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Phytochemical study of *Barteria fustolisa mast* bark traditionally used against infantile colics by local population in Gabon

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ABSTRACT

Barteria fistulosa mast is a tree found from western Nigeria to the Equatorial forest of Central Africa, which bark is empirically used in Gabon by local population to relieve infantile colics. This study consists of a phytochemical analysis of ethanolic and aqueous extracts of *Barteria fistulosa mast* bark from Gabon. We have identified secondary metabolites present in the extracts of the bark. The results of phytochemical screening revealed the presence of alkaloids, polyphenols, flavonoids, and saponins in the different extracts. The results of quantitative analysis showed that aqueous extract is more concentrated (127.1 ± 10.1mg GAE/100g) in total polyphenonols than ethanolic extract (35.7 ± 6.80 mg GAE/100g) of dry matter. Concerning total flavonoids, ethanolic extract shows a concentration of 4.99 ± 0.08 mg QE/100g of dry matter, while for the aqueous extract we note no quantitative detection by the Genesis10 Uv–Vis spectrophotometer. Finally, the presence of several groups of active molecules would justify its use in traditional medicine, particulary against infantile colics in Gabon.

Introduction

Gabon is covered by 80 % of forest, with a diversity of plants used in traditional medicine (Raponda-Walker, 1995). The knowledge and the application of these potentially therapeutic plants is important in Africa and in Gabon in particular. This study focuses on the mastery and valorisation of medicinal plants, with a specific concern regarding *Barteria fistulosa mast*, which bark maceration is empirically used to relieve infantile colics by local population in Gabon. Infantile colics are defined as paroxysms, irritability, agitation and crying of babies, lasting more than three hours a day and occuring more than three days a week (Silvia Salvatore et *al*, 2016).. These are thought to be due to spasms of the intestine and tend to occur in the first trimester after birth (Ferrer et al, 2000). This period of colics appears very difficult for babies who cry abnormally and for parents whose sleep is often severly disrupted, with a risk of postpartum depression and an altered bond between them and babies. Unfortunately, as painful as colics may be for babies and their parents, there is currently no modern medical treatment for that. As the trouble is not clearly defined, there are no international recommendation to guide its management (Bruvas-Bertholon et al, 2012). No medication has been proven to be effective and some, such as simethicone or dicyclomine which were previously used, are not recommended because of their side effects (Bellaiche, 2009). Thus, as infantile colics do not benefit of formal medical treatment, traditional or empirical approaches are often use to try to relieve the babies and to reassure parents. In the western word, probiotics or food supplements containing bacteria, constitute an approach to relieve babies suffering from colics. They are the focus of more studies and we know that they can modify the intestinal flora of children and reduce the intestinal inflammation, potentially involved in babies' pain. Indeed, clinical studies have shown that the intake of probiotics such as *Lactobacillus reuteri* reduced crying in babies whit colics (Sung et al, 2012). Similary, fennel based herbal teas, a plant found mainly in temperate or in mediterranean basin, would also be a solution to colics and gas for children. Few studies have shown the superiority of fennel extract over placebo in reducing babies' crying (Perry et *al*, 2011), whether used alone or in tea containing chamomile, verbena, liquorice and lemon balm, up to three times a day at 150 mL per dose (Weizman et al, 1993).

Gabon, a country located in central Africa where the above mentioned species are not found, some parents in distress regarding infantile colics have recours to *Barteria fistulosa mast* bark maceration. Local users reveal that taking this maceration causes the affected babies to have abundan feces in less than 24 hours; with result that crying stops, followed by sleep as to express the babies relief. However, to the best of our knowledge, no scientific study has been conducted to confront these assertions from local users.

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Nevertheless, the World Health Organisation estimates that 40 % of medicine have natural substance as active ingredient and about 80% of the worlds' population uses plants to treat themselves (WHO, 2022).

In view of the above mentioned, the purpose of this study is the phytochemical screening of ethanolic and aqueous extracts from *Barteria fistulosa mast* bark, followed by the quantitative analysis of total polyphenols and flavonoids in the extracts of interest. In order to establish a state of knowledge on the main secondary metabolites present in *Barteria fistulosa mast* bark from Gabon, with potentially therapeutic benefits against infantile colics. This, with the concern to preserve the empirical knowledge in traditional medicine from Africa and to contribute to the regulation of the modes of consumption, with a scientist proven, effectiveness and an optimal acceptability of *Barteria fistulosa mast* bark against infantile colics.

Materials and Methods

Barteria fistulosa mast

Barteria fistulosa mast is a hollow-trunked tree of *Passifloraceae* family and genus *Barteria*, which can grow up to 15 m high (Keay, 1954). It lives in symbiosis with ants, so it is also called ants tree (Figure 1). In fact, its hollow branches house the species *Tetraponera aethiops*, aggressive ants that in return defend the tree against herbivores by dropping onto these predators and stinging them; stings that can cause fainting and death.



Figure 1: Tree and leaves of *Barteria fistulosa mast (Collected from online)*

In addition, *Barteria fistulosa mast* is found from western Nigeria to the Equatorial forest of central Africa: Cameroon, Central Africa Republic, Gabon, Congo and towards the south of the Democratic Republic of Congo (Keay, 1954). Its roots, stems and leaves have been used for traditional medicine (Gassita et al, 1982; Akendengué and Louis, 1994; Breteler, 1999).

Bark collection

The barks of *Barteria fistulosa mast* were collected at the beginning of the dry season (May 2021) at breath height (2 m) from the ground in the commune of Ntoum in the Estuary Province of Gabon. The plant was authenticated in the National Herbarium of Gabon at the Institute of Pharmacopeia and the Traditional Medicine (IPHAMETRA), in Libreville (Gabon). After air drying, the bark was ground into a fine powder using a Retsch type electric grinder, and sieved to collect only 1-2 millimeter pellets, in accordance with ASTM n° 1105 (1996) in terms of granulometry for quantifying the content of wood extracts prior to the chemical analysis.

Extraction by maceration

Extraction method using maceration has been applied. More precisely, 10 g of powder was transferred to a 250 mL Erlenmeyer flask, then 100 mL of extract solvent was added and a bar magnet was used to homogenise the mixture. Then the Erlenmeyer flask was sealed with glass stopper and completely covered with aluminium foil to prevent possible degradation of light sensitive molecules. The mixture obtained was agitated for 24 h using a Pierron type stirrer. Then filtered under vacuum using a suction pump, Büchner funnel and a whatman filter no.1. The ethanol solvent was evaporation under vacuum using a rotary evaporator, while the aqueous filtrate was concentrate by lyophylisation. The extracts were then dried under vacuum in a desiccators in the presence of P_2O_5 and weighed regularly until constant mass. Each extraction was carried out in triplicate.

Phytochemical screening tests

Phytochemical screening allowing qualitative analysis of secondary metabolites, was based either on the formation of coloured complexes using coloring reaction, or on the formation of insoluble complexes obtained by precipitation (Badiaga, 2011). The different tests applied in this study were carried out in triplicate.

The detection of Polyphenols was achieved by adding a drop of 10% aqueous iron perchloride solution in 2 mL of extracts solution(1 g/L) involving an intense blackish color appearance.

Flavonoids presence determination was done by dissolution of 2 mg of extracts in 2mL of 95% ethanol with a few drops of hydrochloric acid and 0.5 g of magnesium chips, leading to a cherry pink color taken by the solution to indicate flavonoids presence or not.

Alkaloids were detected as follow: 20 mg of extracts and 10 mL of a dilute 10% sulfuric acid solution were poured into a test tube. The mixture was strong stirred for two minutes and some drops of Mayer's reagent were then added. The appearance of a yellowish precipitate was characteristic of the presence of alkaloids.

The Saponins were identified by mixing 50 mg of extracts with 30 mL of distilled water in a water bath at 30°C for 5 minutes. After cooling, 10 mL of this solution were introduced into a test tube and vigorously vortex-shaken for 10 seconds. The presence of 1 cm thick persistent foam indicates the presence of saponins.

Sterols and Terpenes detection was carried out by mixing 20 mg of extracts, 2 drops of oleum,10 drops of acetic anhydride into 3 mL of chloroform causing the appearance of a purple ring, turning blue and then green in the test tube.

Total polyphenols content

The quantitative determination of total phenols was carried out using the Folin-Ciocalteu colorimetric method (Nsi et al, 2013) with a slight modification. First, a six point Gallic acid calibration curve (0 - 200 mg/L) was performed. The different extracts were dissolved in methanol. To carry out the assay, 0.5 mL of solution of extract dissolved in methanol and 2.5mL of Folin-Ciocalteu reagent (diluted 10 times in distilled water) were successively introduced into a test tube. Then, 30 seconds to 8 minutes after adding the Folin-Ciocalteu reagent, 2 mL of sodium carbonate Na_2CO_3 (0.7 M) were added. A reaction blank containing no phenolic compounds is also produced. The reaction mixtures are stirred and incubated for 5 min at 50°C in a water bath. After this reaction time, all the samples were transferred to a cold water bath. The reaction mixtures are centrifuged at 4 rpm for 10 minutes. Then using a UV-Visible spectrophotometer (Genesys 10 Uv~~S~~) the absorbance is measured at 765 nm. The test was carried out in triplicate. The content of total phenols determined by means of the Gallic acid calibration curve (0,011x + 0,029; r^2 = 0.9997) by calculating the average concentration polyphenols present in extracts in mg equivalent of gallic acid/100g of dry extract.

Total flavonoids

The flavonoid assay was carried out using the aluminum chloride colorimetric method described by Arvouet – Grant et al (1994) with small modifications. First, a six points quercetin calibration curve was performed. The different extracts were then diluted in methanol. To carry out the assay, 0.5 mL of the extract solution diluted in methanol, 2 mL of distilled water and 1 mL of aluminum chloride (10%) were successively introduced into a test tube, and leaved for 6 minutes. Then, 1 mL of 1 M potassium acetate was added. After incubation at room temperature for 30 minutes, the absorbance of all the reaction mixtures corresponding to each concentration in the calibration curve and the samples were measured at 415 nm using a UV-visible spectrophotometer (Genesys 10 UV~~S~~).The test was carried out in triplicate and the flavonoid content determined using the quercetin calibration curve (0,025x + 0,043;r^2 = 0.9980) by calculating the average concentration of flavonoids present in extracts in mg equivalent of quercetin/100 mg of dry extract.

Statistical analysis

The data were expressed as Mean ± Standard Deviation (SD) of three determinations. Statistical analysis (ANOVA with a statistical significance level set at $p<0.05$ and linear regression) was carried out with Microsoft Excel.

Results

Phytochemical screening

Sterols and terpenes were not detected in ethanol extracts and water (Table 1). Alkaloids, polyphenols, flavonoids and saponins were detected in all extracts (Table 1).

Total phenols contents vary from one solvent to another (Table 2). The aqueous extract is more concentrated in total polyphenols than the ethanolic extract, with 127.1 ± 10.1 mg EGA/100g of dry extract, against 35.5 ± 6.80 mg EGA/100g of dry extract respectively. The total flavonoids quantification was not detected in water extract by the Genesys 10 UV-Vis spectrophotometer (down the

quantification limit). While for total flavonoids, the ethanolic extract shows a low concentration of about 4.99 ± 0.08 mg QE/100 g of dry extract.

Table 1: Phytochemical screening of the main groups in *Barteria fistulosa mast* bark

Active compounds	Test procedure	Extraction solvent[a]	
		Ethanol	Water
Alkaloids	Mayer's reagent	+	+
Flavonoids	Shinoda test	+	+
Polyphenols	Iron (III) chloride	+	+
Saponins	Aphrogenic power	+	+
Sterols and terpenes	Liebermann-Bouchard	-	-

+/− = presence/absence of the groups

Table 2: Total polyphenols and flavonoids contents in the different extracts of *Barteria fistulosa mast* bark

	Extraction solvent	
	Ethanol	Water
Total phenols (mg EGA/100g)[a]	35,7 ± 6,80[c]	127,1 ± 10,1[c]
Total flavonoids(mg EQ/100g)[b]	4,99 ± 0,08[c]	Nd

[a]mg EGA/100g: Milligram equivalent of gallic acid per 100 gram of dry extrac.
[b]mg EQ/100g:Milligram equivalent of quercetin per 100 gram of dry extract.
[c]Mean value of three replicates ± standard deviation.
Nd: not detected

Discussion

The phytochemical are known to have potential antioxidant, antimicrobial, anti-inflammatory and antifungal activities (Breteler, 1999; Bruneton, 1999 ; Schmelzer, 2008; Bafor, 2009; Djakpo, 2010; Goyal, 2013; Katarzyna, 2021 and Wang, 2022). Alkaloids are known to play an important role in protecting trees from predatory attacks by others organisms such as bacteria, fungi, insects and help their own survival in the ecosystem (Goyal, 2013). Polyphenols are active against viruses and bacteria (Bruneton, 1999).

Alkaloids have insecticidal, antifungal and antibacterial properties (Bruneton, 1999). Tannins are used for thier antiseptic and bactericidal properties and as antidiarrheal agents (Djakpo, 2010; Wang, 2022). Breteler et al. (1999) reveal that the stem bark, roots and leaves of *Barteria fistulosa mast* are believed to treat fever, intestinal and back pain. The potential therapeutic activities of the secondary metabolites detected in *Barteria fistulosa mast* bark from Gabon, would justify the ethnobotanical success of the bark of this tree in the empirical treatment of infantile colics, by local population in Gabon

The highest contents of phenolic compounds were obtained with the water extracts and the lowest with the ethanol extracts. This is easily explained by the polarity of the solvent. The higher polarity of water allowing to extract higher polarity compounds, while ethanol is known to extract mainly phenolic compounds (Brennan et al, 2020). Finally, the concentrations of total polyphenols and flavonoids resulting from extracts of *Barteria fistulosa mast* bark from Gabon obtained in this study are higher, in comparison to some extracts of bark from other medicinal plants such as *Embella ribes Burm.f* (26.59 mg GEA/100g ; 01.35 mg QE/100g), *Gmelina arborea Roxb* (29.43 mg GEA/100g ; 02.65 mg QE/100g) and *Ficus racemosa* L (12.36 mg GEA/100g ; 0.86 mg QE/100g) (Sulaiman and Balachandran, 2012).

Conclusion

This study was performed by motivation from the observation in our daily environment of the potential benefit of *Barteria fistulosa mast* bark from Gabon against infantile colics.. We focused on the phytochemical screening of aqueous and ethanolic extracts of the bark in order to know the secondary metabolites present in the bark of *Barteria fistulosa mast*. The phytochemical screening revealed the presence of polyphenols, alkaloids, saponins, flavonoids coumpounds. In addition, the results revealed higher concentrations of polyphenols in the aqueous extract compared to those of the ethanolic extract. As for total flavonoids, they are more concentrated in the ethanolic extract and their detection in the aqueous extract is below the detection limit of the Genesys 10 UV-Visible spectrophotometer. Further studies are needed to-

Test the antioxidant, antibacterial and antifungal potential of *Barteria fistulosa mast* bark extracts, regarding families of secondary metabolites indentified in this study

Characterize and isolate the potebtial active molecules present in *Barteria fistulosa mast* bark, then évaluate the toxicity of extracts from this bark;

Initiate rigorous blinded clinical trials of infantile colics, requiring collaboration with clinical

paediatricians and families among others; with specific aim of studying behaviour of newborns afected by colic, befor, during and after the *Barteria fistulosa mast* and placebo trials.

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