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Fractionation and bioassay-guided isolation of Loganin from the bark of *Breonia perrieri* Homolle, an endemic Rubiaceae from Madagascar

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Abstract
For the valorization of endemic plants of Madagascar's flora, the study of plant *Breonia perrieri* (Rubiaceae) was performed. This study aimed to assess the potential antioxidant and antimicrobial activities from the bark of *Breonia perrieri*. The methanolic crude extract, the fractions resulting from the fractionation of the methanolic extract and the isolated compound from the active extract were tested. The antimicrobial activities were evaluated by disc diffusion method and microdilution technic and the potential antioxidant by the method of DPPH assay. *Streptococcus pneumonia* is the most sensitive bacterium tested with crude methanolic extract and soluble ethyl acetat (IZD = 11mm, MIC = 250 µg/mL). Soluble ethyl acetat showed the most powerful extract in scavenging ability on DPPH free radicals (IC_{50} = 5.43 ± 0.15µg/mL). The bioassay-guided of the ethyl acetate extract resulted in the isolation of iridoid glycoside namely Loganin. Its structure was established by spectroscopic methods. This compound exhibited strong DPPH scavenging activity with IC_{50} value of 1.50 µg/mL and showed antibacterial activity against *Streptococcus pneumonia* (IZD = 10 ± 0.5 mm). This is the first work of chemical and biological studies on *B. perrieri.*

Keywords: *Rubiaceae, Breonia perrieri*, bark extracts, Loganin, antioxidant, antibacterial

1. Introduction
The use of natural products as therapeutic agents has become increasingly popular. The World Health Organization (WHO) estimates that up to 80% of the world's developing countries depends on locally available plant resources for their primary healthcare because they are easily accessible and less expensive [1]. The Rubiaceae family has played a significant role in drug discovery by providing molecules used as templates for the development of new drug such as iridoids, indole alkaloids, anthraquinones, terpenoids (diterpenes and triterpenes), flavonoids and other phenolic derivatives [2, 3]. The genus *Breonia* is endemic to Madagascar, includes some larges trees attaining 30 m, and occupies a wide habitat ranging from eastern rainforest to western deciduous dry forest [4, 5]. Razafimandimbison recognizes 20 species in this genus including eight new species [6]. *B. perrieri* has various local uses such as for construction, carpentry and furniture, for making boats and handicrafts and for general construction purposes because of its high quality wood [7, 8]. The pulp of infructescence is edible. This plant has not been intensively studied, to determine their biological characteristics, which are the reason of the lack of informations about his medicinal virtues [9, 10]. Unfortunately, *B. perrieri* is actually endangered because of rapid deforestation, slash and burn cultivation that affect plants habitat in the Western part of the Island [11, 12]. In fact, this research was conducted for the first time, with the aims to understand the importance of plant species as remedies and to document the knowledge on their uses by local population.

2. Materials and Methods
2.1 General
Silica gel (Macherey Nagel, 70-270 mesh) was used for column chromatography and PTLC and Silica gel 60F254 was used for analytical TLC, which was viewed by UV illumination at 254 and 366 ηm and by spraying with vanillin spray reagent [13].

2.2 Plant material and description

The bark of *Breonia perrieri* was collected on April 2018 in the New Protected Area Mahavavy Kinkony Complex at Analamanitra, in the Boeny region of Madagascar latitude 16° 03' 03.7''S and longitude 045° 48' 33.0''E. The species was later identified by the botanist at the department of Botany and Ethnobotany of the National Center of Applied Pharmaceutical Research (CNARP), Antananarivo, Madagascar. A voucher specimen (RFM35-BK1) has been also deposited in the Herbarium.

B. perrieri belongs to the Rubiaceae family with common names Valotsy, Valotra [15]. It is a deciduous small tree up to 15-25 m tall. The bark surfaces are longitudinally fissured and grey. The leaves are decussately opposite, simple and entire. The inflorescence is solitary, axillary and globose head 2.5-3cm in diameter. The peduncle length is 2.5 -3.5cm with node near apex [16].

The plant is characterized by flowers bisexual, fruit berry and seeds ellipsoid. *B. perrieri* flowers in November–January and bears fruits in January–March. The tree loses its leaves in the dry season and new leaves appear after the start of the rainy season.

2.3 Extraction and isolation

Dried barks of *B. perrieri* were reduced to a fine powder with a mechanical grinder. The powdered plant material (200 g) was extracted by maceration with methanol (1800 ml) at room temperature for 24 x 3 hours. After concentration under reduced pressure, the methanolic extract (23 g) was suspended in hot water and then partitioned sequentially using hexane and ethyl acetate furnishing respectively hexanic, ethyl acetate and aqueous extract. These extracts were used for assessment of antioxidant and antibacterial activities and the active extract was selected to subsequent separation.

The ethyl acetate extract (2.79 g) was chromatographed on a silica gel column (11.64 g), eluting successively with a gradient solvent system of hexane- DCM (100:0 → 0:100) and a solvent system of DCM-methanol (100:0 → 95:5); 850 aliquots of 10 ml each were collected and analyzed by thin layer chromatography (TLC). Five microliters of aliquots were deposited on TLC plates and eluted with mobile phase DCM-methanol (99:1).

The plates were dried and separated compounds were detected under UV lamp and by spraying with freshly prepared vanillin spray reagent and then heated at 110 °C for 1 min. The spots exhibiting the same R*f* on TLC were combined. All the fractions collected were used for assessment of antioxidant and antibacterial activities.

The fraction G_{10} obtained with DCM-methanol (98:2) was subjected to Preparative Thin Layer Chromatography (PTLC) with mobile phase DCM-methanol (95:5) to give a pure product. Its structure was elucidated by NMR spectral data (1D- and 2D-NMR). This product was also used for assessment of antioxidant and antibacterial activities.

2.4 Phytochemical Analysis

The reactions of the detection of chemical groups were carried out using standard methods as developed by Fong *et al*. (1977) [17] and Marini-Bettolo *et al*. (1981) [18].

2.5 Spectral analysis

The NMR spectra were recorded at 400 MHz for ^{1}H and 2D experiments on a Bruker Avance III 400 spectrometer and 75 MHz for ^{13}C on a Bruker Avance 300 spectrometer, using Bruker pulse programs. NMR Fourier transform, integration and peak picking were done with Bruker Top Spin software version 3.2 or with MestReNova software version 6. Chemical shifts are reported in ppm, coupling constants (*J*) in Hertz.

2.6 Pharmacological experiments

2.6.1 Experimental animals

Male Swiss mice (6-8 weeks) were used for acute toxicity study. They were provided by IMVAVET (Institut Malgache des Vaccins Vétérinaires) and were acclimatized to laboratory conditions for 3 days before the experiments were conducted.

2.6.2 Acute toxicity

The acute toxicity study of the bark of *B. perrieri* was carried out according to the guidelines (423, 425) of the Organization for Economic Co-operation and Development (OCDE) [19, 20]. Thirty male Swiss were randomly divided into ten groups of three mice and deprived of food for 18 h. Group 1 received 1 mL/100 g of distilled water while Group 2 until Group 10 received 250, 500, 750, 1000, 1500, 2000, 2500, 3000 and 4000 mg/Kg of crude methanolic extract respectively. The mortality and findings including variation in color of skin, membrane, and pupils of eyes were recorded within the first 30 min, during the sixth hour, thereafter every one hour for 24 h and later daily for 3 days [21-23]. The main observation like body postures, movements, rearing, tremors, absorbance were observed and additionally the result of dose on pain, touch response, righting reflex, and so on were recorded. The median lethal dose (LD_{50}) of the crude extract was calculated as the geometric mean of the dose that did not produce mortality and the highest dose that produced mortality where there was mortality but estimated to be above the highest dose exposure if no mortality was recorded [24]. Changes in body weights monitored during the experiment were also used as index of toxicity [25].

2.6.3 Antioxidant properties

The antioxidant activity of the bark extract was determined by the method of DPPH assay, based on the degradation of DPPH radical (2, 2 Diphenyl-1Picrylhydrazyl) [26] with some modifications. The addition of antioxidant reduces the DPPH radical and causes the mixture to discolor [27].

2.6.3.1 Qualitative evaluation of the free-radical scavenging

The qualitative assay was performed according to the method of Takao *et al*. (1994) [28] and Ruiz-Terán *et al*. (2008) [29]. Two milligrams of the dried extracts or compound were diluted with 1 mL of the methanol then, 20 (µL) aliquot of each dilution of the extracts or compound was carefully loaded individually onto the baseline of the TLC plates (20 cm x 10 cm) and the sample was allowed to dry. DCM-methanol (95:5) was used as mobile phases. Once dried, the plate was sprayed with a 25% solution of radical-DPPH in methanol. Compounds with radical-scavenging activity showed a yellow-on-purple spot due to the discoloration of DPPH.

2.6.3.2 Quantitative evaluation of the free-radical scavenging

The antiradical activity of the plant extract samples was evaluated using a spectrophotometer, via Popovici *et al*. (2009) [30] method with slight modifications. A solution of DPPH 4.5% in methanol was made, and then, 3.800 mL of this solution was blended with 0.200 mL of the plant extract

samples in methanol including 3.125- 50 µg/mL of the sample. The reaction mixtures were placed for 30 min in the dark at room temperature. The mixtures absorbance was recorded spectrophotometrically at 517 nm [31-33]. α-tocopherol (3.125 µg – 50 µg) was used as positive control. The capability to scavenge DPPH radical was determined using the following formula:

$$\% \text{ inhibition} = [1 - (A_x / A_c)] \times 100$$ [30, 34, 35]

A_x is the absorbance of DPPH radical in the presence of the sample and A_c is the absorbance of DPPH (control solution).

DPPH scavenging activity of extract was expressed by the concentration providing 50% of inhibition (IC_{50}) [36]. The IC_{50} value was calculated by linear regression equation between the percentage of inhibition and the concentration of the sample.
As cited by Abas *et al*. (2006) [37] and Ahmad *et al*. (2010) [38] extract with an IC_{50} value of less than 30 µg/ml, between 30 - 100 µg/ml and more than 100 µg/ml is considered to possess strong, moderate, and weak free radical scavenging activity, respectively [37, 38]. Each sample was measured in triplicate and the mean value of absorbance was obtained.

2.6.4 Antibacterial activity
The *in vitro* antibacterial activity of the plant extracts was qualitatively and quantitatively assessed by the presence or absence of inhibition zones, zone diameters and Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values.

2.6.4.1 Microorganism strains
The microorganisms used in this study included 13 strains of pathogen microorganisms: six Gram-positive (*Staphylococcus aureus* ATCC 11632, *Bacillus cereus* LMG 6910, *Listeria monocytogenes* ATCC 19114TM, *Streptococcus pyogenes* ATCC 19615, *Streptococcus pneumoniae* ATCC 6305), six Gram- negative (*Salmonella enterica Subsp* ATCC 1376, *Shigella flexnerii* ATCC 12022, *Proteus mirabilis* ATCC 35659, *Yersinia enterocolitica* ATCC 23715, *Enterobacter cloacae* ATCC 13047, *Enterobacter aerogenes* ATCC 13048) and one yeast (*Candida albicans* ATCC 10231). These microorganisms were provided from the collection of the Microbiology Unit of CNARP. They were maintained on agar slant at 4 °C before cultured on a fresh appropriate agar plate during 24 h prior to antimicrobial tests.

2.6.4.2 Disc diffusion method
The *in vitro* antimicrobial activity of the plant extract samples was determined using disc diffusion method described by Andriamampianina *et al*. (2016) [39]. Two mL of inoculum corresponding to 0.5 MacFarland (10^8 CFU/ml) was uniformly spread on the surface of Mueller-Hinton Agar for the bacteria and Sabouraud Agar for yeast. Sterilized filter paper discs 6 mm diameter were impregnated with 10 µL of each sample to the concentration of 1 mg/disc. Negative controls were prepared by using the same solvents employed to dissolve the plant extract samples while Neomycin and Miconazole were used as positive controls at the concentration of 30 µg/mL and 50 µg/mL respectively. The soaked discs were then placed on the surface of the agar, and incubated at 37 °C during 24 h for bacteria, or at 25 °C for yeast. The inhibition zone diameter (IZD) was measured. The results were interpreted by means of the scale used by Ponce *et al*. (2003) [40], Moreira *et al*. (2005) [41] and Celikel and Kavas (2008) [42] stating that microorganism are not sensitive for IZD less than 8 mm, sensitive for IZD of 9 to 14 mm, very sensitive for IZD of 15 to 19 mm and extremely sensitive for IZD larger than 20 mm. Tests were performed in triplicate and the results were expressed as mean values ± standard deviations (mm ± SD).

2.6.4.3 Micro-well dilution assay
The MIC and MBC values were evaluated using the method of Ozturk and Ercisli (2006) [43], Kuete *et al*., (2011) [44]. Briefly, 95 µL of culture medium (Mueller-Hinton Broth, Becton Dickinson, Sparks, MD, USA) were placed inside wells. Then, 100µL of each extract to be tested (1000 mg/mL) were placed in wells A1, A2 and A3. Thus, 100 µL of each extract stock solution was taken for serial 2-fold dilutions up to the twelfth column, the last 100 µL (column 12) are removed. Finally, 5 µL of the inoculum (10^6 CFU/ mL) are aseptically removed with a micropipette and added to all wells of the microplate except for the wells which used as control for the bacterial growth and wells for control of sterility of the medium. Microplates were incubated at 37 °C for 24 h for bacteria and 48 h for yeast (as yeast require a longer time for growth). After this period, 40 µL of a 0, 4 mg/mL solution of MTT (3-(4, 5-dimethylthiazolil-2)-2, 5-dipheniltetrazolium bromid) was added to each well as an indicator of microbial growth. The microplates were kept for a second incubation at appropriate temperature for 30 min (bacteria) and 24 h (yeast). Then, the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) was identified.
The MIC value was visually determined. It is considered as the lowest concentration of each concentration sample displaying no visible growth. The concentration that inhibited bacteria or yeast growth completely (the first clear well) was taken as the MIC value [45]. The Minimum Bactericidal Concentration (MBC) was recorded as a lowest extract concentration killing 99,9% of the bacterial innocula after 24 h incubation at 37 °C. Ten microliters were taken from the well obtained from the MIC value and the wells above the MIC value well and spread it on Mueller-Hinton Agar (MHA) plates. The number of colony was counted after 24 h of incubation at 37 °C. The concentration of sample that produces < 10 colonies was considered as MBC value. Each experiment was repeated at least three times.
For each extract and fractions, the MIC and MBC were determined on susceptible strains only. The standards used to interpret MIC results are those of Dalmarco *et al*. (2010) [46]. Crude extracts and fractions could be said to have significant activity if the MIC value is 100 µg/mL or lower, moderate from 100 to 500 µg/mL, weak from 500 to 1000 µg/mL and as inactive over if 1000 µg/mL. An isolated compound is said to possess significant antimicrobial activity (MIC ≤ 10µg/mL), moderate (10 < MIC ≤ 100 µg/mL) and low or negligible (MIC > 100 µg/mL) [47].

2.6.5 Statistical analysis
The results are expressed as mean ± standard error mean. The significance of the differences of the means was evaluated by the test "T" of Student by using the software "R studio". The values of *p* lower than 0.05 were regarded as statistically significant.

3. Results

3.1 Results of extraction and structure of isolated compound

Extraction by methanolic maceration at room temperature for 24x3 hours of powder from bark of *B. perrieri* gave 8.25 g (20.63%) of methanolic extract. Partitioning of methanolic extract (23 g) yielded 0.82 g (3.49%) of hexanic extract, 2.91 g (12.41%) of ethyl acetate extract and 2.64 g (11.26%) of aqueous extract. Fractionation of ethyl acetate extract (2.79 g) gave 850 aliquots grouped in 15 fractions (G_1 to G_{15}). After fractionation in PTLC, the fraction G_{10} gave 7 mg of a pure compound ($G_{10\text{-}2}$). By concerted use of one and two-dimensional NMR spectroscopy, proton and carbon signals were assigned totally and this compound was identified as a Loganin (Figure 1) [48]. The assignments of protons and carbons (Table 1) were found in accordance with reported values in literature [49].

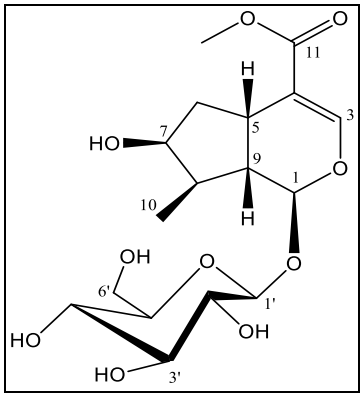

Fig 1: Structure of Loganin ($C_{17}H_{26}O_{10}$) isolated from the bark of *Breonia perrieri*

Table 1: NMR data of isolated compound $G_{10\text{-}2}$ (^{1}H 400 MHz, ^{13}C 75 MHz, Methanol-d_4)

Position	δ_H	δ_C	Cosy	HMBC (H => C)
1	5.27	97.35	H-9	8, 7, 9
3	7.38	151.69		
4		113.76		
5	3.10	31.94	H- 6, H-1	4, 3, 6, 5
6	1.62/2.22	42.49	H-5, H-7	7, 8, 9, 4
7	4.04	74.84	H-6, H-8	
8	1.88	41.97	H-7, H-10	
9	2.03	46.36	H-1, H-5	
10	1.09	13.31	H-8	8, 7, 9
11		169.25		
1’	4.64	99.82	H-2’	5
2’	3.19	74.50	H-1’, H-3’	1’
3’	3.37	77.84	H-2’	
4’	3.27	71.31		
5’	3.29	78.19		
6’	3.66/3.89	62.54	H-5’	
11-OOMe	3.69	51.46		11

3.2 Phytochemical Analysis

The major secondary metabolites detected in the crude methanolic extracts are registred in Table 1.

To the best of our knowledge, coumarins, antharaquinones, anthracenosides, tannins, steroids, triterpenoids, flavanols, flavonols, cardenolides, heterosides, leucoanthocyanins, iridoids and saponins are first detected from the bark of *Breonia perrieri*.

Table 2: Phytochemical screening of *Breonia perrieri* crud extract

Metabolites	Crude extract	Metabolites	Crude extract
Alkaloids Dragendorf	-	Flavanones	-
Alkaloids Wagner	-	Flavanes	+
Alkaloids Mayer	-	Anthocyanins	-
Saponins	+	Leucoanthocyanins	++
Coumarins		Cardenolides	++
Steroids	+	Polysaccharides	+++
Triterpenoids	++	Sugar	-
Flavonoids	+++	Unsaturated Sterols	++
Iridoids	++	Lactonic Steroid	-
Heterosides	+++	Flavanones/ Flananonols	-
Tannins	+++	Flavanes	+
Polyphenols	-	Flavanonols	-

(+): low; (++): moderately present; (+++): highly present; (-): absent

3.3 Acute toxicity

The acute toxicity studies performed with the doses ranging from 250 mg/Kg to 4000 mg/Kg did not resulted in death of any animal until 72 h of observation (Table 5). Since no mortality were observed at this dose rate, the median lethal dose of crude extract of *B. perrieri* intraperitoneal injection, is therefore greater than 4000 mg/Kg. According to OECD guidance (OECD, 2008) [50], this extract may be assigned to be the lowest toxicity class 5 (LD_{50} > 2000mg/Kg). The animals did not reveal any signs of acute toxicity or any major effect on normal behavior. Moreover, insignificant differences in body weight were observed between the initial and final body weight of the mice treated with crude extract and control group (Table 6).

It’s clearly demonstrate that the all doses did not cause any sensitivity. The signs like changes in locomotion, and piloerection were found to be chiefly traditional in teams to stating associate degree illation that the drug concentrations were found to be utterly safer to be used and non-toxic beneath acute toxicity study.

Table 3: Mortality pattern in Swiss mice in intraperitoneally route of crude extract from of the *B. perrieri* bark

Dose per group* (mg/Kg)	Mortality of mice					
	30 min	1h	6h	12h	24h	72h
Control**	0/3	0/3	0/3	0/3	0/3	0/3
250	0/3	0/3	0/3	0/3	0/3	0/3
500	0/3	0/3	0/3	0/3	0/3	0/3
750	0/3	0/3	0/3	0/3	0/3	0/3
1000	0/3	0/3	0/3	0/3	0/3	0/3
1500	0/3	0/3	0/3	0/3	0/3	0/3
2000	0/3	0/3	0/3	0/3	0/3	0/3
2500	0/3	0/3	0/3	0/3	0/3	0/3
3000	0/3	0/3	0/3	0/3	0/3	0/3
4000	0/3	0/3	0/3	0/3	0/3	0/3

*: 3 mice per exposed group; **: No treated group

Table 4: The effect of crude extract from *B. perrieri* bark on body weight of the exposed Swiss mice in acute intraperitoneal toxicity

Treatment	Body weight (g)				
Control	Dose* (mg/Kg)	Initial (0 days)	Final (3 days)	t-value	p-value
Treated group	250	28.66 ± 1.15	28.76 ± 1.02	0.5664	0.915
	500	21.66 ± 0.57	22.10 ± 2.50	- 0.3450	0.7920
	750	22.33 ± 1.52	22.44 ± 1.36	- 0.0930	0.9301
	1000	32.33 ± 0.57	33.20 ± 1.01	- 1.8386	0.2806
	1500	32.83 ± 0.76	33.85 ± 1.84	- 2.2827	0.4476
	2000	30.66 ± 0.57	30.71 ± 0.53	- 0.1029	0.5709
	2500	29.50 ± 0.50	29.40 ± 0.66	- 2.1881	0.9048
	3000	29.50 ± 0.50	29.51 ± 0.28	- 0.0402	0.9777
	3500	32.33 ± 0.57	32.40 ± 0.49	- 0.1599	0.8798
	4000	31.66 ± 0.57	32.03 ± 0.13	- 1.0631	0.3781

*: 3 mice per exposed group; Statistical significance according to Student's *t* test $p < 0.05$;

3.3 Antioxidant activity of the extracts from the bark of *B. perrieri*

All the extracts from the bark of *B. perrieri* excepted hexanic extract was showed antioxidant activity (Table 4). The TLC-bioautography revealed that crude methanolic extract, ethyl acetate soluble and water soluble had intensive yellow spot formation (Table 4). This result indicate that ethyl acetate soluble had the most powerful antioxidant activity based on the IC_{50} value (5.43 ± 0.151µg/mL), the smaller concentration of the fraction were needed to inhibit 50% of free radicals. In comparison, ethyl acetate soluble has better activity than α-tocopherol used as positive control (IC_{50} value = 11.43 ± 0.1 µg/mL). These results suggest that the bark of *B. perrieri* has health benefits for their antioxidative property.

Table 5: DPPH scavenging activity of the extracts from *B. perrieri* bark

Extract	TLC – DPPH test	DPPH RSA at 50 µg/mL (%)	IC_{50} µg/mL
Crude methanolic extract	+++	95.43	6.56 ± 0.20
Hexane soluble	-	-	-
Ethyl acetate soluble	+++	95.64	5.43 ± 0.15
Water soluble	++	76.05	33.31 ± 0.10
α- tocopherol	+++	NT	11.43 ± 0.10

RSA: Radical Scavenging Activity; Values are mean ± standard deviation (n=3); +++: high intensity; ++: medium intensity; +: low intensity; -: absent; NT: Not tested

3.4 Antimicrobial assay of extracts from *B. perrieri* bark

The antimicrobial properties of methanolic crude extract from the bark of *B. perrieri* are presented in Table 3. The plant extracts exhibit specific antibacterial activity against Gram + *S. pneumoniae*. This was the only strain for which a zone of growth inhibition around the well was observed (crude extract: IZD =11 ± 0.7 mm, ethyl acetate soluble: IZD = 10 ± 0.65 mm). Crude methanolic extract and ethyl acetat solubles have both a moderate antibacterial activity against *S. pneumonia* with MIC = 250 µg/mL. Positives controls (Neomycin and Miconazol) showed significantly high inhibition against all tested microorganisms. Gram-positive strain is more sensitive than Gram-negative and *Streptococcus pneumoniae* was the most sensitive bacteria. In short, isolation of the responsible element is necessary for fully elucidating the antibacterial activity of the bark of this species. This might also provide insight about their possible use against bacterial infection.

Table 6: *In vitro* antimicrobial activity of the different extracts from *B. perrieri* bark

Test organisms	Inhibition Zone Diameter (mm), MCI , MCB value (µg/ mL),					
	Crude methanolic extract	Hexane soluble	Ethyl acetate soluble	Water soluble	Standards	
					C_1	C_2
Staphylococcus aureus	-	-	-	-	23 ± 0	NA
Bacillus cereus	-	-	-	-	25 ± 0.33	NA
Listeria monocytogenes	-	-	-	-	20 ± 0	NA
Streptococcus pyogenes	-	-	-	-	22 ± 0.6	NA

Streptococcus pneumoniae	11[a] ± 0.70* 250[b] > 1000[c]	7 ± 0.84*	11 ± 0.1* 250 >1000	0 ± 0	21 ± 0.15	NA
Clostridium perfringens	-	-	-	-	20 ± 0.1	NA
Salmonella enterica	-	-	-	-	20 ± 0	NA
Shigella flexnerii	-	-	-	-	27 ± 0.5	NA
Proteus mirabilis	-	-	-	-	22 ± 0.15	NA
Yersinia enterocolitica	-	-	-	-	27 ± 0.33	NA
Enterobacter cloacae	-	-	-	-	22 ± 0.1	NA
Enterobacter aerogenes	-	-	-	-	13 ± 0.15	NA
Candida albicans	-	-	-	-	NA	11 ± 0.5

*: Mean ± SD, n= 3; - : Inactive; Control antimicrobial agents: C_1 (Neomycin 30 µg for bacteria), C_2 (Miconazol 50 µg for yeast). ; NA: Not Applicable

3.5 Antioxidant activity and Antimicrobial activities of Loganin

Loganin isolated from the bark of *B. perrieri* has been proved to have antioxidant and antibacterial activities as showed in Table 7. From these results, it was evidenced that *B. perrieri* displays antibacterial and antioxidant activities due to the presence of Loganin.

Table 7: Antioxidant and Antimicrobial activities of Loganin isolated from the bark of *B. perrieri*

Compound isolated	TLC – DPPH test	DPPH RSA (IC_{50}[a] µg/mL)	Antibacterial activity against *S. pneumonia*, IZD (mm)[b]
Loganin	+++	1.50 ± 0.3	10 ± 0.5

Values are mean ± standard deviation (n=3); +++: high intensity; ++: medium intensity; +: low intensity;
-: absent; NT: Not tested; a: Concentration providing 50% of inhibition; b: Inhibition Zone diameter (mm);

4. Discussions

The results of this study are a contribution to the valorization of *B. perrieri* species. Therefore, this investigation can be evaluated as the first report about their pharmacological and chemical studies. The study showed that the crude methanolic extract from the bark of *B. perrieri* contained major secondary metabolites which are a common feature of the Rubiaceae family such as coumarins, anthraquinones, anthracenosides, tannins, steroids, triterpenoids, flavanols, flavonols, cardenolides, heterosides, leucoanthocyanins, iridoids and saponins. The extraction and fractionation with series of solvents with increasing order of polarity, have given highest yield of ethyl acetate fraction (12.41%) followed by water soluble (11.26%) and hexane fraction (3.49%). Most of the researches focus on optimizing and advancing the extraction methods so as to extract maximum biological active material. However, assessing the toxicity profile of such products is of vital importance [51]. Acute toxicity study is a preliminary, yet an important study which could shed light on the general safety of the substance. The 72 h acute toxicity study of crude methanolic extract did not cause any mortality or behavioral changes when injected intraperitoneally in mice. The monitoring of body weight consumption of the experimental animals is important while studying the toxicity and safety of natural product since hints at the physiological and metabolic status of the animals and gets rid of the researcher from deriving any false observations due to nutritional abnormalities of the mice. In the current study, none of the experimental groups suffered loss in weight or gained overweight, which suggested that the crude extract from the bark of *B. perrieri* did not induce significant changes in the appetite and did not exert any deleterious effects on the general health status and metabolic growth of the mice. The pattern of body weight was not altered significantly. It suggested that the plant is relatively nontoxic and the no-observed-adverse-effect level (NOAEL) of crude extract was determined as 4000 mg/Kg of body weight. Then, the plant is free from serious side effects which the synthetic drugs possesses drives people towards using the natural product. Hence, further investigations of toxicity assessment such as subacute, chronic, or genotoxic studies using repeated and prolonged exposures need to be conducted to confirm its safety.

The bio guided assay with pharmacological evaluations including antioxidant and antibacterial study was performed to isolate the active product of the plant. Using the disc diffusion method at 1mg/disc, the antibacterial profiles of the crude methanolic extract and the ethyl acetate solubles were identical for *Streptocuccus pneumonia* the most sensitive tested organism (IZD =11 mm; MIC= 250 µg/mL). In reference to Dalmarco *et al*. (2010), this activity is considered as a moderate antibacterial activity. The main factors molding the antimicrobial activity of plants involve the structure of main bioactive compound and type of pathogen. In general, bacteria like Gram-positive are more sensitive than Gram-negative ones because their membrane are rich in lipopolysaccharides and form an impassable barrier to the bioactive compounds [52]. The antibacterial activity of the extract may be attributed to the presence of flavonoids, which have been reported to be involved in inhibition nucleic acid biosynthesis and other metabolic processes [53]. Furthermore, the partially hydrophobic nature of phenolic compounds has also been reported to be responsible for their antimicrobial activity [54]. This might also provide insight about their possible use against bacterial infection. Some studies have found that *S. pneumoniae* is the causative agent in approximately 1% to 11% of neonatal sepsis case and a cause of morbidity and mortality in children aged less than 60 days infected with community-acquired pneumonia (meningitis, otitis, sinusitis, septic arthritis, osteomyelitis, peritonitis and endocarditis) [55]. For these reasons, *B. perrieri* could constitute one good alternative to synthetic antibiotics, particularly against Pneumococcal infections caused by *Streptococcus pneumoniae*.

In the DPPH method, the antioxidant potential of the plant extracts was assessed on the basis of their scavenging activity of the stable DPPH free radical. Among the sample tested, the ethyl acetat solubles was found to be strong free radical scavengers with IC_{50} value of 5.43 ± 0.15 µg/mL, less than 30 µg/mL in reference to Ahmad *et al.* (2010). It is significantly high when compared to the standard α-tocopherol used as positive control (IC_{50}=11.43 ± 0.1 µg/mL) because compared

with control, the IC_{50} value is inversely proportional to the free radical scavenging activity. That means a smaller IC_{50} represents higher activity or vice versa. Antioxidant activity is often correlated to the high phenolic content which may be in the form of flavonoids, simple phenolics, terpens or tannins because these compounds have the same polarity with the ethyl acetate solvent, that can dissolve sterols, alkaloids, glycosides and terpenoids [56]. Flavonoids are highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various other free radicals implicated in several diseases by suppressing reactive oxygen formation, chelating trace elements involved in free- radical production, scavenging reactive species and up-regulating protect antioxidants defense [57, 58]. As well, iridoids exhibit high antioxydant property modulated by phenolic compounds [59]. These components are good for the management of cardiovascular diseases and oxidative stress which has been linked to cancer, aging, inflammation, and neurodegenerative diseases [60]. This result suggests that the bark of *B. perrieri* has health benefits for their antioxydative property. To complete the above antioxidant strategies, it is advisable to combine *in vitro* and *in vivo* antioxidant studies for more precise therapeutic value to plant antioxydant entities.

Since natural products such as iridoids glycoside have long been known to be of chemotaxonomic significance in the family Rubiaceae as reported by Ahmad *et al*. (2010), whether the antioxidant and antibacterial potential reported in this study can be attributed to the iridoids alone, or to any of the natural product, or to a synergistic effect of the compounds present. In order to verify these hypotheses, investigations of the ethyl acetate solubles was conducted for further study of separation and isolation of active compound. The study results in the isolation of Loganin, a monoterpenoid iridoid glycoside belongs to bicyclic monoterpens with a cyclopentane-pyran ring in his structure. This isolated compound was also subjected to antibacterial and antioxidant assays as and have revealed again strong antioxidant capacity with IC_{50} value of 1.50 ± 0.3 µg/mL and antibacterial activity against *Streptococcus pneumoniae* (IZD = 10 ± 0.5 mm). From the above results, it was evident that *B. perrieri* displays antibacterial and antioxidant activities do to the presence of this bioactive compound. Loganin was proven to have different activities: anti-inflammatory, neuroprotective, hypotensive, antibiotic, sedative, antidiabetic, hepatoprotective, immune-stimulating, leishmanicidal, prevent osteoporosis effects, cytotoxic activities and have neuroprotection effect in several models of neurodegerenation, including Parkinson's disease [61, 62]. It can also attenuate MPP^+ (1-methyl-4-phenylpyridinium) induced apoptotic death, neurite damage, and oxidative stress through enhancement of neurotrophic signaling [63] and may exert antiamnesic activity *in vivo* through acetylcholinesterase inhibition [64]. Plants rich in this type of monoterpene include Aponacynaceae, Lamiaceae, Loganiaceae, Scrophulariaceae, Verbenaceae and Rubiaceae [65]. This report has demonstrated the antioxydant and antibacterial activities of Loganin isolated from the bark of *B. perrieri*.

5. Conclusion

Overall, our results provide evidence indicating that *Breonia perrieri* may be a potential source of novel agents for treating diseases due to its range of phytochemicals, antibacterial and antioxidant properties. For the first time, this study demonstrated that *B. perrieri* could be considered as a natural source of bioactive compounds, particularly phenolics and iridoids, and confirmed its high potential for the pharmaceutical industry. It had demonstrated the safe of his methanolic extract. The antibacterial and antioxidant activity of Loganin as well as compound isolated, suggesting that the bark of *B. perrieri* could also be potential sources for prevention of cancer and other diseases associated with oxidative stress. The secondary metabolites that were identified in this genus are a common feature of the Rubiaceae family; however, they have first investigated in this species. Further studies are necessary to investigate its mechanism of action, bioavailability, *in vivo* effects and other pharmacological applications. This review endeavors to provide a comprehensive and up-to-date compilation of documented biological activities and the phytochemistry of the *Breonia* genus. However, more attention should be paid to this plant of its medicinal properties.

6. References

1. World Health Organization. Traditional Medicine Strategy 2002-2005. Role of Traditional Medicine in Primary Health Care, Geneva, World Health Report 2009, 724-743.
2. Razafimandimbison SG. A systematic revision of Breonia (Rubuaceae- Naucleeae). Annals of the Missouri Botanical Garden 2002;89(1):1-37.
3. Ngwenya NM. Biological and phytochemical screening of major compounds in *Cephalanthus natalensis*, Thesis submitted for master's in science: University of Johannesburg 2008.
4. Martins D, Nunez CV. Secondary metabolites from Rubiaceae species. Molecules 2015;20(7):13422-95.
5. Gurib-Fakeem AB. The-medicinal and aromatic plants of the Indian Ocean Islands. Boca Raton: CRC Press 2004, 331-2.
6. Homolle AM. Le genre Breonia de Madagascar, Bulletin de la Société Botanique de France 1937;84(4):457-462.
7. Annals of the Missouri Botanical Garden 2002, 89(1).
8. Rakotovao G, Rabevohitra AR, Collas de Chantelperron P, Guibal D, Gerard J. Atlas des bois de Madagascar. Quae Cirad 2012, 413.
9. Davis AC, Bridson D, Goodman S, Jonathan P, Benstead J. The Rubiaceae- Introduction in the Natural History of Madagascar. University of Chicago Press, Chicago 2003, 431-434.
10. Direction des Eaux et Forêts. Inventaire écologique forestier national: recueil botanique de 200 espèces forestières. Antananarivo: « EEDR Mamokara FTM », 1996, 7-477.
11. The IUCN Red List of Threatened Species. Version 2019-3. http://. Iucnredlist.org. 9 October 2020.
12. Beech E, Rivers MC, Andriambololonera S, Lantoarisoa F *et al*. Red List of Dry Forest Trees of Madagascar. Botanic Gardens Conservation International. Richmond, UK 2020, 3-14.
13. Eloff JN, Famakin JO, Katerere DRP. Isolation of an antibacterial stilbene from *Combretum woodii* (Combretaceae) leaves. Afr. J Biotechnology 2005;4(10):1167-1171.
14. Silverstein RM, Basler GC, Morill TC. Identification spectrométrique des composés organiques. Edn 5, Emmanuël Laure 1991, 261.
15. Boiteau P. Dictionnaire de noms Malgaches de végétaux, Microédition no. 85 04 10, Archive des documents, Museum Nationale d'Histoire Naturelle, Paris 1985.

16. Brink M. *Breonia perrieri* Homolle. Record from PROTA4U. Lemmens RHMJ, Louppe D. & Oteng-Amoako AA. PROTA (Plant Resources of Tropical Africa / Ressources végétales de l'Afrique tropicale), Wageningen, Netherlands 2012.
17. Fong EHS, Tin-Wa M, Farnsworth NR, Dobberstein RH. Phytochemical screening methods. Rev. Department of pharmacognosy and pharmacology. College of pharmacy. University of Illinois 1977.
18. Marini-Bettolo GB, Nicoletti S, Patami M. Plant screening by chemical and chromatographic procedure under field conditions. J Chromatogr 1981;218:113-217.
19. OCDE. Sous-comité d'experts du système général harmonisé de classification et d'étiquetage des produits chimiques: dangers pour la santé et l'environnement - toxicité aiguë. UN/SCEGHS/2/INF 2001, 11-13.
20. OCDE. Ligne directrice de l'OCDE pour les essais de produits chimiques: toxicité orale aiguë - méthode de l'ajustement des doses. OCDE. 425, 2006, 29.
21. Dragstedt A, Lang B. Etude de la toxicité par administration unique d'un nouveau médicament. Annales Pharmaceutiques Français 1957, 11.
22. Dupont C. Le cout des études toxicologiques d'un nouveau médicament. Lett. Pharma 1987;1:1.
23. Rajemiarimoelisoa CF, Rakoto DAD, Randrianarivo HR, Jeannoda VL. Purification and toxicity study of a saponin from seeds of *Albizia odorata*, a Fabaceae from Madagascar, JPS 2015;3(5):264-271.
24. Teo S, Stirling D, Thomas S, Hoberman A, Kiorpes A. A 90 days oral gavage toxicity study of D-methylphenidate and D, L-methylphenidate in Sprague-Dawley rats, Toxicol 2002;179(3):183-196.
25. Lee M, Seo C, Cha S, Sim H. Safety assessment of So-cheong-ryong- tang: Subchronic toxicity study in Crl: CD Sprague Dawley rats, Mol. Med. Rep 2014;9:2273-2282.
26. Bentabet N, Boucherit-Otmani Z, Boucherit K. Composition chimique et activité antioxydante des extraits organiques des racines de *Fredolia aretioides* de la région de Béchar en Algérie. Phytothérapie 2014;12(6):364-371.
27. Brand-Williams W, Cuvelier ME, Berset C. Use of free radical method to evaluate antioxidant activity. Lebensm. Wiss. Technol 1995;28:25-30.
28. Takao T, Kitatani F, Watanabe N, Yagi A, Sakata K. A Simple Screening Method for Antioxidants and Isolation of Several Antioxidants Produced by Marine Bacteria from Fish and Shellfish, BBB 1994;58(10):1780-1783.
29. Ruiz-Terán F, Medrano-martinez A, Navarro-Ocaña. Antioxidant and free radical scavenging activities of plant extracts used in traditional medecine in Mexico. Afr. J Biothechnol 2008, 7.
30. Popovici, Saykova I, Tylkowski B. Evaluation de l'activité antioxydant des composés phénoliques par la réactivité avec le radical libre DPPH, ERGI 2009;4:25-39.
31. Awika JM, Rooney LW, Wu X, Prior RL, Cisneros-Zevallos L. Screening method to measure antioxidant activity of sorghum (*Sorghum bicolor*) and sorghum products, J Agri. Food Chem 2003;51(23):6657-6662.
32. Calvin A. Investigation phytochimique de trois plantes indonésiennes aux propriétés antioxydantes et antiradicalaires. *Tinospora crispa*, *Merremia emarginata* et Oropheaenneandra. Thesis of Lausanne University 2001, 243.
33. Kapepula PM, Mungitshi PM, Franck T, Ngoyi DM, Kalenda PDT, Ngombe NK *et al*. Antioxidant potentiality of three herbal teas consumed in Bandundu rural areas of Congo. Nat. Prod. Res 2017;31(16):1940-1943.
34. Ngbolua KN, Bishola TT, Mpiana TP, Mudogo V, Tshibangu DST, Ngombe KN *et al*. Gbolo Ethno-botanical survey, *in votre* antisickling and free radical scavenging activities of *Garcinia punctata* Oliv. (Clusiaceae). J adv. botany zool 2014a;1(2):1-8.
35. Yoo KM, Lee CH, Lee H, Moon B, Lee CY. Relative antioxidant and cytoprotective activities of common herbs. Food Chem 2008;106(3):929-936.
36. Bongo G, Inkoto C, Masengo C, Tshiama C, Lengbuye E, Djolu R *et al*. Antisickling, Antioxydant and Antibacterial Activities of *Afromomum alboviolaceum* (Ridley) K. Schum, *Annona senegalensis* Pers. and *Mondia whitei* (Hook. f.) Skeels. AJLM 2017;2(4):52-59.
37. Abas F, Lajis NH, Israf DA, Khozirah S, Umi Kalsom Y. Antioxidant and nitric oxide inhibition activities of selected Malay traditional vegetables. Food Chem 2006;95:566-573.
38. Ahmad R, Mahbob ENM, Noor ZM, Ismail NH, Lajis NH, Shaari K. Evaluation of antioxidant potential of medicinal plants from Malaysian Rubiaceae (subfamily Rubioideae). Afr. J Biotechnol 2010;9(46):7948-7954.
39. Andriamampianina HL, Rakoto DAD, Thomat P, Ramanankierana H. Antimicrobial activity of extracts from *Crotalaria bernieri* Baill (Fabaceae). Afr. J microbiol. Res 2016;10(31):1229-1239.
40. Ponce AG, Fritz R, Del Valle C, Roura SI. Antimicrobial activity of essential oils on native microflora of organic Swiss chard. LWT 2003;36:679-684.
41. Moreira MR, Ponce AG, Del Valle CE, Roura SI. Inhibitory parameters of essential oils to reduce a food borne pathogen. LWT 2005;38:565-570.
42. Celikel N, Kavas G. Anticrobial properties of some essential oils against some pathogenic microorganisms. Czech J Food Sci 2008;26:174-181.
43. Ozturk S, Ercisli S. Chemical composition and *in vitro* antibacterial activity of *Seseli libanotis*. World J Microbiol Biotechnol 2006;22:261-5.
44. Kuete V, Kamga J, Sandjo LP, Ngameni B, Poumale HM, Ambassa P *et al*. Antimicrobial activities of the methanol extract, fractions and compounds from *Ficus polita* Vahl. (Moraceae). BMC Complement. Altern. Med 2011;11:6.
45. Razafintsalama VE, Rakotoarison RA, Rakotoarisoa MA, Rakoto-Ranoromalala DAD, Ranarivelo LR, Randriamialinoro F *et al*. Etudes des activités antimicrobienne et antioxydante de l'extrait de racine *d'Anthospermum perrieri* (RUBIACEAE). Madahary 2016;5:12-18.
46. Dalmarco JB, Dalmarco EM, Koelzer J, Pizzolatti MG, Frode TS. Isolation and identification of bioactive compounds responsible for the antibacterial efficacy of *Lotus corniculatus* var. São Gabriel. IJGP 2010;4:108-114.
47. Kuete V. Potential of Cameroonian plants and derived natural products against microbial infections: a review. Planta Med 2010;76(14):1479-91.
48. Patil SP, Laddha KS. Simple method of isolation of loganin from *Nux vomica* fruits and its quantification by HPLC. Int J Pharmacognosy 2017;4(1):39-42.
49. Calis I, Lahloub MF, Sticher O. Loganin, loganic acid and periclymenoside, a new biosidic ester iridoid

glucoside from *Lonicera periclymenum* L. (*Caprifoliaceae*). HCA 1984;67:160-165.
50. OECD Guidelines for the Testing of Chemicals. Test no. 425, Acute oral toxicity: Up-and down procedure. OECD Publishing 2008, 1-12.
51. Newman DJ, Cragg GM. Natural products as sources of new drugs over the 30 years from 1981 to 2010. J Nat. Prod 2012;75(3):311-335.
52. Seow YX, Yeo CR, Chung HL, Yuk HG. Plant essential oils as active anti- microbial agents. Crit. Rev. Food Sci. Nutr 2014;54:625-644.
53. Harborne JB, Williams CA. Advances in flavonoid research since 1992, Phytochemistry 2000;55:481-504.
54. Favier A. Le stress oxydant: Intérêt conceptuel et expérimental dans la compréhension des mécanismes des maladies et potentiel thérapeutique. L'actualité chimique 2003, 108-115.
55. Hoffman JA, Masson EO, Schutze EG, Tan TQ, Barson WJ, Givner LB *et al*. *Streptococcus pneumoniae* infections in the neonate. Pediatrics 2003;112:1095-102.
56. Bajpai VK, Majumder R, Park JG. Isolation and purification of plant secondary metabolites using column-chromatographic technique. Bangladesh J Pharmacol 2016;11(4):844-848.
57. Agati G, Azzarello E, Pollastri S, Tattini M. Flavonoids as antioxidants in plants: location and functional significance, Plant Sci 2012;196:67-76.
58. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: An overview. Sci. World J 2013, 1-8.
59. Ramírez-Cisneros MA, Rios MY, Aguilar-Guadarrama AB, Rao PPN, Aburto-Amar R, Rodríguez-López V. *In vitro* COX-1 and COX-2 enzyme inhibitory activities of iridoids from *Penstemon barbatus*, *Castilleja tenuiflora*, *Cresentiaalata* and *Vitex mollis*. Bioorg. Med. Chem. Lett 2015;25:4505-4508.
60. Sharif S, Kitaz A, Al-Kayali R. TLC Screening and Evaluation of Antioxydant, Antibacterial Activity of *Onopordon macrocephalum* by Bioautography Method. IJPS 2016;12(2):1-8.
61. Xu YD, Cui C, Sun MF, Zhu YL, Chu M, Shi YW *et al*. Neuroprotective effects of loganin on MPTP-induced Parkinson's disease mice: Neurochemistry glial reaction and autophagy studies. J Cell. Biochem 2017;118:3495-3510.
62. Tseng YT, Lin WJ, Chang WH, Lo YC. The novel protective effects of loganin against 1-methyl-4-phenylpyridinium- induced neurotoxicity: Enhancement of neurotrophic signaling, activation of IGF-1R/GLP-1R, and inhibition of RhoA/ROCK pathway. Phytother Res 2019;33:690-70.
63. Lee KY, Sung SH, Kim SH, Jang YP, Oh TH, Kim YC. Cognitive-enhancing activity of loganin isolated from *Cornus officinalis* in scopolamine-induced amnesic mice. Arch. Pharm. Res 2009;32:677-683.
64. Simmons DL, Botting RM, Hla T. Cyclooxygenase isozymes: The biology of prostaglandin synthesis and inhibition. Pharmacol. Rev 2004;56:387.
65. Berger A, Kostyan MK, Klose SI, Gastegger M, Lorbeer E, Brecker L *et al*. Loganin and secologanin derived tryptamine – iridoid alkaloids from *Palicourea crocea* and *Palicourea padifolia* (Rubiaceae). Phytochemistry 2015;116:162-169.