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# Secoiridoids and Iridoids from *Bremeria hymenopogonoides* Baker Rubiaceae leaves

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**ABSTRACT:** *Bremeria hymenopogonoides* Baker is a small tree endemic of Madagascar. The infusion of its leaves is used in traditional medicine for the treatment of yellow fever, snakebite, syphilis, gonorrhoea, splenomegaly and rat hypertrophy. This work aims to evaluate the chemical constituents, antioxidant and antimicrobial activities of this plant. The chemical constituents of the crude ethanolic, hexane, ethyl acetate and methanolic extracts of the leaves were studied. Column chromatography and TLC methods allowed the isolation of products. The structures of the isolated products were established using spectroscopic methods including nuclear magnetic resonance (NMR) and high resolution mass spectrometry, and by comparison with reported data in the literature. The hexane, ethyl acetate and methanolic extracts were used for biological activity. The antimicrobial activity was tested by diffusimetric method and the antioxidant activity of the extracts was evaluated using DPPH° free radical scavenging. According to this method, crude ethanolic extract of *Bremeria hymenopogonoides* leaves exhibited an antioxidant capacity (IC<sub>50</sub>= 22.43 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 methanolic extract (IC<sub>50</sub>= 44.42 µg/mL). The results of antimicrobial activity showed the ineffectiveness of the extracts against most of the strains tested. The fractionation of the ethyl acetate and methanolic extracts led to the isolation of eight products with its three new 2, 3-secoiridoids bremesecoiridoate esters A 6, B 7 and C 8 and five known compounds trioleine 1, acylated β-sitosterol 2, morisecoiridoic acid A 3, 8-acetyl-1-epishanzigenin methyl ester 4 and 8-acetylshanzigenin methyl ester 5.

**KEYWORDS:** Iridoids, NMR, Antioxidant, Antimicrobial, *Bremeria hymenopogonoides*.

## I. INTRODUCTION

*Bremeria* is an important genus that belongs to the Ixoroideae subfamily in the Rubiaceae family, these being known for the biosynthesis of iridoïdes as main secondary metabolites [1]. The genus *Bremeria* comprises about 18 species in Madagascar [2], mainly distributed in the inter-tropical zone. *Bremeria hymenopogonoides* Baker, endemic to Madagascar is a small tree with 4 m tall, diameter 6 cm, bark semi-rough grey, Calyx green, corolla pink, corolla lobes white inside. It has a limited distribution in Antsiranana, Toamasina, Fianarantsoa provinces. The plant is classified as a vulnerable species in the International Union of Conservation of Nature (IUCN) Red List of Threatened Plant Species [3]. *Bremeria hymenopogonoides*, locally known as “fatorialahy” in Madagascar, is used in folk medicine to treat yellow fever, snakebite, syphilis, gonorrhoea, splenomegaly and rat hypertrophy. In continuation of our research on *Bremeria* species [4,5], we report for the first time the isolation and structural identification of three new secoiridoids (6-8) and five known compounds, one secoiridoid (3), two iridoids (4 and 5), one triglyceride (1) and one acylated

sterol (2) from the leaves extracts of *Bremeria hymenopogonoides* Baker by mass and NMR spectra analysis, together with antioxidant capacity on DPPH and antimicrobial activity by disk diffusion method.

## II. MATERIALS AND METHODS

### II. 1. General procedures

Silica gel 60 (Merck, 0.04-0.063 mm) and sephadex LH 20 were 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 ( $^1\text{H}$ ,  $^{13}\text{C}$ , DEPT) and 2D ( $^1\text{H}$ - $^1\text{H}$  COSY,  $^1\text{H}$ - $^{13}\text{C}$  HSQC,  $^1\text{H}$ - $^{13}\text{C}$  HMBC) are recorded on the Bruker 600 NMR apparatus operating at 600.19 MHz and 125.78 MHz using  $\text{CD}_3\text{OD}$ ,  $\text{CDCl}_3$  and DMSO as solvents and TMS as internal reference.

### II. 2. Plant material

The leaves of *Bremeria hymenopogonoides* Baker were harvested in Mahasoabe, Haute Matsiatra region from Fianarantsoa in the south-eastern part of Madagascar in October 2021. 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.

### II. 3. Extraction

The dried leaves of *Bremeria hymenopogonoides* Baker were ground into powder (50 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 powder of dried leaves (350 g) was subjected to successive maceration with solvent in increased polarity hexane, ethyl acetate and methanol. The solutions obtained are evaporated under reduced pressure to give hexane, ethyl acetate and methanol extracts.

### II. 4. Phytochemical screening

The detection of main classes of phyto-constituents such as alkaloids, flavonoids, saponins, anthraquinones, triterpenoids, steroids, leucoanthocyanins, tannins and iridoids were conducted on the crude ethanolic extract according to methods previously published [6]. Appearance of specific colors or precipitates indicates the presence of the targeted metabolites.

### II. 5. Antimicrobial test

The crude ethanolic extract and hexane, ethyl acetate, and methanolic extracts, of *Bremeria hymenopogonoides* Baker 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 [7], in Petri dishes. Sterile discs of 6 mm in diameter (Biomérieux, Marcy l'Etoile, France) impregnated with 10  $\mu\text{l}$  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  $\mu\text{g}$  / 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.

## II. 6. 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. The chromatoplate is sprayed with a solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol (2 mg/1 ml). An active product has a yellow spot on a purple back ground antioxidant test.

### Quantitative test

The quantification of the antioxidant product is carried out according to the method of Brand Williams et al (1995) and Sanchez Moreno et al (1998) with some modifications [7,8,9]. 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  $\mu$ l of each concentration were respectively mixed with 3800  $\mu$ l of the 4.5 % DPPH solution. Blanks consisting of 3800  $\mu$ l of the 4.5 % DPPH solution and 200  $\mu$ l 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 ( $\alpha$ -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).

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

**Ac** = the absorbance of the control

**As** = the absorbance of the sample.

## II. 7. Isolation

The ethyl acetate extract (3 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 240 fractions of 10 ml. Purification by crystallization of fractions having the same TLC appearance was carried out. Purification of fractions 62-89 (80 mg, yellow oil, eluted with hexane/AcOEt 10/90) on silica column yielded, two products **A** (44 mg, yellow oil) and **B** (10 mg, white powder), respectively from subfractions 19-22 eluted with Hexane / Dichloromethane 70 / 30 and 50-59 eluted with Dichloromethane / AcOEt 70 / 30. Product **C** (125 mg, white amorphous powder) was obtained from fractions 92- 135 eluted with hexane / AcOEt 60/40.

The methanol extract (2 g) was separated by chromatography on LH 20 column (60 g LH 20, 80x2 cm) using the eluent methanol to give 31 fractions of 10 ml. Purification by crystallization of fractions having the same TLC appearance was carried out. Three products **D** (49 mg, brown powder), **E** (13 mg, yellow powder) and **F** (12 mg, green powder), respectively from fractions 5-13, 15 and 17 were obtained.

Their structures were determined by means of spectroscopic analysis and by comparison of their NMR data with those reported in the literature.

## III. EXPERIMENTAL RESULTS

### III. 1. Extraction

Maceration in EtOH of 50 g of the plant material yielded 19.28 g (38.56 %) of crude ethanolic extract. Maceration in solvent in increasing polarity of 300 g of the plant material furnished 1.83 g (0.61 %) of hexane, 8.83 g (2.94 %) of ethyl acetate and 83.24 g (27.74 %) of methanol extracts.

### III. 2. Phytochemical screening

The phytochemical screening was performed on the crude ethanolic extract by means of different chemical assays. It revealed the presence of triterpenoids, steroids, flavonoids, tannins and iridoids.

### III. 3. Free radical scavenging activity on DPPH

With the four extracts crude ethanolic (HA), hexane (Hex), ethyl acetate (Acetate) and methanol (MeOH) extracts of *Bremeria hymenopogonoides* Baker leaves, the crude ethanolic (HA) and methanol (MeOH) extracts contain antioxidant products (Fig 1).



Stationary phase : Silica gel 60  
Developer : DPPH/ MeOH (2 mg/1 ml)

Fig 1: Qualitative antioxidant test of *Bremeria hymenopogonoides* Baker 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 hymenopogonoides* Baker leaves extracts (crude ethanolic, ethyl acetate, methanol), and standard antioxidant, vitamin E is presented in the figure 2.

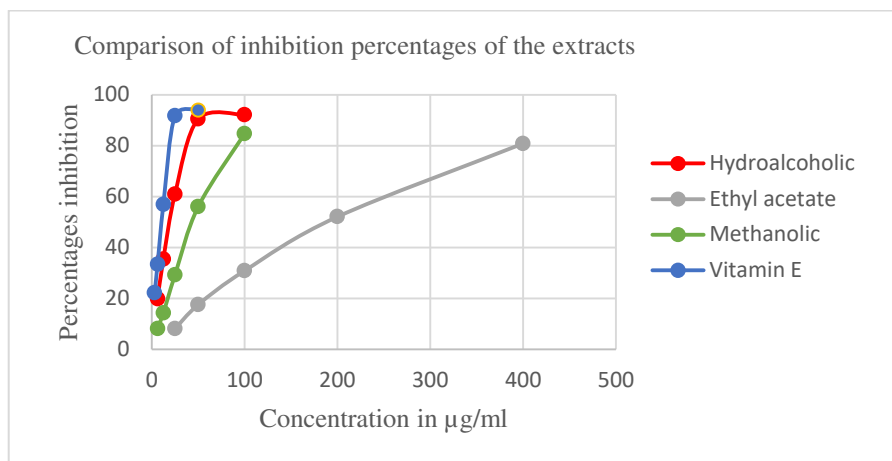


Fig 2: DPPH radical scavenging activity of extracts of leaves.

The scavenging abilities of the extracts of species *Bremeria hymenopogonoides* Baker leaves were concentration dependent, usually expressed as IC<sub>50</sub> values, the amount of antioxidant necessary to decrease the initial concentration of DPPH by 50 %. Lower IC<sub>50</sub> value indicates a higher antioxidant activity. The IC<sub>50</sub> values of each extract are compared with the IC<sub>50</sub> value of  $\alpha$ -tocopherol (Table 1).

Table 1: Inhibition concentration IC<sub>50</sub> of the sample tested.

Sample	CI <sub>50</sub> (µg/ml)
Crude ethanolic	22,43
Ethyl acetate	189,2
Methanol	44,42
$\alpha$ -tocopherol (Vitamin E)	10,67

In this study, DPPH radical scavenging activity of the tested samples was in the order  $\alpha$ -tocopherol > crude ethanolic > Methanol > Ethyl acetate; the crude ethanolic extract of leaves showed high antioxidant capacity. According to this

method, ethanolic extract and methanolic of *Bremeria hymenopogonoides* Baker leaves exhibited a high antioxidant capacity ( $IC_{50} \leq 50 \mu\text{g/ml}$ ), and ethyl acetate extract of *Bremeria hymenopogonoides* Baker has a low antioxidant activity ( $100 \mu\text{g/ml} < IC_{50}$ ). The antioxidant capacity in *Bremeria hymenopogonoides* Baker leaves is due to polar constituent.

### III. 4. Assay of antimicrobial activity

The antibacterial and antifungal activities of the crude ethanol extract, hexane and ethyl acetate of *Bremeria hymenopogonoides* Baker leaves are shown in the table 2. The extracts were investigated for them 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  $\geq 20$  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 hymenopogonoides* Baker.

Samples	Zone of inhibition (mm)				
	<i>Bacillus cereus</i>	<i>Streptococcus pneumoniae</i>	<i>Bacillus anthracis</i>	<i>Enterobacter cloacae</i>	<i>Candida albicans</i>
Crude ethanolic	0	0	7±0.2	0	0
Hexane	0	0	7±0.2	0	0
Ethyl acetate	8±0.2	09±02	11±0.2	0	0
Gentamicin	16±1	18±1	19±3	14±2	0

Notes: The values represent the average of three determinations  $\pm$  standard deviations. Gentamicin (10  $\mu\text{g/well}$ ) was used as a positive control.

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

### III. 5. Spectral data

The structures of the isolated products were identified by concerted analysis of the  $^1\text{H}$ ,  $^{13}\text{C}$ , DEPT, HMQC, HMBC NMR spectra and by comparison with data from the literature. Compound **A** is a triglyceride which is a trioleine **1** [4, 12]. Compound **B** is an acylated sterol has been identified as acylated  $\beta$ -sitosterol **2** [4], compound **C** is a secoiridoid identified to morisecoiridoic acid **3** [13], compound **E** is a mixture of an isomer of iridoide identified to 8-acetyl-1-epishanzigenin methyl ester **4** and 8-acetylshanzigenin methyl ester **5** [13, 14, 15], compound **F** is a mixture of bremesecoiridoate esters **A 6**, **B 7** and **C 8**. Their chemical structures are shown in figure 1.

#### Product 1: Appearance: yellow oil

$\delta$  (ppm)  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 600 MHz): 5.35 (6H, H-9, H-10); 5.24 (1H, H-2 glycerol); 4.30 (2H, H-1 $\beta$ , H-3  $\beta$  glycerol); 4.16 (2H, H-1 $\alpha$ , H-3 $\alpha$  glycerol); 2.31 (6H, H-2); 2.02 (12H, H-8, H-11); 1.62 (6H, H-3); 1.28 (6H, H-4); 0.90 (9H, H-18);

$\delta$  (ppm)  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125.78 MHz): 173.0 (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); 32.0 (C-16); 29.7 (C-4, C-5, C-6, C-7, C-12, C-13, C-14, C-15); 27.2 (C-8, C-11); 24.9 (C-3); 22.6 (C-17); 14.1 (C-18).

**Product 2: white cottony**

$\delta$  (ppm)  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 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 ( $\text{CH}_2$ ) n; 0.90 ( $\text{CH}_3$  ter).

$\delta$  (ppm)  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 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 ( $\text{CH}_2$ ) n; 21.0 ( $\text{CH}_2$ - $\text{CH}_3$  ter); 14.0 ( $\text{CH}_3$  ter).

**Product 3: Appearance: white powder**

$\delta$  (ppm)  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 600 MHz): 4.40 (1H, m, H-4); 3.98 (1H, dd, H-7 $\beta$ ); 3.86 (1H, dd, H-7 $\alpha$ ); 3.69 (3H, s, H-9); 2.88 (1H, m, H-6); 2.77 (1H, d, H-1); 2.71 (1H, m, H-5); 2.41 (1H, dd, H-3 $\beta$ ); 1.70 (1H, dd, H-3 $\alpha$ ); 1.29 (3H, s, H-11).

$\delta$  (ppm)  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 125.78 MHz): 174.6 (C-8); 174.0 (C-10); 78.5 (C-2); 75.5 (C-4); 62.6 (C-7); 60.5 (C-1); 50.6 (C-9); 49.7 (C-6); 48.9 (C-5); 48.3 (C-3); 24.2 (C-11).  $[\text{M}+\text{Na}]^+$  m/z 286.2 (calculated for  $\text{C}_{11}\text{H}_{18}\text{O}_7\text{Na}^+$  286.2)

**Product 4: Appearance: white powder**

$\delta$  (ppm)  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 600 MHz): 7.47 (1H, s, H-3); 5.59 (1H, d, H-1); 4.26 (1H, t, H-6); 3.74 (3H, s, H-11), 3.06 (1H, d, H-5); 2.87 (1H, dd, H-9); 2.16 (2H, s, H-7 $\beta$ ); 2.09 (2H, d, H-7 $\alpha$ ); 2.02 (3H, m, H-14); 1.53 (3H, s, H-12).

$\delta$  (ppm)  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 125.78 MHz): 171.3 (C-13); 168.0 (C-10); 153.2 (C-3); 107.0 (C-4); 92.8 (C-1); 88.4 (C-8); 75.0 (C-6); 50.4 (C-11); 49.3 (C-9); 45.5 (C-7); 41.2 (C-5); 21.4 (C-14); 20.5 (C-12).  $[\text{M}+\text{Na}]^+$  m/z 309.4 (calculated for  $\text{C}_{13}\text{H}_{18}\text{O}_7\text{Na}^+$  309.4)

**Product 5: Appearance: white powder**

$\delta$  (ppm)  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 600 MHz): 7.45 (1H, s, H-3); 5.49 (1H, d, H-1); 4.27 (1H, q, H-6); 3.78 (3H, s, H-11); 2.94 (1H, t, H-5); 2.75 (1H, d, H-9); 2.34 (1H, d, H-7 $\beta$ ); 2.16 (1H, t, H-7 $\alpha$ ); 2.03 (3H, s, H-14); 1.67 (3H, s, H-12).

$\delta$  (ppm)  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 125.78 MHz): 171.3 (C-13); 170.0 (C-10); 151.6 (C-3); 108.0 (C-4); 90.1 (C-1); 90.7 (C-8); 77.9 (C-6); 50.5 (C-11); 48.8 (C-9); 45.9 (C-7); 42.2 (C-5); 21.4 (C-14); 18.5 (C-12).  $[\text{M}+\text{Na}]^+$  m/z 309.4 (calculated for  $\text{C}_{13}\text{H}_{18}\text{O}_7\text{Na}^+$  309.4)

**Products 6, 7, 8: Appearance: green powder**

The structures of compounds **6**, **7** and **8** are identified by concerted analysis of his NMR spectra of **F**. Product **F**, a metabolite of the leaf extracts, was obtained as a green solid from methanol extract. The existence of a double pics in there  $^{13}\text{C}$  NMR suggests that **B** is a mixture of compounds **6**, **7** and **8**. The  $^1\text{H}$  NMR data of **F** (Table 3) showed signals for sets of diastereotopic protons at  $\delta_{\text{H}}$  2.45 (H-3a, 3H), 2.28 (H-3b, 3H);  $\delta_{\text{H}}$  1.94 (H-6a/6, 1H), 1.71 (H-6b/6, 1H) and  $\delta_{\text{H}}$  1.89 (H-6a/7/8, 2H), 1.75 (H-6b/7/8, 2H), for methine protons at  $\delta_{\text{H}}$  4.67 (H-7/7, 1H), 4.60 (H-7/8, 1H), 4.49 (H-7/6, 1H), 4.23 (H-4, 3H), 3.33 (H-1/6, 1H), 3.32 (H-1/7, 1H), 3.35 (H-1/8, 1H), 2.45 (H-5/8, 1H), 2.40 (H-5/7, 1H) and 2.30 (H-5/6, 1H), methyl group protons at  $\delta_{\text{H}}$  2.03 (H-10, H-13, 18H) and 1.59 (H-11, 9H), methoxy group protons at  $\delta_{\text{H}}$  3.67 (H-15/6, 7, 8, 18H) and 3.37 (H-8/6, 3H). The COSY coupling patterns of these signals suggested the presence of a three structures portions; thus, H-5/6 ( $\delta_{\text{H}}$  2.30) couples to H-6a/b/6 ( $\delta_{\text{H}}$  1.94 and 1.71), H-4 ( $\delta_{\text{H}}$  4.23), H-1/6 ( $\delta_{\text{H}}$  3.33), H-4 to H-3a/b ( $\delta_{\text{H}}$  2.45 and 2.28) and H-6a/b/6 to H-7/6 ( $\delta_{\text{H}}$  4.49).

H-5/7 ( $\delta_{\text{H}}$  2.40) couples to H-6a/b/7 ( $\delta_{\text{H}}$  1.89 and 1.75), H-4 ( $\delta_{\text{H}}$  4.23), H-1/7 ( $\delta_{\text{H}}$  3.32), H-4 to H-3a/b ( $\delta_{\text{H}}$  2.45 and 2.28) and H-6a/b/7 to H-7/7 ( $\delta_{\text{H}}$  4.67).

H-5/8 ( $\delta_{\text{H}}$  2.45) couples to H-6a/b/8 ( $\delta_{\text{H}}$  1.89 and 1.75), H-4 ( $\delta_{\text{H}}$  4.23), H-1/8 ( $\delta_{\text{H}}$  3.35), H-4 to H-3a/b ( $\delta_{\text{H}}$  2.45 and 2.28) and H-6a/b/8 to H-7/8 ( $\delta_{\text{H}}$  4.60).

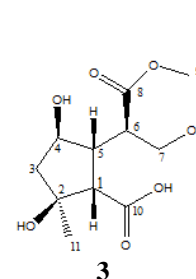
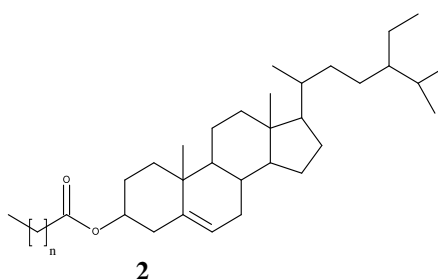
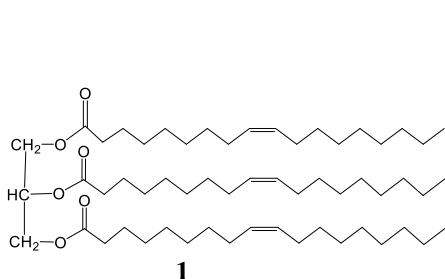
The  $^{13}\text{C}$  NMR spectrum of product **F** showed signals of carbonyls at  $\delta_{\text{C}}$  171.0 (C-9, C-12) and 173.5 (C-14) with their chemical shifts being compatible with ester functionalities. An oxygenated tertiary carbon at  $\delta_{\text{C}}$  88.9 (C-2), four additional oxygenated carbons at  $\delta_{\text{C}}$  103.4 (C-7/6),  $\delta_{\text{C}}$  97.6 (C-7/7),  $\delta_{\text{C}}$  97.2 (C-7/8), 75.9 (C-4),  $\delta_{\text{C}}$  50.0 (C-15) and  $\delta_{\text{C}}$  48.5 (C-8/6) methyl carbons at  $\delta_{\text{C}}$  21.0 (C-10, C-13) and 20.4 (C-11) and aliphatic carbons at  $\delta_{\text{C}}$  36.5 (C-6/7), 36.4 (C-6/8), 32.2 (C-6/6), 45.7 (C-3), 45.2 (C-5/6), 45.0 (C-5/7), 44.7 (C-5/8), 58.5 (C-1/6), 58.3 (C-1/7) and 58.2 (C-1/8) were also detected and confirmed by multiplicity-edited HSQC data. The cyclic structures of each compounds were confirmed by the HMBC cross-peaks of H-11 ( $\delta_{\text{H}}$  1.52) with the oxygenated tertiary carbon C-2 ( $\delta_{\text{C}}$  88.9), methine

carbons C-1/6 ( $\delta_C$  58.5), C-1/7 ( $\delta_C$  58.3) (C-1/7), C-1/8 ( $\delta_C$  58.2) and methylene carbon C-3 ( $\delta_C$  45.7). After the C-7–C-6–C-5–C-4–C-3–C-2–C-1–C-5 connectivity were established based on COSY, HSQC and HMBC data. Hence, HMBC cross-peaks were observed between H-3a/b ( $\delta_H$  2.45, 2.28) with C-2 ( $\delta_C$  88.9) and C-4 ( $\delta_C$  75.9), while H-1 ( $\delta_H$  3.35/3.33/3.32) of each compounds showed HMBC correlation to C-14 ( $\delta_C$  173.5), C-2 ( $\delta_C$  88.9), each C-5 ( $\delta_C$  45.2, 45.0, 44.7) and C-6 ( $\delta_C$  75.9). The positions of CH<sub>3</sub>-10 or CH<sub>3</sub>-13 ( $\delta_H$  2.03) were revealed by their HMBC correlation to C-12 or C-9 ( $\delta_C$  171.0), indicating the presence of two acetyl moiety in each compounds. The correlation of the methoxy 3.67/50.0 (H-15/C-15) with the carbonyl 173.5 (C-14) indicates the ester structure of each compounds. The position of the ester carbonyl C-14 ( $\delta_C$  173.5) was confirmed by its HMBC correlation to the methine protons H-1 ( $\delta_H$  3.35/3.33/3.32) and H-5 ( $\delta_H$  2.45/2.40/2.30). The methoxy 3.37/48.5 (H-8/C-8) showed a correlation with the C-7/6. Hence compound 6 has a methoxy group at C-7 but compounds 7 and 8 are isomers with hydroxyl group at C-7. The hydroxyl group at C-7 of compound 7 has  $\beta$ -configuration but compound 8 has  $\alpha$ -configuration at C-7. The methoxy 6.67/50.0 (H-15/C-15) showed a correlation with the carbonyl C-14(173.5). This correlation showed that the compounds are secoiridoids ester.

These findings were also supported by <sup>13</sup>C chemical shift values analyzed for 6, 7 and 8 in comparison with those observed for secoiridoid 5 model compounds used to establish the structure [13]. But, there are many differences. Confirmation of the stereochemistry of its stereogenic centers was achieved by comparison with <sup>13</sup>C NMR literature data, especially those for chiral centers at C-1, C-2, C-4, C-5 and C-7 which indicated the  $\beta$ -configuration of the hydroxyl group at C-4 [13, 14].

Table 3. <sup>1</sup>H, <sup>13</sup>C, and HMBC NMR Spectroscopic Data for compounds 6, 7 and 8 (600 MHz, Methanol-d<sub>4</sub>)

Position	Type	COSY H-H	HMBC H- C	Compound 6		Compound 7		Compound 8	
				$\delta_H$	$\delta_C$	$\delta_H$	$\delta_C$	$\delta_H$	$\delta_C$
1	HC-COO	H-5	C-1, C-15, C-2, C-4	3.33 (m)	58.5	3.32 (m)	58.3	3.35 (m)	58.2
2					88.9		88.9		88.9
3	CH <sub>2</sub>	H-4	C-4, C-5, C-2, C-11	2.45 (d) 2.28 (d)	45.6	2.45 (d) 2.28 (d)	45.6	2.45 (d) 2.28 (d)	45.6
4	CH-O	H-3, H-5		4,23	75.9	4,23	75.9	4,23	75.9
5	CH	H-4,H-6, H-1	C-1, C-14, C-6, C-4, C-7	2.30 (m)	45.2	2.40 (m)	45.0	2.45 (m)	44.7
6	CH <sub>2</sub>	H-7, H-5	C-5, C-1, C-7	1.94 (m) 1.71	32.2	1.89 (m) 1.75	36.5	1.89 (m) 1.75	36.4
7	O-CH-O	H-6	C-10, C-5, C-6, C-8, C-9	4.49 (t)	104.3	4.67 (t)	97.6	4.63 (t)	97.0
8	CH <sub>3</sub> -O		C-7/6	3.37	48.5				
9	OC=O				171.0		171.0		171.0
10	CH <sub>3</sub>		C-9	2.03 (s)	21.0	2.03 (s)	21.0	2.03 (s)	21.0
11	CH <sub>3</sub>		C-1, C-2, C-3	1.59 (s)	20.4	1.59 (s)	20.4	1.59 (s)	20.4
12	OC=O				171.0		171.0		171.0
13	CH <sub>3</sub>		C-12	2.03 (s)	21.0	2.03 (s)	21.0	2.03 (s)	21.0
14	C=O				173.5		173.5		173.5
15	CH <sub>3</sub> -OCO		C-14	3.67 (s)	50.0	3.67 (s)	50.0	3.67 (s)	50.0





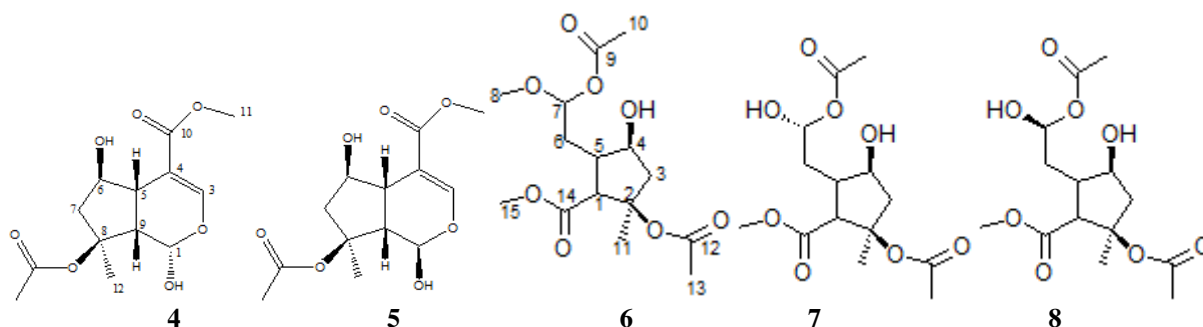


Fig 3: Chemical structures of compounds 1 to 8 isolated from *Bremeria hymenopogonoides* Baker leaves.

#### IV. DISCUSSION

One triglyceride, one acylated sterol, four secoiridoids and two aglycon iridoids have been isolated from *Bremeria hymenopogonoides* Baker leaves. Trioleine and acylated  $\beta$ -sitosterol have been reported in species of *Bremeria* genus.[5] Morisecoic acid A **3** have previously been reported from the genus *Morinda* only, [13] and thus this is the first report of their occurrence in the *Bremeria* genus. 8-acetyl-1-epishanzigenin methyl ester **4** and 8-acetylshanzigenin methyl ester **5** were obtained as an isomeric mixture. Compounds **4** and **5** have previously been reported from the family Lamiaceae and *Morinda* genus family Rubiaceae, [13,14,15] and thus this is the first report of their occurrence in the *Bremeria* genus Rubiaceae family.

Diverse biological activities have been described for some isolated compounds. Morisecoic acid A **3** showed mild antiparasitic activities against the chloroquine-sensitive malaria parasite *Plasmodium falciparum*. [13] Trioleine is a natural triglyceride. Triglycerides are anhydrous energy reserves, so there is no overweight for the body. [18] Sterols, more specifically acylated  $\beta$ -sitosterol, are endowed with antioxidant properties, which explains why this plant can reduce the loss of vitamins or essential fatty acids and above all increase the levels of antioxidants present in the body, protecting it as well as degenerative diseases. [19, 20] The amount of antioxidant necessary to decrease the initial concentration of DPPH by 50 %  $IC_{50}$  value = 22.43  $\mu$ g/ml of the ethanolic extract of *Bremeria hymenopogonoides* which is much higher than that of the ethanolic extract of *Bremeria arachnocarpa* whose  $IC_{50}$  = 10.30  $\mu$ g/ml [5]. This allows us to say that the extract of *B. arachnocarpa* has a strong antioxidant activity compared to the extract of *B. hymenopogonoides*.

All six aglycone iridoids (**3-8**) were isolated and identified in the leaves of *B. hymenopogonoides*, confirming that the family Rubiaceae is known for iridoid biosynthesis.

From the traditional uses of iridoid plants, many studies have focused on the biological and pharmacological activities of iridoids. [21, 22, 23]. Despite all these activities, no drug containing iridoids or based on the iridoid skeleton is on the market. Indeed, there are many activities but very low is the specificity of these monoterpenes. This is why these compounds are of little interest from a pharmacological point of view, but we will see that they can be used as chiral raw material for the design of bioactive molecules either by nature or in synthesis.

#### V. CONCLUSION

The results presented here constitute the first information on the chemistry study, the antioxidant and antimicrobial activity of *Bremeria hymenopogonoides* Baker 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 hymenopogonoides* Baker leaves showed the strongest DPPH scavenging activity. Its antioxidant activity is focalised in methanol extract. Via this research, a known trioleine **1**, acylated  $\beta$ -sitosterol **2**, morisecoic acid A **3**, 8-acetylshanzigenin methyl ester **5**, 8-acetyl-1-epishanzigenin methyl ester **4**, bremeseicoic acid esters A **6**, B **7** and C **8**, were isolated from *Bremeria hymenopogonoides* Baker for the first time.

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