ORIGINAL ARTICLE

ANTIOXIDANT, ANTIBACTERIAL AND PHYTOCHEMICAL EVALUATIONS OF *Bremeria arachnocarpa* Wernham Rubiaceae

| Safidy Pierret Rivoandry [1,4*] | Manitriniaina Rajemiarimiraho [2*] | Léa Herilala Rasoanaivo [4] | Anne Wadouachi [3] | Roger Marie Rafanomezantsoa [1] | and | Amélie Raharisololalao [4] |

[1.] University of Fianarantsoa | Ecole Doctorale Géochimie et Chimie Médicinale (GEOCHIMED) | Fianarantsoa 301 | Madagascar |
[2.] University of Antananarivo | Mention Génie Rural | Ecole Supérieure Polytechnique | Madagascar |
[3.] University of Picardie | Laboratoire de Glycochimie, des Antimicrobiens et des Agroressources FRE CNRS 3517 | Amiens | France |
[4.] University of Antananarivo | Laboratoire de Chimie des Substances Naturelles et Chimie Organique Biologique (LCSN/COB) | Antananarivo | Madagascar |

| Received December 02, 2021 | | Accepted December 12, 2021 | | Published December 22, 2021 | | ID Article | Rivoandry-Ref04-ajiras021221 |

ABSTRACT

Background: *Bremeria arachnocarpa* Wernham is a small tree endemic of Madagascar. The decoction of its leaves is used in traditional medicine for the treatment of yellow fever, in case of poisoning. **Aim of the study:** This work aims to evaluate the chemical constituents, antioxidant and antimicrobial activity of this plant. **Materials and methods:** The chemical constituents of the hexane-soluble and ethyl acetate-soluble parts of the crude ethanolic extract of the leaves were studied. Column chromatography and TLC methods allow the isolation of products. The structures of the isolated products were identified by concerted analysis of the 1D and 2D NMR spectra and by comparison with the literatures. The crude ethanolic extract and hexane-soluble, ethyl acetate-soluble and butanol-soluble parts of the crude ethanolic extract were used for biological activity. The antimicrobial activity was test by diffusimetric method and the antioxidant activity of the extracts was evaluated using DPPH° free radical scavenging. **Results:** According to this method, crude ethanolic extract of *Bremeria arachnocarpa* leaves exhibited a high antioxidant capacity (IC_{50}= 10.3 µg / ml), the antioxidant capacity being more powerful than of α-tocopherol (10.67 µg / ml), used as standard drug. Its antioxidant capacity is focalised in butanol-soluble part of the crude ethanolic extract (IC_{50}= 12.34 µg/ml). The results of antimicrobial activity showed the ineffectiveness of the extracts against most of the strains tested. The fractionation of the hexane-soluble and ethyl acetate-soluble parts of the crude ethanolic extract led to the isolation of three known compounds trioleine 1, acylated β-sitosterol 2 and ursolic acid 3.
Keywords: *Trioleine, ursolic acid, acylated β-sitosterol, Antioxidant, Antimicrobial, Bremeria arachnocarpa, NMR.*

1. INTRODUCTION

Bremeria (Rubiaceae) is a new endemic genus of Madagascar and Mascarenes. It comprises only 28 species [1,2]. These species were initially placed in the genus *Mussaenda* (Rubiaceae) before being recently transferred into *Bremeria*. The present report is focusing on *Bremeria arachnocarpa*, which is one of the eigthing species endemics in Madagascar, encountered in the east part of Madagascar and locally known under the vernacular name "fatoralahy". This plant is traditionally used for anti-yellow fever, antipiretic, in case of poisoning. To the best of our knowledge, no previous phytochemical and pharmacological investigations have been performed on this species. Herein, we report for the first time the isolation and identification of isolated products obtained from leaves of this plant species by analysis of NMR spectra, together with some biological properties, namely the antioxidant capacity on DPPH and the antimicrobial activity by disk-diffusion method.

2. MATERIALS AND METHODS

2.1 General procedures

Silica gel 60 (Merck, 0.04-0.063 mm) was used for column chromatography. The thin layer chromatography was carried out on silica plates on a plastic or aluminum sheet (Macherey-Nagel, SIL G / UV254, 0.20 mm). All solvents are distilled before use. The 1D NMR spectra (^{1}H, ^{13}C, DEPT) and 2D (^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC) are recorded on the Bruker 600 NMR apparatus operating at 600.19 MHz and 125.78 MHz using CD_3OD solvents, $CDCl_3$ and DMSO and TMS as internal reference.

2.2 Plant material:

The leaves of of *Bremeria arachnocarpa* Wernham was harvested in the Ambositra, Amoron'I Mania region, 400 km from Fianarantsoa in the south-eastern part of Madagascar in November 2020. The species was identified by botanists at the botanical and zoological park of Tsimbazaza Antananarivo and a voucher specimen has been deposited in the LCSN / COB laboratory.

2.3 Extraction and isolation

The dried leaves of *Bremeria arachnocarpa* Wernham were ground into powder (300 g) and extracted with EtOH 80° by maceration for 48 hours at room temperature, resulting in the crude ethanolic extract after evaporation under reduced pressure of the solvent. The crude ethanolic extract was subjected to successive liquid-liquid partitioning between hexane

Corresponding author Author & Copyright Author © 2021: | Safidy Pierret Rivoandry * |

and water, ethyl acetate and water, and n-butanol and water to furnish hexane-soluble, ethyl acetate-soluble, butanol-soluble and water-soluble extracts. The solutions obtained are evaporated under reduced pressure to give hexane-soluble, ethyl acetate-soluble, butanol-soluble extracts.

The hexane-soluble part of crude ethanolic extract (1.3 g) was separated by chromatography on a silica column (60 g of silica gel 60, 80x2 cm) using the eluent hexane in gradient with dichloromethane to give 190 fractions of 20 ml. Purification by crystallization of fractions having the same TLC appearance was carried out. Two products 1 (54 mg, yellow oil) and 2 (4.1 mg, white amorphous powder), respectively from fractions 19-22 eluted with hexane / dichloromethane 90 / 10 and 65-69 eluted with hexane / dichloromethane 60/40 were obtained.
The ethyl acetate extract (2 g) was separated by chromatography on a silica column (60 g silica gel 60, 80x2 cm) using the eluent hexane in gradient with ethyl acetate to give 180 fractions of 20 ml. Purification by crystallization of fractions having the same TLC appearance was carried out. One product 3 (4 mg, white amorphous powder) from fractions 34-45 eluted with Hexane / ethyl acetate 60 / 40 was obtained. Its structures were determined by means of spectroscopic analysis and by comparison of their NMR data with those reported in the literature.

2.4 Antimicrobial test

The crude ethanolic extract and hexane-soluble, ethyl acetate-soluble, and butanol-soluble parts of the crude ethanolic extract, of *Bremeria arachnocarpa* Wernham leaves were tested for antimicrobial activity against three Gram-positive bacterial strains: *Bacillus cereus*, *Bacillus anthracis*, *Streptococcus pneumoniae*, against one Gram-negative bacterial strain: *Enterobacter cloacae* and one fungal strain: *Candida albicans*, by a previously described disk diffusion method [3], in Petri dishes. Sterile discs of 6 mm in diameter (Biomérieux, Marcy l'Etoile, France) impregnated with 10 µl of extracts with a concentration equal to 100 mg / ml or 1 mg / disc are placed on the surface of the seeded agars. The petri dishes are then incubated at 37° C. The diameter of the inhibition zone (mm) around each disc is measured after 24 h. The reference antibiotic Gentamycin (30 µg / disc) is used as a positive control. All tests were performed in triplicate, and clear halos greater than 7 mm were considered as positive results.

2.5 Antioxidant assay

Qualitative test

The qualitative antioxidant test was carried out according to the bioautography method. Briefly, the extracts to be tested are deposited in solution on a silica plate. After development of the chromatoplate in an appropriate solvent, it is sprayed with a 25% solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol. An active product has a yellow spot on a purple back ground antioxidant test.

Quantitative test

The quantification of the antioxidant power is carried out according to the method of Brand Williams et al (1995) and Sanchez Moreno et al (1998) with some modifications [4,5,6]. The DPPH (25 mg) is dissolved in 100 ml of methanol. This preparation is stored in the dark. Ten milliliters of this solution are added with 45 ml of methanol. Cascade concentrations of the extract to be tested ranging from (2 mg / ml to 0.125 mg / ml) were prepared. In dry tubes, 200 µl of each concentration were respectively mixed with 3800 µl of the 4.5% DPPH solution. Blanks consisting of 3800 µl of the 4.5% DPPH solution and 200 µl of methanol are also prepared. The test is repeated in triplicate and incubated in the dark for 60 min. The same procedure is applied to the control consisting of vitamin E (α-tocopherol). For the reading, the absorbance is measured using a spectrophotometer at the wavelength of 517 nm. The antioxidant activity which expresses the capacity to scavenge the free radical is estimated by the percentage of discoloration (percentage of inhibition) of DPPH in solution in methanol. The percent inhibition is calculated using formula (1).

$$\text{Percent inhibition (\%)} = [(Ac - As) / (Ac)] \times 100 \quad \textbf{(1)}$$

Ac = the absorbance of the control
As = the absorbance of the sample.

3. RESULTS

3.1 Extraction

Maceration in EtOH of 300 g of the plant material yielded 37.88 g (12.62%) of crude ethanolic extract. Liquid-liquid partitioning of the crude ethanolic extract furnished 1.59 g (0.53%) of hexane-soluble, 1.036 g (0.34%) of ethyl acetate-soluble and 1.16 g (0.38%) of butanol-soluble extracts.

3.2 Spectral data

The structures of the isolated products were identified by concerted analysis of the ^{1}H, ^{13}C, DEPT, HMQC, HMBC NMR spectra and by comparison with data from the literature. Compound 1 is a triglyceride which is a trioleine [7]. Compound 2 is an acylated sterol has been identified as acylated β-sitosterol [8] and compound 3 is a triterpene identified to ursolic acid [9]. Their chemical structures are shown in figure 1.

Product 1: Appearance: yellow oil

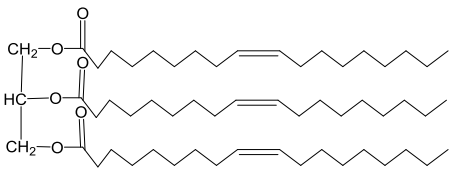
δ (ppm)^{1}H NMR ($CDCl_3$, 600 MHz): 5.35 (4H, H-9, H-10); 5.24 (1H, H-2 glycerol); 4.30 (2H, H-1β, H-3 β glycerol); 4.16 (2H, H-1α, H-3α glycerol); 2.31 (12H, H-8, H-11); 1.62 (6H, H-3); 1.28 (6H, H-4); 0.90 (9H, H-18);
δ (ppm)^{13}C NMR ($CDCl_3$,125.78 MHz): 173.2 (C-1, C-1 "); 172.9 (C-1 '); 129.6 (C-9, C-10); 68.8 (C-2 glycerol); 62.0 (C-1, C-3 glycerol); 34.0 (C-2); 31.9 (C-8, C-11); 29.7 (C-4, C-5, C-6, C-7 , C-12, C-13, C-14,C-15); 27.2 (C-3); 24.9 (C-16); 22.6 (C-17); 14.1 (C-18).

Product 2: white cottony

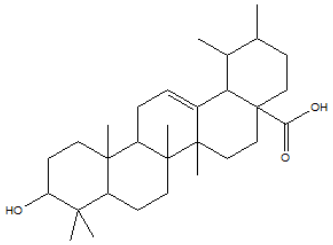
δ (ppm) ^{1}H NMR (CD_3OD, 600 MHz): 5.33(1H,H-6); 4.62 (1H, H-3); 1.04 (3H, s, H-18); 0.94 (3H, d, H-21); 0.83(3H, d, H-26); 0,70 (3H,s,H-19). Long chain: 2.34(H-2'); 1.27 (CH_2)n; 0.90 (CH_3 ter).
δ (ppm)^{13}C NMR (CD_3OD, 125.78 MHz):139.6 (C-5); 122.6 (C-6) ; 73.6 (C-3); 56.7 (C-14); 56.0 (C-17); 50.0(C-9); 45.8 (C-24); 42.3 (C-13); 39.7 (C-12); 38.2 (C-4); 37.0 (C-1); 36.6(C-10); 33.9 (C-22); 31.9 (C-7); 31.8 (C-8); 36,1 (C-20); 29.1 (C-25); 28.2 (C-16); 26.1 (C-23); 24.3 (C-15); 23.0 (C-28); 22.7 (C-11); 19.8 (C-26); 19.3 (C-19); 19.0 (C-27); 18.7 (C-21); 11.9 (C-29); 11.8 (C-18);
Long chain: 173.5 (C-1'); 34.7 (C-2'), 29.2 (CH_2)n; 21.0 (CH_2- CH_3 ter); 14.0 (CH_3 ter).

Product 3: Appearance: white powder

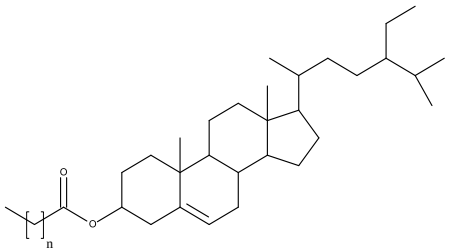
δ (ppm) ^{1}H NMR (CD_3OD, 600 MHz): 5.11 (1H, br.s, H-12); 3.12 (1H, dd, H-3); 2.63 (1H, d, H-18); 1.24 (3H, s, H-23); 1.23 (3H, s, H-27); 1.05 (3H, s, H-25); 1.03 (3H, s, H-26) ; 1.00 (3H, br.s, H-29); 0.96 (3H, s, H -30); 0.90(3H, s, H-24)
δ (ppm) ^{13}C NMR (CD_3OD, 125.78 MHz): 181.7 (C-28); 139.3 (C-13); 126.5 (C-12); 79.4 (C-3); 55.4 (C-5); 53.9 (C-18); 48.7 (C-9); 43.2 (C-17); 42.9 (C-14); 40.5 (C-19), 40.2 (C-20), 39.8 (C-8), 39.7 (C -1), 38.0 (C-4), 37.8 (C-22), 34.2 (C-10), 31.7 (C-7); 29.1 (C-21); 28.7 (C-23); 27.6 (C- 2); 25.2 (C-16); 24.3 (C-27); 21.6 (C-11); 19.4 (C-30); 17.8 (C-6); 17.7 (C-29); 16.4 (C-26); 16.2 (C-24); 15.7 (C-25).



1



3



2

Figure 1: Chemical structures of compounds 1 to 3 isolated from *Bremeria arachnocarpa* leaves.

3.3 Free radical scavenging activity on DPPH·

The four extracts crude ethanolic (HA), hexane-soluble (Hex), ethyl acetate-soluble AcOEt, butanol-soluble (BuOH) parts of ethanolic extract of *Bremeria arachnocarpa* Wernham leaves contain antioxidant products (Figure 2).

Stationary phase: Silica gel 60

Developer : DPPH/ MeOH(2 mg/1 ml)

Figure 2: Qualitative antioxidant test of *Bremeria arachnocarpa* (Wernham) leaves extracts.

The DPPH is a stable radical with a maximum absorption at 517 nm that can readily undergo scavenging by antioxidant. It has been widely used to test the ability of compounds as free-radical scavengers or hydrogen donors and to evaluate the antioxidant activity of plant extracts and foods [10]. The DPPH radical scavenging activity of *Bremeria arachnocarpa* leaves extracts, and standard antioxidant, vitamin E is presented in the figure 3.

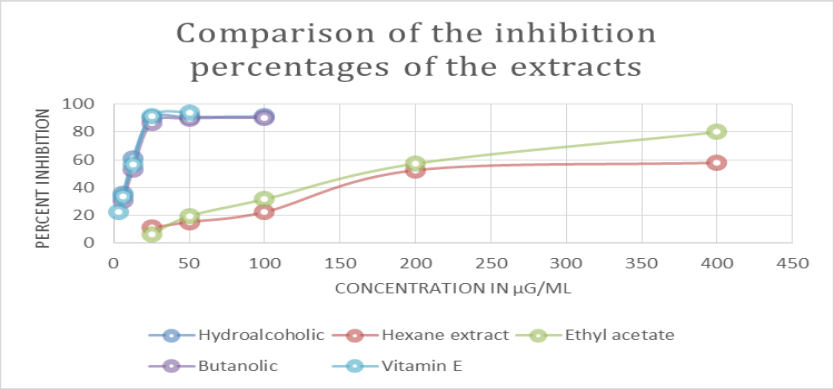


Figure 3: DPPH radical scavenging activity of extracts of leaves.

The scavenging abilities of the extracts of species *Bremeria arachnocarpa* Wernham leaves were concentration dependent, usually expressed as IC_{50} values, the amount of antioxidant necessary to decrease the initial concentration of DPPH by 50%. Lower IC_{50} value indicates a higher antioxidant activity. The IC_{50} values of each extract are compared with the IC_{50} value of α-tocopherol (Table 1).

Table 1: Inhibition concentration IC_{50} of the sample tested.

Sample	**CI_{50} (µg/ml)**
Crude ethanolic	10.30
Hexane-soluble	194.87
Ethyl acetate-soluble	172.82
Butanol-soluble	12.34
α-tocopherol (Vitamin E)	10.67

In this study, DPPH radical scavenging activity of the tested samples was in the order crude ethanolic > α-tocopherol > butanol-soluble > Ethyl acetate-soluble> Hexane-soluble; the crude ethanolic extract of leaves showed high antioxidant capacity. According to this method, ethanolic extract and butanolic-soluble of *Bremeria arachnocarpa* leaves exhibited a high antioxidant capacity (IC50 ≤50 µg/ml), and the hexane-soluble and ethyl acetate-soluble parts of crude ethanol extract of *Bremeria arachnocarpa* has a low antioxidant activity (100 µg/ml < IC50). The antioxidant capacity in *Bremeria arachnocarpa* leaves is due to polar constituent.

3.4 Assay of antimicrobial activity

The antibacterial and antifungal activities of the crude ethanol extract, hexane-soluble and ethyl acetate-soluble parts of crude ethanolic of *Bremeria arachnocarpa* leaves are shown in the table 2. The extracts were investigated for their in vitro antimicrobial properties using a disk-diffusion method against *Bacillus cereus*, *Bacillus anthracis*, *Streptococcus pneumoniae*, *Enterobacter cloacae* and *Candida albicans*. The antibacterial activity is ranked from no activity (inhibition diameter < 8 mm), low (inhibition diameter between 9 and 14 mm), moderate (inhibition diameter between 15 and 19 mm) and high activity (diameter inhibition ≥ 20 mm) [11]. After incubation, all plates were examined for any zones of growth inhibition, and the diameters of these zones were measured in millimetres.

Table 2: Antimicrobial activity (inhibition zone expressed in mm) of three investigated extracts of *Bremeria arachnocarpa* Wernham.

Samples	**Zone of inhibition (mm)**				
	Bacillus cereus	***Streptococcus pneumoniae***	***Bacillus anthracis***	***Enterobacter cloacae***	***Candida albicans***
Crude ethanolic	0	6±0.2	6±0.2	0	0
Hexane-soluble	0	7±0.2	7±0.2	0	0
Ethyl acetate-soluble	0	10±02	6±0.2	0	0
Gentamicin	16±1	18±1	19±3	14±2	0

Notes: The values represent the average of three determinations ± standard deviations. Gentamicin (10 µg/well) was used as a positive control.

The crude ethanolic extract and hexane- soluble part of crude ethanolic extract of *Bremeria arachnocarpa* leaves showed inactive against of the strains tested (inhibition diameter < 10 mm). Ethyl acetate- soluble part of crude ethanolic extract showed a low activity towards *S. pneumonia* (inhibition diameter between 9 and 14 mm). The results of the present investigation suggest that *Bremeria arachnocarpa* leaves showed the ineffectiveness of the extracts against most of the strains tested.

4. DISCUSSION

One triterpene acid, one acylated sterol and one triglyceride have been isolated from *Bremeria arachnocarpa* leaves. Trioleine and acylated β-sitosterol are isolated for the first time from the genus *Bremeria*. Ursolic acid has been reported in *Bremeria erectiloba* and in the other species of Musaenda such as *Mussaenda pubscens, Mussaenda hainanensis*, *Mussaenda frontosa* [12,13,14]. Diverse biological activities have been described for isolated compounds. Ursolic acid has demonstrated multiple biological properties, such as anti-inflammatory activities due to its anti-cylooxygenase and anti-lipoxygenase, antiviral [15] and anti-tumor potential [16]; it showed strong cytotoxic activity on colon cancer lines [17]. Ursolic acid inhibits the activity of these enzymes in HL60 leukemia cells in vitro [18]. This triterpene is also used in cosmetology, because it has a potential to inhibit the induction of MMPs [19,20,21], which could explain the beneficial role of this molecule in preventing skin aging. Phytosterol products have been added into various commercial foods and drink products as a cholesterol-lowering agent [22,23,24].

5. CONCLUSION

The results presented here constitute the first information on the chemistry study, the antioxidant and antimicrobial activity of *Bremeria arachnocarpa* leaves extracts. Low antimicrobial activity is detected of the extracts against most of the strains tested. This study reveals that the crude ethanolic extract of *Bremeria arachnocarpa* Wernham leaves showed the strongest DPPH scavenging activity. Its antioxidant activity is focalised in butanol-soluble part of crude ethanolic extract. Via this research, a known trioleine, acylated β-sitosterol and ursolic acid, were isolated from *Bremeria arachnocarpa* for the first time.

Acknowledgment: We would like the National Center for Applied Pharmaceutical Research (CNARP) Antananarivo, Madagascar for the biological tests.

6. REFERENCES

1. Davis.A.P., Razafimandimbison.S.G. Et Andriambololonera.S. "Nomenclatural changes in the genus *Bremeria* (Rubiaceae)". *Blumea*. 2011; 56: 4-5. DOI: 10.3767/000651911X557686
2. Alejandro GD, Razafimandimbison SG, Liede-Schumann S. Polyphyly of Mussaenda inferred from ITS and TrnTF data and its implication for generic limits in Mussaendeae (Rubiaceae). *American Journal of Botany*. 2005; 92: 544-557. DOI: 10.3732/ajb.82.3.544
3. Kil HY, Seong ES, Ghimire BK, Chung IM, Kwon SS, Goh EJ *et al*. Antioxidant and antimicrobial activities of crude sorghum extract. *Food Chemistry*. 2009; 115(4):1234–1239. DOI: 10.1016/j.foodchem.2009.01.032
4. Brand-Williams W., Cuvelier M. E., Berset C. Use of a free radical method to evaluate antioxidant activity, LWT-*Food Science and Technology*. 1995; 1: 25-30. DOI : 10.1016/S0023-6438(95)80008-5
5. Sánchez-Moreno, C., Larrauri, J. A., & Saura-Calixto, F. A procedure to measure the antiradical efficiency of polyphenols. *Journal of the Science of Food and Agriculture*. 1998; 76(2): 270–276. Available on: http://dx.doi.org/10.1002/(SICI)1097-0010(199802)76:2%3C270::AID-JSFA945%3E3.3.CO;2-0
6. Julio Hervé Andriamadio, Volasoa Herilalaina Victorine Rambeloson, Anne Wadouachi, Amélie Raharisololalao and Léa Herilala Rasoanaivo, Biflavonoids isolated from the stem bark of *Garcinia chapelieri* H. Perr. Clusiaceae, *American Journal of Innovative Research and Applied Sciences*. 2021; 12(5): 181-187. Available on: https://american-jiras.com/Andriamadio%E2%80%93Ref4-ajira070521.pdf
7.Mohamed El Mahad Ibrahim Abdou, Olivier Rakotoarison, Louise Faramalala Rabemandroso, Lahadson Stanislas Fulgence, Lovasoa Rakotondramasy, Anne Wadouachi and Léa Herilala Rasoanaivo **;** Chemical study of *Phylloxylon xylophylloides* Baker and assessment of its anti-hyperglycaemic activity in Glucose-loaded Normal Rabbits; *Journal of Pharmacognosy and Phytochemistry*. 2020; 9(2): 2278-2282. Available on: http://www.phytojournal.com
8.Daniela B, Laurian V, Ilioara O, Augustin CM, Grigore D, Daniela H et al. Polyphenolic Composition, Antioxidant and Antibacterial Activities for Two Romanian Subspecies of *Achillea distans* Waldst. et Kit. ex Willd. *Molecules*. 2013; 18, 8725-8739. DOI: 10.3390/molecules18088725
9. Bolzani VS, Yong MCM, Furlan M, Cavalheiro AJ, Araujo AR, Silva DHS et al. Secondary Metabolites from Brazilian Rubiaceae Plant Species: Chemotaxonomical and Biological Significance. *Recent Research Developments in Phytochemistry*. 2001; 5:19-3. Available on: http://pascal-francis.inist.fr/vibad/index.php?action=getRecordDetail&idt=14194350
10. Kandhasamy S, Sun CK. Free radical scavenging activity from different extracts of leaves of *Bauhinia vahlii* Wight and Arn. *Saudi Journal of Biological Sciences*. 2013; 20, 319–325. Available on: https://doi.org/10.1016/j.sjbs.2012.12.005
11.Reeves DS, White LO. Principles of methods of assaying antibiotics. In: Hugo WB, Russel AD, editors. Pharmaceutical microbiology. 3rd ed, Oxford, UK: Blackwel Scientific Publication; 1983, 140-162.
12. Ke Yuan, Wen-Tao Qiao, Ming-Wen Yin. Phytochemical Studies of *Mussaenda hainanensis* Merr. *Asian Journal of Chemistry*. 2009; 21(9):7138-7142. Available on: https://asianjournalofchemistry.co.in/User/ViewFreeArticle.aspx?ArticleID=21_9_76
13.Zhou Zhonglin, Sun Jiyan, Pan Liming; Wang Hui. Studies on chemical constituents from *Mussaenda pubescens* Ait. F. *Journal of Guangdong Pharmaceutical University*, 2017, 2.
14. Daniel SS, Anne W, Amélie R and Léa HR. A new iridolactone from *Bremeria erectiloba* Wernham (Rubiaceae). *International Journal of Chemical Studies*, 2018; 6(5): 78-80. Available on: https://www.chemijournal.com/archives/2018/vol6issue5/PartB/6-4-550-301.pdf
15. Quere L., Wenger T., and Schramm H.J. Triterpenes as potential dimerization inhibitors of HIV- 1 protease. *Biochem Biophys Res Commun*. 1996; 227 (2): 484–488. https://doi.org/10.1006/bbrc.1996.1533
16. Huang M., Ho C.T., Wang Z.Y., Ferraro T., Lou Y.R., Stauber K., Ma W., Georgiadis C., Laskin J.D., and Conney A.H. Inhibition of skin tumorigenesis by rosemary and its constituents carnosol and ursolic acid. *Cancer Research*. 1994; 54(3): 701–708. Available on: https://cancerres.aacrjournals.org/content/54/3/701.full-text.pdf
17. Andersson D., Liu J.J., Nilsson A., and Duan R.D. Ursolic Acid Inhibits Proliferation and Stimulates Apoptosis in HT29 Cells Following Activation of Alkaline Sphingomyelinase. *Anticancer Research*. 2003; 23 (4): 3317–3322. Available on: https://pubmed.ncbi.nlm.nih.gov/12926069/
18. Najid A., Simon A., Cook J., Chable-Rabinovitch H., Delage C., Chulia A.J., and Riguad M. Characterization of ursolic acid as a lipoxygenase and cyclooxygenase inhibitor using macrophages, platelets and differentiated HL60 leukemic cells. *FEBS Letters*. 1992; 299(3): 213-217. Available on: https://doi.org/10.1016/0014-5793(92)80117-Y
19.Shishodia S., Majumdar S., Banerjee S., and Aggarwal B.B. Ursolic acid inhibits nuclear factor-kappaB activation induced by carcinogenic agents through suppression of IkappaBalpha kinase and p.65 phosphorylation: correlation with down-regulation of cyclooxygenase 2, matrix metalloproteinase 9, and cyclin D1. *Cancer Research*. 2003; 63 (15): 4375-4383. Available on: https://pubmed.ncbi.nlm.nih.gov/12907607/
20. Lee Y.S., Jin D.Q., Beak S.M., Lee E.S., and Kim J.A. Inhibition of ultraviolet A-modulated signaling pathways by asiatic and ursolic in HaCaT human keratinocytes. *European Journal of Pharmacology*. 2003; 476(3): 173-178. DOI: 10.1016/s0014-2999(03)02177-0

21. Cha H.J., Park M.T., Chung H.Y., Kim N.D., Sato H., Seiki M., and Kim K.W. Ursolic acid-induced down-regulation of MMP-9 gene is mediated through the nuclear translocation of glucocorticoid receptor in HT1080 human fibrosarcoma cells. *Oncogene*. 1998; 16: 771-778. Available on: https://doi.org/10.1038/sj.onc.1201587
22. F. Marangoni, A. Poli, Review: phytosterols and cardiovascular health. *Pharmacological Research*. 2010; 61: 193–199. DOI: 10.1016/j.phrs.2010.01.001
23. N. Weber, P. Weitkamp, K.D. Mukherjee, Cholesterol-lowering food additives: lipase-catalysed preparation of phytosterol and phytostanol esters, Food Research International. 2002; 35: 177–181. DOI:10.1016/S0963-9969(01)00180-6
24. M. Noakes, P.M. Clifton, A.M. Doornbos, E.A. Trautwein. Plant sterol ester enriched milk and yoghurt effectively reduce serum cholesterol in moderately hypercholesterolemic subjects, European Journal of Nutrition. 2005; 44: 214–222. DOI:10.1007/s00394-004-0513-z

Cite this article: Safidy Pierret Rivoandry, Manitriniaina Rajemiarimiraho, Léa Herilala Rasoanaivo, Anne Wadouachi, Roger Marie Rafanomezantsoa, Amélie Raharisololalao. ANTIOXIDANT, ANTIBACTERIAL AND PHYTOCHEMICAL EVALUATIONS OF *Bremeria Arachnocarpa* Wernham Rubiaceae. *Am. J. innov. res. appl. sci.* 2021; 13(6): 572-577.