depends. According to the WWF (World Wild Foundation) and IUCN (International Union for Conservation of Nature) programmes, *C. boivinianum* is included in the TRAFFIC list or, more accurately, in the network of the surveillance of the trade of the species of flora and fauna. This taxon has not yet been assessed for the IUCN Red List, but is in the Catalogue of Life.

Thus, deeper assessment, valorisation and conservation strategies of *C. boivinianum* are becoming ever more crucial. The objective of this study was to determine the phenolic and organic compounds in different famelona plant parts (leaves, stems) in relation to their biological activities and local preparation and use.

2. Materials and methods

2.1. Plant material

The plant material collection was carried out in August 2016 in the Tsiazopaniry forest, in the Andramasina district, and in east central Madagascar. The geographical coordinates of the collection material were 19° 20′ 19.6″ S, 47° 52′ 3.9″ E at an altitude of 1496 m above sea level. The branches of the *C. boivinianum* trees were cut to collect leaf and twig samples. This method is not destructive to the rest of the plant. Plant material was directly pretreated in the field by cleaning the dust, removing water droplets and drying in the sun (OMS, 2013). The purpose of this pretreatment was to eliminate the undesirable

Fig. 3. Fruits of *C. boivinianum*.

materials and to avoid the degradation of plant material. In the laboratory, the leaves and stems were dried in an oven at 40 °C for 2 days (Fig. 5). A herbarium specimen was prepared in the field and was identified by the botanist at the Botanical and Zoological Park of Tsimbazaza, Madagascar (Fig. 2).

2.2. Sample preparation

2.2.1. Drying the samples

The leaves and stems of *C. boivinianum* were dried in an oven at 40 °C for 48 h (Fig. 6). This method avoids the rotting or fermentation of the plant material in order to maintain the compounds (OMS, 2013).

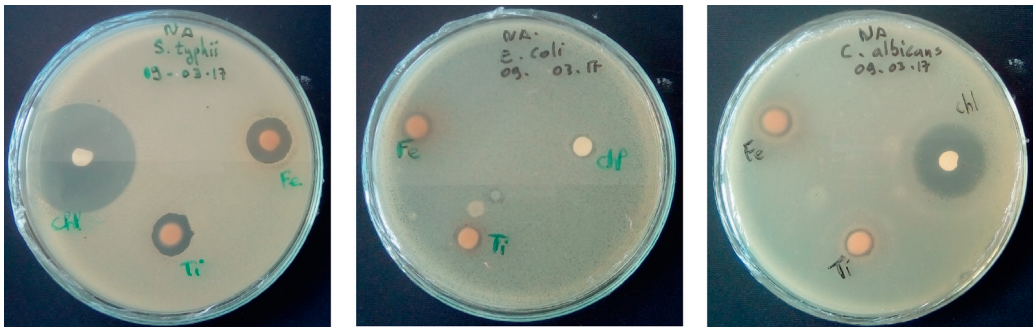
2.2.2. Extractions of leaves and stems by solvents

Each piece of plant material was cut into several pieces. Approximately 5 g of leaves and 10 g of stem pieces were weighed using a Mettler electronic balance (PM460, Delta Range). Three replicates were performed for each leaf and stem sample. Quantities of 500 mL, 23.8 mL and 1.4 mL of methanol, water and chloridric acid, respectively, were mixed to obtain an extraction solvent (Donno et al., 2012). The quantity of reagents used for the extraction of leaves and stems were 125 mL and 75 mL, respectively. The whole extraction mixture (solvent and sample) was macerated for 24 h in the dark. It was then mixed and homogenised using a mixer (Ultra Turrax T25 basic, IKA-Werke), after which it was placed in darkness for 72 h to enable good maceration. The samples were then mixed for 3 min for leaves and 5 min for stems. Each sample was again placed in the dark for 24 h, followed by the first filtration (Whatman Filter Paper, Hardened Ashless Circles, 185 mm Ø). The filtrate was stored at 4 °C and a relative humidity of 95%.

A second extraction was performed by adding 50 mL of extraction reagent to the plant material. The whole extraction mixture was placed in the dark for 72 h and then filtered through the same type of Whatman filter paper. The plant material was then pressed in a presser to obtain the maximum extract. The extract was again filtered through a Whatman filter paper. At the end, the final filtrate was added to the first and second filtrates and stored in normal conditions.

Fig. 4. Seeds of *C. boivinianum*.

Fig. 5. Drying of *C. boivinianum*.



a. Antimicrobial activity of *S. typhii*

b. Antimicrobial activity of *E. coli*

c. Antimicrobial activity of *C. albicans*

Fig. 6. Strains and antimicrobial activity of *Salmonella typhii*, *Escherichia coli* and *Candida albicans*. a. Antimicrobial activity of *S. typhii.* b. Antimicrobial activity of *E. coli.* c. Antimicrobial activity of *C. albicans.*

2.2.3. Leaf infusions and stem decoctions of C. boivinianum

The leaves were cut into several pieces to facilitate the extraction of compounds. Five grams of leaves were weighed and added to 200 mL of boiling water at 80 °C for 10 min. Each sample was filtered (Whatman Filter Paper, 185 mm Ø), and the supernatant was stored under normal conditions at 4 °C and a relative humidity of 95% until analysis (Donno et al., 2016).

The stems were also cut into small pieces. Twenty grams of plant material was weighed and then added to 200 mL of water in a beaker. The whole mixture was brought to a boil for 20 min at 100 °C. Each sample was then filtered (Whatman Filter Paper, 185 mm Ø) to obtain the extract, and the supernatant was stored under normal conditions at 4 °C and a relative humidity of 95% until analysis (Donno et al., 2016).

2.3. Antimicrobial activity

The gram-positive bacterial strain *Salmonella typhii*, the gram-negative bacterial strain `Escherichia coli` and the yeast strain `Candida albicans` were tested with the extracts from the solvent extraction of the leaves and stems of *C. boivinianum*. The antimicrobial activity test was carried out in the Laboratory of Microbiology of the Malagasy Institute of Applied Research (IMRA) in Madagascar.

2.3.1. Disc diffusion method

The goal of this method is to identify the antimicrobial activities of the plant extracts on the strains. The disc diffusion method can detect the sensitivity of the strains tested.

Regarding the preparation of the inoculum, two or three colonies isolated for 24 h were inoculated into 25 mL of nutrient broth medium for bacteria and Sabouraud broth medium for yeast. The suspensions of bacteria and yeast were incubated at 37 °C and 25 °C, respectively, for 24 h (Akter et al., 2016). The bacterial density was measured using a densitometer (Densicheck, BioMérieux Inc., France). The suspension was prepared using sterile physiological water and 0.5 MacFarland standards, corresponding to an inoculum of 10^8 cfu/mL for bacteria and 10^7 cfu/mL for yeast. After dilution, concentrations of 10^6 cfu/mL of each strain were used for testing. The inoculum was uniformly spread on the surface of the nutrient agar for bacteria and on the surface of Sabouraud agar for yeast. Sterile filter paper discs (Whatman 6 mm in diameter) were impregnated with 20 μL of plant extracts. A concentration of 40 mg/mL of extracts was used. For the positive control, 0.5 mg/mL chloramphenicol was used. Each Petri dish was incubated at 37 °C for bacteria and 25 °C for yeast for 24 h. The activity of the extracts in the media inhibited the growth of the strains tested. The presence of an inhibition zone (clear zone) in the media around the discs indicated that the samples were active. The efficacy of the extracts was determined by measuring the diameter of the inhibition zone (Rajaonarivelo, 2006).

2.4. Spectrophotometric analysis

2.4.1. Antioxidant activity

The antioxidant activity of the leaves and stems of *C. boivinianum* was assessed using the ferric reducing antioxidant power (FRAP) method. This method is based on the reduction of ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) in a solution of 2,4,6-tripiridil-S-triazine (Benzie and Strain, 1999). The optical density was read by a UV/Vis spectrophotometer (1600 - PC, VWR International) at a wavelength of 595 nm (Donno et al., 2015). The results were expressed as millimoles of Fe^{2+} equivalents per kilogramme of fresh weight (Donno et al., 2016).

2.4.2. Total polyphenolic compounds (TPC)

The method used to determine the TPC is based on the Folin–Ciocalteu reagent and the reading of the optical density at 750 nm by a spectrophotometer (Slinkard and Singleton, 1977). The results were expressed as milligrammes of gallic acid equivalents (GAE) per 100 g of fresh weight (Donno et al., 2016).

2.5. Chromatographic analysis

A chromatographic technique (high performance liquid chromatography – diode array detector, HPLC – DAD) was used to separate the phytochemical components in the plant extracts.

2.5.1. Chromatographic apparatus

An Agilent 1200 High-Performance Liquid Chromatograph was used for the analysis. The system was equipped with a G1311A quaternary pump, a manual injection valve and a 20-μL sample loop coupled to an Agilent GI315D UV/Vis diode array detector. Five different chromatographic methods were used to analyse the samples: two for polyphenols and one for terpenic compounds; one for organic acids; and one for vitamin C. In all of the used methods, bioactive compound separation was achieved on a Phenomenex Kinetex C18 column (4.6 × 150 mm, 5 μm, Agilent Technologies) (Donno et al., 2015).

2.5.2. Identification and quantification of compounds

All substances were identified by the comparison and combination of retention times and UV spectra. The standard external method was used for quantitative determination (Donno et al., 2015). Five polyphenolic classes were analysed, which were characterised by cinnamic acids, flavonols, benzoic acids, catechins and tannins (Donno et al., 2016).

2.5.3. Analysis of polyphenols by HPLC

2.5.3.1. Preparation of extracts for polyphenol analysis. Polyphenol analysis required filtration to separate the polyphenols from vitamin C. After extraction, 2 mL of each extract was added to a 2-mL test tube. The whole mixture was centrifuged at 12,000 rpm at a temperature of 4 °C for 5 min. The purpose of this preparation was to obtain a good homogeneous extract without the pellet. The purification of polyphenols by filtration was then carried out. The filtration allowed separation of the polyphenol and vitamin C on a C18 cartridge for solid phase extraction (Sep-Pak® C-18, Waters). Approximately 2 mL of methanol was used to recover the polyphenols. The samples were stored under normal conditions at 4 °C and a relative humidity of 95% until analysis by HPLC (Donno et al., 2016).

2.5.3.2. Conditions for analysis of cinnamic acid and flavonols. Two solvents were used as the mobile phase for the analysis of cinnamic acid and flavonols by HPLC: the first mobile phase was acetonitrile, and the second was 10 mM potassium phosphate (KH_2PO_4) solution. The analysis was carried out at a flow rate of 1.5 mL/min, a post-time of 2 min and a duration of 20 min. Cinnamic acid and flavonols were detected at a wavelength of 330 nm (Donno et al., 2016).

2.5.3.3. Conditions for analysis of benzoic acids, catechins and tannins. Two mobile phases were used for the analysis. The first mobile phase was composed of a water:methanol:formic acid solution (5:95:0.1; v/v/v) with a pH of 2.5, and the second mobile phase was a mixture of methanol and formic acid (100:0.1; v/v). The flow rate was 0.6 mL/min with an analysis time of 23 min. The compounds were detected at a wavelength of 280 nm (Donno et al., 2016).

2.5.3.4. Conditions for the analysis of organic acids. Organic acid extracts were analysed directly by HPLC after their extraction. Two mobile phases were used for this analysis. A 10 mM aqueous solution of potassium phosphate (KH_2PO_4) with a pH of 2.8 adjusted by phosphoric acid was used. The second mobile phase consisted of acetonitrile (CH_3CN) at 14.4% (Donno et al., 2015). The flow rate was 0.6 mL/min, and the analysis time was 20 min. The organic acids were read at a wavelength of 214 nm.

2.5.3.5. Conditions for analysis of monoterpenes. The extract was also analysed directly after extraction. Water and methanol were the two mobile phases used for monoterpene analysis. The flow rate was 1.0 mL/min, with an analysis time of 17 min. The wavelengths used were 201, 220, 235 and 250 nm (Donno et al., 2015).

2.5.3.6. Conditions for analysis of vitamin C. A quantity of 2 mL of each extract was added to a test tube or vial. The whole extract was centrifuged at 12,000 rpm at a temperature of 4 °C for 5 min.

The supernatants were then filtered through a 0.45-μm filter (Titan 2 HPLC filter 17-mm polytetrafluoroethylene - PTFE membrane); polyphenolic compounds were absorbed on a C18 cartridge for solid phase extraction (Waters Corporation, Sep-Pak® C-18). Polyphenols were recupered with 2 mL of methanol and stored under normal conditions at 4 °C and a relative humidity of 95% until analysis.

The analysis of vitamin C by HPLC requires a specific treatment in order to separate ascorbic acid and dehydroascorbic acid (DHAA) (Donno et al., 2015). The separation of vitamin C was carried out in a 2-mL test tube. Two hundred fifty microlitres of O-phenylenediamine (OPDA) solution was added to 750 μL of extract sample for DHAA derivatisation into the fluorophore 3-(1,2-dihydroxyethyl)furo[3,4-b] quinoxalina-1-one. After 37 min in the dark, the samples were analysed using an HPLC/DAD system.

It is important that the reagent for the separation of vitamin C be prepared daily. Mixing before use and storage at 4 °C in the dark were highly recommended.

Two mobile phases were used for the analysis of vitamin C by HPLC. The first mobile phase consisted of 50 mM potassium phosphate solution and 5 mM cetrimide in a water:methanol (5:95; v/v) solution. The second mobile phase was methanol. The analysis was carried out at a flow rate of 0.9 mL/min for 15 min. Vitamin C was detected at both 261 nm and 348 nm. The retention times for hydroascorbic acid and ascorbic acid were 2.6 min and 3.6 min, respectively. The duration of the analysis was 10 min (Donno et al., 2016).

2.6. Statistical analysis

All samples were prepared and analysed in triplicate. The results were statistically tested by the Student t-test, the analysis of variance (ANOVA) and the Tukey multiple range test ($P < 0.05$) using the statistical software SPSS (v. 22.0, IBM Analytics, USA).

3. Results

3.1. Spectrophotometric analysis

3.1.1. Antioxidant activity

Leaf extracts and leaf infusions were statistically different in antioxidant activities ($P < 0.05$). The leaf infusions had higher antioxidant activity than leaf extracts, with 49.67 ± 0.45 mmol Fe^{2+}/kg_{DW} and 27.60 ± 0.32 mmol Fe^{2+}/kg_{DW}, respectively. On the other hand, the stem extracts had higher antioxidant activity than did the stem decoctions, with 19.86 ± 7.35 mmol Fe^{2+}/kg_{DW} and 12.53 ± 0.15 mmol Fe^{2+}/kg_{DW}, respectively. From these results, the leaf infusions showed better antioxidant activity than did the leaf extracts. The stem extracts and the stem decoctions had lower antioxidant activities (Table 1).

3.1.2. Total polyphenolic compounds (TPC)

The TPC of the *C. boivinianum* leaf extracts were very high compared with those of leaf infusions, with values of 805.16 ± 1.08 $mg_{GAE}/100\ g_{DW}$ and 477.87 ± 38.49 $mg_{GAE}/100\ g_{DW}$, respectively; these differences were statistically significant ($P < 0.05$). The same results were found for *C. boivinianum* stems. The TPC obtained in the stem extracts were higher than those of the stem decoctions, with values of 249.12 ± 7.11 $mg_{GAE}/100\ g_{DW}$ and 191.66 ± 14.89 $mg_{GAE}/100\ g_{DW}$, respectively. However, the stems contained lower amounts of TPC (Table 1).

Table 1
Antioxidant activity and total polyphenolic content.

	Leaf extract	Leaf infusion	Stem extract	Stem decoction
Total polyphenolic content ($mg_{GAE}/100\ g_{DW}$)	805.16 ± 1.08	477.87 ± 38.49	249.12 ± 7.11	191.66 ± 14.88
Antioxidant activity (mmol Fe^{2+}/kg_{DW})	27.60 ± 0.32	49.67 ± 0.45	19.86 ± 7.35	12.53 ± 0.15

Mean values ± standard deviation (N = 3).
GAE = gallic acid equivalents.
DW = dried weight.

Table 2
Cinnamic acids in *C. boivinianum*.

	Cinnamic acids			
	Caffeic acid	Chlorogenic acid	Coumaric acid	Ferulic acid
	(mg/100 g_{DW})	(mg/100 g_{DW})	(mg/100 g_{DW})	(mg/100 g_{DW})
Leaf extract	1.70 ± 1.80	145.69 ± 36.34	5.56 ± 4.54	1214.93 ± 272.28
Leaf infusion	13.02 ± 0.15	217.08 ± 2.89	n.d.	n.q.
Stem extract	n.d.	n.d.	n.d.	n.d.
Stem decoction	n.q.	n.d.	n.d.	n.d.

Mean values ± standard deviation (N = 3).
DW = dried weight.
n.d. = not detected.
n.q. = not quantified.

3.2. Chromatographic analysis

3.2.1. Polyphenols

Chromatographic analysis showed that the leaf extracts were rich in chlorogenic acid (145.69 ± 36.34 mg/100 g_{DW}) and ferulic acid (1214.93 ± 272.28 mg/100 g_{DW}). The leaf infusion contained chlorogenic acid and caffeic acid, and their amounts were much higher at 217.08 ± 2.89 mg/100 g_{DW} and 13.02 ± 0.15 mg/100 g_{DW} than those of leaf extracts, respectively; the difference was significant ($P < 0.05$). Cinnamic acids were not detected in the stem samples (Table 2).

All samples were poor in terms of flavonol content. Only the flavonol hyperoside was found in leaf extracts, at a concentration of 8.98 ± 2.60 mg/100 g_{DW} (Table 3).

Leaf extracts showed very high values of epicatechin and vescalagin at 1400.58 ± 180.23 mg/100 g_{DW} and 2734.90 ± 472.90 mg/100 g_{DW}, respectively. Leaf extracts were also rich in ellagic acid and castalagin, with values of 385.15 ± 112.28 mg/100 g_{DW} and 413.82 ± 102.84 mg/100 g_{DW}, respectively. These values presented statistically significant differences according to the Student t-test at $P < 0.05$. Moreover, the leaf infusions were also rich in ellagic acid, castalagin and vescalagin, which had values of 298.01 ± 91.99 mg/100 g_{DW}, 168.28 ± 87.97 mg/100 g_{DW} and 276.11 ± 141.64 mg/100 g_{DW}, respectively. The stem extracts and the stem decoctions showed lower amounts of benzoic acid, catechins and tannins (Table 4).

3.2.2. Monoterpenes

The leaf and stem extracts of *C. boivinianum* contained lower values of monoterpenes than did leaf infusions and stem decoctions. Phellandrene (82.19 ± 11.88 mg/100 g_{DW}), sabinene (210.10 ± 9.98 mg/100 g_{DW}) and terpinolene (115.37 ± 9.65 mg/100 g_{DW}) were the major monoterpenes in leaf infusions with statistically different contents compared with those of leaf extracts.

Stem decoctions showed higher values of limonene (989.56 ± 416.01 mg/100 g_{DW}) than did stem extracts (170.09 ± 34.42 mg/100 g_{DW}). Sabinene and γ-terpinene were not detected in the stem extracts, but they were identified in the decoctions, with respective values of 24.12 ± 28.37 mg/100 g_{DW} and 421.25 ± 258.00 mg/100 g_{DW}, respectively. Indeed, infusion and decoction were the best methods for the extraction of monoterpenes (Table 5).

3.2.3. Organic acids

Leaf extracts were rich in citric acid (70.89 ± 18.87 mg/100 g_{DW}) and tartaric acid (327.52 ± 99.58 mg/100 g_{DW}). On the other hand, leaf infusions were rich in quinic acid (188.62 ± 27.95 mg/100 g_{DW}), oxalic acid (61.52 ± 28.58 mg/100 g_{DW}) and succinic acid (86.44 ± 34.39 mg/100 g_{DW}). Stem extracts and stem decoctions were poor in organic acids (Table 6).

3.2.4. Vitamin C

Statistically significant differences ($P < 0.05$) in vitamin C were observed in the leaf and stem extracts, with values of 33.89 ± 1.24 mg/100 g_{DW} and 29.11 ± 0.87 mg/100 g_{DW}, respectively. The two plant materials presented high rates of vitamin C, but the stem extracts exhibited higher levels of vitamin C. On the other hand, the values of vitamin C detected in the leaf extracts were higher than the values in leaf infusions (29.11 ± 0.87 mg/100 g_{DW} and 13.32 ± 0.48 mg/100 g_{DW}, respectively). Finally, the stem decoctions showed a lower vitamin C amount than did the other extracts (3.40 ± 0.10 mg/100 g_{DW}) (Table 7).

3.3. Antimicrobial activity

Strains grew well after 24 h of incubation. Leaf and stem extracts had some antimicrobial activities. The antimicrobial activities of these two extracts were not statistically different for each strain, but the diameter of the clear zone indicated activity (Table 8). A clear zone between 8 mm and 9 mm of diameter indicated that the samples were active, and a clear zone between 9 mm and 14 mm of diameter indicated that the samples had high antimicrobial activity. Past 14 mm of diameter, the samples had very high antimicrobial activity (Andriamampianina et al., 2016).

Table 3
Flavonols in *C. boivinianum*.

	Flavonols				
	Hyperoside	Isoquercitrin	Quercetin	Quercitrin	Rutin
	(mg/100 g_{DW})	(mg/100 g_{DW})	(mg/100 g_{DW})	(mg/100 g_{DW})	(mg/100 g_{DW})
Leaf extract	8.98 ± 2.60	n.d.	n.d.	n.d.	n.d.
Leaf infusion	n.q.	n.q.	n.d.	n.d.	n.d.
Stem extract	n.d.	n.q.	n.d.	n.d.	n.d.
Stem decoction	n.d.	n.d.	n.d.	n.d.	n.d.

Mean values ± standard deviation (N = 3).
DW = dried weight.
n.d. = not detected.
n.q. = not quantified.

Table 4
Benzoic acids, catechins and tannins in *C. boivinianum*.

	Benzoic acids		Catechins		Tannins	
	Ellagic acid	Gallic acid	Catechin	Epicatechin	Castalagin	Vescalagin
	mg/100 g_{DW}	mg/100 g_{DW}	mg/100 g_{DW}	mg/100 g_{DW}	mg/100 g_{DW}	mg/100 g_{DW}
Leaf extracts	385.15 ± 112.28	4.40 ± 2.49	n.d.	1400.58 ± 180.23	413.82 ± 102.84	2734.90 ± 472.90
Leaf infusion	298.01 ± 91.99	15.19 ± 1.63	n.d.	61.69 ± 2.44	168.28 ± 87.97	276.11 ± 141.64
Stem extract	167.32 ± 34.6	n.d.	164.13 ± 1.95	24.54 ± 2.64	n.d.	111.78 ± 18.30
Stem decoction	29.42 ± 13.23	4.15 ± 0.72	1.64 ± 1.73	23.59 ± 20.78	13.90 ± 0.71	123.59 ± 16.9

Mean values ± standard deviation (N = 3).
DW = dried weight.
n.d. = not detected.
n.q. = not quantified.

Table 5
Monoterpenes in *C. boivinianum*.

	Monoterpenes				
	Limonene	Phellandrene	Sabinene	γ-Terpinene	Terpinolene
	mg/100 g_{DW}	mg/100 g_{DW}	mg/100 g_{DW}	mg/100 g_{DW}	mg/100 g_{DW}
Leaf extract	n.d.	55.53 ± 11.01	n.q.	n.d.	58.38 ± 24.59
Leaf infusion	n.q.	82.19 ± 11.88	210.10 ± 9.98	n.q.	115.37 ± 9.65
Stem extract	170.09 ± 34.42	25.17 ± 3.17	n.q.	n.d.	39.03 ± 1.71
Stem decoction	989.56 ± 416.01	92.01 ± 70.27	24.12 ± 28.37	421.25 ± 258.00	28.50 ± 1.34

Mean values ± standard deviation (N = 3).
DW = dried weight.
n.d. = not detected.
n.q. = not quantified.

For *S. typhii*, leaf and stem extracts resulted in a clear zone diameter of 15.50 ± 0.70 mm and 11.75 ± 1.76 mm, respectively. A clear zone larger than 9 mm in diameter indicated that the samples had high antimicrobial activity. Chloramphenicol had very strong antimicrobial activity, resulting in approximately 31.00 ± 2.82 mm of clear zone diameter (Fig. 6a).

Regarding *E. coli*, the antimicrobial activity was positive but lower than that of *S. typhii* (Fig. 6b). The clear zone was 9.00 ± 0.70 mm in diameter from the leaf extract and 8.00 ± 0.70 mm from the stem extract. Chloramphenicol had a smaller clear zone diameter of 8.00 ± 0.70 mm.

For *C. albicans*, leaf extracts had higher antimicrobial activity than did stem extracts. The clear zone diameter was 9.50 ± 0.70 mm from the leaf extract and 7.75 ± 0.35 mm from the stem extract (Fig. 6c).

4. Discussion

The leaf extracts of *C. boivinianum* contained very high TPC (805.16 ± 1.08 mg_{GAE}/100 g_{DW}). Approximately twice the TPC in leaf infusions (477.87 ± 38.49 mg_{GAE}/100 g_{DW}) and more than four times the TPC in the decoctions (191.66 ± 14.88 mg_{GAE}/100 g_{DW}) were found in the leaf extracts. This finding is due to the richness in chlorogenic acid (145.69 ± 36.34 mg/100 g_{DW}) and ferulic acid (1214.93 ± 272.28 mg/100 g_{DW}) in the leaf extracts.

Leaf infusions also had a higher amount of TPC (477.87 ± 38.49 mg_{GAE}/100 g_{DW}) than stem extracts and stem decoctions. This richness of TPC in the leaf infusions is characterised by the presence of high rates of chlorogenic acid and caffeic acid, with values of 217.08 ± 2.89 mg_{GAE}/100 g_{DW} and 13.02 ± 0.15 mg_{GAE}/100 g_{DW}, respectively. The leaf infusions showed higher antioxidant activity than did leaf extracts, even if the latter presented a high rate of TPC: the high rates of chlorogenic acid and caffeic acid in the leaf infusions influenced antioxidant activity more than ferulic acid content did in the leaf extracts. Chlorogenic acid is a thermally stable compound and has a very high antioxidant activity (Agar et al., 2015; Uchida et al., 2017). Also, caffeic acid has strong antioxidant properties and is present in higher amounts in leaf infusions than in leaf extracts (Marinova et al., 2009). For this reason, leaf infusions had higher antioxidant activity than the others. Polyphenols are among the compounds synthesised by plants. They consist of an aromatic nucleus with several hydroxyl functions and have better antioxidant properties (Chen et al., 2004; Ghedadba et al., 2015). According to the FRAP method, the leaf infusions showed a significant reduction in ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), which is represented by higher antioxidant activity (49.67 ± 0.45 mmol

Table 6
Organic acids in *C. boivinianum*.

	Organic acids					
	Citric acid mg/100 g_{DW}	Malic acid mg/100 g_{DW}	Oxalic acid mg/100 g_{DW}	Quinic acid mg/100 g_{DW}	Succinic acid mg/100 g_{DW}	Tartaric acid mg/100 g_{DW}
Leaf extract	70.89 ± 18.87	n.d.	n.d.	25.15 ± 3.24	48.98 ± 10.52	327.52 ± 99.58
Leaf infusion	4.95 ± 1.19	16.36 ± 1.20	61.52 ± 28.58	188.62 ± 27.95	86.44 ± 34.39	3.84 ± 0.55
Stem extract	n.q.	n.q.	n.d.	15.31 ± 7.67	11.88 ± 0.33	25.31 ± 2.67
Stem decoction	n.q.	2.69 ± 0.50	15.20 ± 1.69	46.33 ± 15.69	3.77 ± 2.17	n.d.

Mean values ± standard deviation (N = 3).
n.d. = not detected.
n.q. = not quantified.

Table 7
Vitamin C in *C. boivinianum*.

	Ascorbic acid (AA)	Dehydroascorbic acid (DHAA)	Vitamin C (AA + DHAA)
	(mg/100 g_{DW})	(mg/100 g_{DW})	(mg/100 g_{DW})
Leaf extract	26.61 ± 1.02	2.5 ± 0.17	29.11 ± 0.87
Leaf infusion	6.91 ± 0.48	6.41 ± 0.008	13.32 ± 0.48
Stem extract	30.54 ± 1.25	3.35 ± 0.01	33.89 ± 1.24
Stem decoction	1.78 ± 0.11	1.62 ± 0.01	3.4 ± 0.1

Mean values ± standard deviation (N = 3).
n.d. = not detected.
n.q. = not quantified.

Fe^{2+}/kg_{DW}) than that in the leaf extracts (27.60 ± 0.32 mmol Fe^{2+}/kg_{DW}) and in the stem extracts (19.86 ± 7.35 mmol Fe^{2+}/kg_{DW}). This higher antioxidant activity could be due to high rates of chlorogenic acid and caffeic acid from the leaf infusions of *C. boivinianum*. Chlorogenic acid is a compound that has high antioxidant activity, which is exploited for the pharmaceutical industry (Uchida et al., 2017). In addition, the leaf infusions were also rich in gallic acid, a bioactive compound recognised for its high antioxidant activity (Tsala et al., 2015). The leaf infusions could therefore also be rich in anthocyanins, which are compounds very rich in antioxidant activity (Azevedo et al., 2010).

If these results are compared with those of *Catharanthus roseus*, which is a highly recognised medicinal plant in Madagascar used in the treatment of cancer, the TPC of leaf extracts were similar to the TPC of methanolic extracts of *C. roseus* at 959.00 ± 11.5 $mg_{GAE}/100\ g_{FW}$. Also, the TPC of leaf infusions were higher than the TPC of ethanolic extracts of *C. roseus* at 270.00 ± 2.4 $mg_{GAE}/100\ g_{FW}$ (Pham et al., 2017).

Regarding the methods, extraction with solvent consisting of methanol, water and hydrochloric acid and coupled with maceration (for a few days) was an effective protocol for the extraction of TPC. The use of this extraction system for nutraceutical analysis of fruits in Italy showed reliable results for TPC (Donno et al., 2012). This solvent was ideal for the extraction of polyphenols from the leaves of *C. boivinianum*. However, leaf infusions showed the best antioxidant activity. Only 5 g of plant material in leaf infusions presented a similar optical density to 10 g of plant material in leaf extracts, with respective values of 2.56 ± 0.02 and 2.85 ± 0.03. For this reason, leaf infusion was the best method for extracting bioactive compounds from medicinal plants.

According to the other studies and ethnobotanical surveys, the local population in Madagascar use the leaves of *C. boivinianum* to treat diseases such as fever and muscle pain (Randrianarivelojosia et al., 2003; Rabearivony et al., 2015). The leaves of *C. boivinianum* are rich in biological compounds, particularly polyphenols, including benzoic acid, catechins and tannins. Moreover, leaf infusion was the best method for extracting compounds with high antioxidant activity and other health-promoting properties.

Leaf extracts and leaf infusions were rich in tannins, represented by castalagin and vescalagin. These compounds have a high astringent property widely used for wound healing (Blazso et al., 2004). For this reason, the local population in Madagascar uses the leaves to treat wounds and/or scorpion bites (Rindraniaina, 2012).

Regarding stem extracts of *C. boivinianum*, despite being poor in polyphenols, the antioxidant activity of the stems showed mildly low values because the activity was influenced by vitamin C content. On the other hand, stem decoctions showed a low antioxidant activity because they were poor both in polyphenols and vitamin C. Indeed, the latter was sensitive to thermal shock during the decoction for 20 min at 100 °C (Massot, 2010).

Leaf and stem extracts had high antimicrobial activities against *S. typhii*. A clear zone diameter between 9 and 14 mm indicated that the extracts were very active and that the strain was sensitive (Andriamampianina et al., 2016). Leaf extracts had very high antimicrobial activity because the clear zone was greater than 14 mm in diameter. This activity was influenced by polyphenols found in the leaves of *C. boivinianum*, with values of 805.16 ± 1.08 $mg_{GAE}/100\ g_{DW}$. *Salmonella typhii* is a gram-positive bacterium; the plasma membrane is covered by a thick peptidoglycan wall, and there is no outer membrane, which makes the bacterium susceptible to phenolic compounds. Chlorogenic acid and caffeic acid in the leaf extract were responsible for the antimicrobial activity (Ghimire et al., 2017). Catechin concentrations were high in the leaf extracts (1400.58 ± 180.23 $mg_{GAE}/100\ g_{DW}$) and had high antimicrobial activity (Wahid et al., 2016). The stem extracts of *C. boivinianum* contained some polyphenols (249.12 ± 7.11 $mg_{GAE}/100\ g_{DW}$), and these polyphenols exhibited antimicrobial activity. Chloramphenicol was a positive control that had very high antimicrobial activity, as it was a reference product and was pure.

For `E. coli`, the antimicrobial activity of the samples was positive but lower than that of *S. typhii*. `Escherichia coli` showed low antimicrobial activity because the diameter of the clear zone was less than 9 mm (Andriamampianina et al., 2016). This low activity is caused by the gram-negative bacterial membrane structure. Gram-negative bacteria have an additional layer independent of the cell membrane called the outer membrane, which consists of phospholipids, proteins and lipopolysaccharides. This membrane structure is impermeable to most molecules (Ghedadba et al., 2015), which explains why the clear zone of chloramphenicol also decreased. `Candida albicans` was susceptible to the leaf extract. It was rich in compounds (chlorogenic acid, caffeic acid and catechins) that can inhibit strain growth (Ghimire et al., 2017).

Table 8
Antimicrobial activities of *C. boivinianum*.

	Salmonella typhii (mm)	*Escherichia coli* (mm)	*Candida albicans* (mm)
Leaf extract	15.5 ± 0.70	9.00 ± 0.70	9.50 ± 0.70
Stem extract	11.75 ± 1.76	8.00 ± 0.70	7.75 ± 0.35
Chloramphenicol	31.00 ± 2.82	8.00 ± 0.70	21.50 ± 2.12

Mean values ± standard deviation (N = 3).

5. Conclusion

Chrysophyllum boivinianum is widely used by the local population to treat many diseases in Madagascar. This study aimed to determine the phenolic and organic compounds in the leaves and stems of *C. boivinianum* in relation to their biological activities and local uses. Leaf extracts and leaf infusions were richer in polyphenols and organic compounds than were stem extracts and decoctions. Regardless of the extraction method used, leaves were rich in polyphenols such as chlorogenic acid, caffeic acid and ferulic acid as well as organic acids, catechins and tannins. In contrast, stems were rich in monoterpenes. Leaf infusion was the best technique for extracting bioactive compounds. For these reasons, the local population uses leaves to treat some diseases. Leaf extracts had much higher antimicrobial activity than did stem extracts for all strains. The stems of *C. boivinianum* showed low rates of polyphenols, which is related to the quality and durability of the wood. Wood that is poor in polyphenols is sensitive to fungal attack, as confirmed by the properties of *C. boivinianum* wood.

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