ORIGINAL ARTICLE

PHYTOCHEMICAL AND ANTIOXIDANT EVALUATIONS OF *Psorospermum cerasifolium* Baker Hypericaceae

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ABSTRACT

Background: *Psorospermum cerasifolium* Baker is a small tree endemic of Madagascar. There leaves are used in traditional medicine for the treatment of diseases related to jaundice, wound and mycosis. **Aim of the study:** This work aims to evaluate the chemical constituents and antioxidant activity of this plant. **Materials and methods:** The chemical constituents of the hexane and ethyl acetate extracts 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 antioxidant activity of the crude ethanolic extract and hexane, ethyl acetate and methanol extracts was evaluated using DPPH° free radical scavenging. **Results:** According to this method, *Psorospermum cerasifolium* AcOEt extract ensure trapping the DPPH at 88.09 %. The value is around 531.25 mM / mg / l of extract as equivalent to α-tocopherol with the concentration of 0.5 mg / ml. The fractionation of the hexane and ethyl acetate extracts led to the isolation of five known pentacyclic triterpene lupeol **1,** taraxerol **2,** 2-hydroxylupeol **7**, acetyl aleuritolic acid **8,** putranjivic acid **9** and four phenolic compounds with one new bisphenol 2,2'-di(benzenecarboxylic) **3,** and three known compounds eriodictyol **4,** 4',7-dihydroxy-5,8-dimethoxyflavone **5** and β,2,3',4,4',6-hexahydroxy-α-(α-L-rhamnopyranosyl)dihydrochalcone **6.**

Keywords: *chalconglycoside/ flavonoids/ bisphenol/triterpenes/ antioxidant / Psorospermum cerasifolium / NMR.*

1. INTRODUCTION

In the event of a natural disease, the remedy is discovered in nature itself. Science is until then powerless to cure diseases such as malaria, HIV, AIDS, cancer and currently Covid-19. Since the existence of living beings, natural substances, such as plants, have been used to prevent and cure diseases. The search for a new drug requires empirical knowledge of drug use followed by scientific validation by chemical, biological and toxicological studies. Moreover, species of genus *Psorospermum* (Hypericaceae) have a long history of traditional use for a wide range of medical conditions, particularly rheumatism, neuralgia, vitiligo [1,2]. *Psorospermum* genus comprises over 50 species distributed mainly in exclusively in Madagascar and tropical oriental Africa. *Psorospermum cerasifolium* locally known by the names "harongampanihy, tsifady, fanerana, hazomafaika" is one of the 26 species endemics in Madagascar [3,4,5]. In traditional medicine of this island, this plant is used against diseases related to jaundice, wound and mycosis [6]. Herein, we report for the first time the isolation and identification of isolated products obtained from leaves of this plant by analysis of NMR spectra, together with the antioxidant capacity on DPPH.

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 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, $CDCl_3$ and CD_3COCD_3 solvents and TMS as internal reference.

2.2 Plant material

The leaves of *Psorospermum cerasifolium* were collected in the region of Diana, Commune of Sajoavato in the north part of Madagascar in November 2014. 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.

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2.3 Extraction

The dried leaves of *Psorospermum cerasifolium* Baker were ground into powder (450 g) and extracted with EtOH 80° by maceration for 72 hours days at room temperature, resulting in the crude ethanolic extract after evaporation under reduced pressure of the solvent. The crude ethanolic extract was subjected to phytochemical screening and antioxidant activity.

In order to separate the chemical family in *Psorospermum cerasifolium*, leaves powder (300 g) were extracted successively with hexane (2.5 L, 8 hours), ethyl acetate (2.5 L, 12 hours) and methanol (2.5 L, 12 hours) by percolation with soxhlet apparatus. The three solutions obtained are evaporated under reduced pressure to give hexane, ethyl acetate and methanol extracts.

2.4 Phytochemical screening

The detection of main classes of phyto-constituents such as alkaloids, flavonoids, saponins, anthraquinones, triterpenoids, steroids, leucoanthocyanins, tannins and coumarins were conducted on the crude ethanolic extract according to methods previously published [6, 7]. Appearance of specific colors or precipitates indicates the presence of the targeted metabolites.

2.5 Antioxidant assay

Qualitative test

The qualitative antioxidant test was carried out according to the bioautography method [8]. 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 solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol (2 mg/ml). 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 [9,10]. 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) [11].

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

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

The results were expressed by the average of 3 measurements ± standard deviation.
The percentage of inhibition thus calculated was brought back to the standard calibration curve for α-tocopherol values between 100 mM to 600 mM to express the result in trolox equivalent mM / mg / l of extract.

2.6 Isolation

The hexane extract (2.0 g) was separated by chromatography on a silica column (60 g of silica gel 60, 80x2 cm) using the eluent hexane in gradient with ethyl acetate to give 400 fractions of 10 ml. Purification by crystallization of fractions having the same TLC appearance was carried out. Two products **1** (10 mg, white amorphous powder) and **2** (4.1 mg, white amorphous powder) respectively from fractions 37 and 44 eluted with hexane / ethyl acetate 90 / 10 were obtained.

The ethyl acetate extract (2.0 g) was separated by chromatography on a silica column (60 g of silica gel 60, 80x2 cm) using the eluent hexane in gradient with ethyl acetate and methanol to give 400 fractions of 10 ml. Purification by crystallization of fractions having the same TLC appearance was carried out. Seven products **7** (10 mg, white amorphous powder), **8** (4.1 mg, white amorphous powder), **9** (4.1 mg, white amorphous powder), **3** (9 mg, white powder), **4** (12 mg, yellow powder), **5** (9.1 mg, yellow powder), **6** (10 mg, yellow powder), respectively from fractions 31-32, 37-38, 55

eluted with hexane / ethyl acetate 90 / 10, 98 eluted with hexane / ethyl acetate 60/40, 135-137 and 151-152 eluted with hexane / ethyl acetate 10/90 and 371-374 eluted with ethyl acetate / methanol 90/10 were obtained.

3. RESULTS AND DISCUSSION

3.1 Extraction

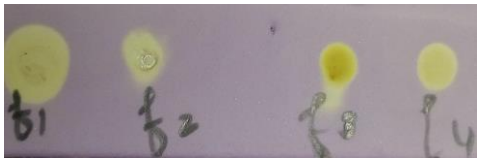
Maceration in ethanol of 450 g of the plant material yielded 47.02 g (10.44%) of crude ethanolic extract. Percolation with solvents in increased polarity of *Psorospermum cerasifolium* leaves (300 g) furnished 7.34 g (2.31%) of hexane extract, 9.94 g (3.31%) of ethyl acetate extract and 43.65 g (14.55%) of methanol extract.

3.2 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 leucoanthocyanins.

3.3 Free radical scavenging activity on DPPH·

The crude ethanolic (f1), hexane (f2), ethyl acetate (f3) and methanol (f4) extracts of *Psorospermum cerasifolium* Baker leaves contains antioxidant products (Figure 1).



Stationary phase : Silica gel 60

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

Figure 1: Qualitative antioxidant test of *Psorospermum cerasifolium* Baker leaves extracts.

The quantification of the antioxidant activity of an extract is measured by referring to the antioxidant activity of α-tocopherol by varying its concentration. This variation in absorbance as a function of α-tocopherol concentration is shown in Figure 2.

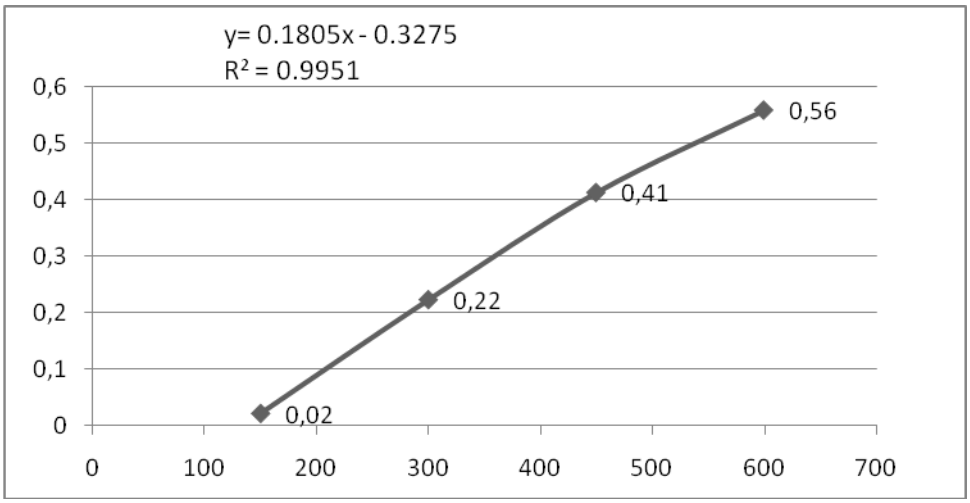


Figure 2: Standard curve of absorbance as a function of α-tocopherol concentration.

The results of the quantification of the crude ethanolic and ethyl acetate extracts are summarized in Table 1.

Table 1: Results of the quantitative test of the antioxidant activity of the crude ethanolic and ethyl acetate extracts.

Extracts	Concentration (mg/ml)	% inhibition	Vitamin E equivalent (mM/mg/l extract)
EtOH	0.5 mg/ml	87.86	529.30
AcOEt	0.5 mg/ml	88.09	531.25
Control	methanol+ DPPH	0	

According to this table, at a concentration of 0.5 mg / ml, the crude ethanolic extract inhibits the free radical of DPPH by 87.86 %. By bringing each DPPH absorbance value into the α-tocopherol trendline equation Y = 0.108X-0.3275 (R^2 = 0.9951), the ability to inhibit the free radical of DPPH for the crude EtOH extract in α-tocopherol equivalent is equal to 529.30 mM / mg / l of extract. The inhibitory activity of the crude EtOH extract is strong because almost of the DPPH is trapped. On the other hand, its antioxidant activity is focalized in the AcOEt part extract. The antioxidant activity of the ethyl acetate extract part at 0.5 mg / ml equivalent to α-tocopherol equal to 531.25 mM / mg / l of extract is very strong

because all 88.09 % of DPPH is trapped. The strong antioxidant activity of AcOEt extract is due to the presence of polyphenols and triterpenes in the plant.

3.4 Identification

The chemical isolation work was continued with the hexane and ethyl acetate extracts. Chromatography on a normal silica column of hexane extract resulted in the isolation of two compounds **1** and **2**. Chromatography on a normal silica column of ethyl acetate extract resulted in the isolation of seven compounds **7**, **8**, **9**, **3**, **4**, **5** and **6**. They are identified by concerted analysis of the recorded NMR spectra and by comparison with data in the literature. Compounds **1**, **2**, **7**, **8** and **9** are known pentacyclic triterpene identified respectively to lupeol **1** [12], taraxerol **2** [13,14], acetyl aleuritolic acid **7** (3β-acetoxy taraxer-4-en-28-oic acid) [15,16,17] , 2-hydroxylupeol **8** (lup-20(29)-en-2,3-diol) [18,19] and putranjivic acid **9** [20]. Compound **3** is a new bisphenol derived identified to 2,2′-di(benzenecarboxylic) **3**. Compounds **4** and **5** are two known flavonoids have been identified respectively as eriodictyol **4** (3′,4′,5,7-tetrahydroxyflavanone) **[21, 22]** and 4′,7-dihydroxy-5,8-dimethoxyflavone **5** [23]. Compound 6 is a known flavonoid glycoside has been identified as β,2,3′,4,4′,6-hexahydroxy-α-(α-L-rhamnopyranosyl)dihydrochalcone **6.** Lupeol and β,2,3′,4,4′,6-hexahydroxy-α-(α-L-rhamnopyranosyl)dihydrochalcone have previously isolated from *Psorospermum androaesafolium* [24]. Their chemical structures are shown in figure 3.

Product 1: white amorphous powder

δ (ppm) ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm : 4.71 (1H,s,H-29); 4.59 (1H, s,H-29); 3.21 (1H,t,H-3); 2.39 (1H, m,H-19); 1.87 (1H,m,H-21β); 1.70 (1H,m,H-15β); 1.65 (1H,m,H-2β); 1.71 (3H,s,H-30); 1.60 (2H,m,H-1β,H-16β); 1.55 (2H,m,H-13); 1.47 (1H, m,H-6β); 1.45 (1H,m,H-2α); 1.35(1H,m,H-12β); 1.32(1H,m,H-6α); 1.30(1H,m,H-21α); 1.28(1H,m,H-18); 1.25 (2H,m,H-7); 1.20 (3H,m,H-9,H-16α,H-22α); 1.15 (1H,m,H-12α); 1.11(1H,m,H-22β); 1.06 (3H,s,H-26); 0.99 (3H,s,H-24); 0.97 (3H,s,H-27); 0.90 (1H,m,H-1α); 0.85 (2H,m,H-11β,H-15α); 0.85 (3H,s,H-25); 0.81 (3H,s,H-28); 0.79 (3H,s,H-23); 0.72(1H,m,H-11 α); 0.59(1H,m,H-5). ^{13}C NMR(125.78MHz, $CDCl_3$) δ ppm (DEPT) : 151.2 (C-20); 109.3 (C-30); 79.0 (C-3); 55.5 (C-5); 50.3 (C-9); 48.3 (C-18); 48.0 (C-19); 42.9 (C-17); 42.8 (C-14); 40.8(C-8); 40.0 (C-22); 38.8 (C-13); 38.7 (C-4); 38.1 (C-1); 37.2 (C-10); 35.6 (C-16); 34.3 (C-7); 29.9 (C-21); 27.9 (C-24); 27.5 (C-2); 27.4 (C-15); 25.1 (C-12); 20.9 (C-11); 19.3 (C-30); 18.3 (C-6); 18.0 (C-28); 16.1(C-26); 15.9(C-25); 15.4 (C-23); 14.7 (C-27).

Product 2: white amorphous powder

δ (ppm): ^{1}H NMR ($CDCl_3$, 600 MHz) δ(ppm): 5.56(1H,t,H-15); 3.23(1H,t,H-3); 2.06(1H,d,H-7β); 1.92(2H,d,H-16); 1.67(2H,d,H-2); 1.63(2H,d,H-1α, H-12β); 1.62(1H,d,H-6β); 1.57(1H,d,H-12α); 1.54(1H,d,H-6α); 1.43(1H,d,H-9); 1.37(1H,m,H-22β); 1.36(1H,d,H-7α); 1.35(1H,d,H-21β); 1.33(1H,d,H-19β); 1.25(1H,d,H-21α); 1.11(3H, s, H-27); 1.02(1H,d,H-22α); 1.00 (3H, s,H-24); 0.99(1H,d,H-19α); 0.97(1H,d,H-1α); 0.96(6H,s,H-18,H-29); 0.94(3H, s,H-25); 0.92(6H, s, H-26,H-30); 0.84(3H, s,H-28); 0.82(3H, s,H-23). ^{13}C NMR ($CDCl_3$, 125.78 MHz) δ(ppm):158.0(C-14); 116.8(C-15); 79.0(C-3); 55.4(C-5); 49.4(C-9); 48.8(C-18); 41.3(C-7); 39.0(C-8); 38.7(C-4); 38.0(C-10); 37.7(C-16); 37.6(C-1); 37.5(C-13); 36.7(C-19); 35.8(C-17); 35.1(C-22); 33.7(C-12); 33.4(C-29); 33.1(C-21); 29.9(C-30); 29.8(C-28); 28.8(C-20); 28.0 (C-24); 27.1(C-2); 25.9(C-27); 21.3(C-26);18.8(C-6); 17.5(C-11); 15.5(C-23); 15.4(C-25).

Product 4: Yellow powder

δ (ppm) ^{1}H NMR (600MHz, CD_3OD): 6.93 (1H, d, H-2′); 6.80 (2H, d, H-5′, H-6′); 5.91 (1H, s, H-8); 5.88 (1H, s, H-6); 5.28 (1H, t, H-2); 3.03 (1H, s, H-3); 2.67 (1H, s, H-3). δ (ppm) ^{13}C NMR (125.78 MHz, CD_3OD): 196.5 (CO); 167.0 (C-7); 164.0 (C-5); 163.4 (C-8a); 145.4 (C-4′); 145.0 (C-3′); 130.3 (C-1′); 117.9 (C-6′); 114.9 (C-5′); 113.3 (C-2′); 101.9 (C-4a) ; 95.6 (C-6); 94.8 (C-8); 79.0 (C-2); 42.7 (C-3).

Product 5: Yellow powder

δ (ppm) ^{1}H NMR (600MHz, $CDCl_3$): 7.56 (2H, d, H-2′,H-6′); 6.73 (2H, d, H-3′, H-5′); 6.37 (1H, s, H-6); 6.36 (1H, s, H-3); 3.74 (3H, s, 5-OCH3); 3.65 (3H, s, 8-OCH3). δ (ppm) ^{13}C NMR (125.78 MHz, $CDCl_3$): 182.4 (CO); 164.9 (C-2); 160.7 (C-4′); 158.7 (C-5); 152.7 (C-7); 152.1 (C-8a); 132.2 (C-8); 128.3 (C-2′, C-6′); 121.9 (C-1′); 115.7 (C-3′; C-5′); 105.5 (C-4a) ; 102.6 (C-3); 90.7 (C-6); 60.6 (8-OCH_3); 56.0 (5-OCH_3).

Product 6: Yellow powder

δ (ppm) ^{1}H NMR (600MHz, CD_3OD): 5.93 (1H, s, H-3); 5.91 (1H, s, H-5); (5.08 (1H, d, H-β); 4.59 (1H, d, H-α)); 6.97 (1H, s, H-2′); 6.86 (1H, d, H-6′); 6.83 (1H, d, H-5′); 4.2 (1H, s, H-5");4.09 (1H, m, H-1");3.63 (1H, m, H-3") ; 3.56 (1H, m, H-2") ; 3.30 (1H, m, H-4");1.20 (3H, m, H-6"). δ (ppm) ^{13}C NMR(125.78 MHz, CD_3OD): 194.3 (C=O); 167.2 (C-4); 164.2 (C-6); 162.6 (C-2)); 101.2 (C-1)); 95.6 (C-3); 94.9 (C-5)); 82.2 (C-β)); 77.4 (C-α); 145.0 (C-3′); 143.2 (C-4′); 127.5 (C-1′); 119.2 (C-6′); 115.1 (C-5′);113.7 (C-2′); 100.4 (C-1");72.2 (C-4");70.6 (C-3");70.2 (C-2");68.5 (C-5");16.4 (C-6").

Product 7: white amorphous powder

δ (ppm): ^{1}H NMR ($CDCl_3$, 600 MHz) δ(ppm): 5.52 (H-15, dd, J = 3.19, 7.77 Hz, 1H); 4.47 (H-3, dd, J = 6.54, 9.18 Hz, 1H); 2.04 (H-2′, s, 3H); 0.95 (H-25, H-26 ,s, 6H); 0.94 (H-29 ,s, 3H); 0.92 (H-30, s, 3H); 0.91 (H-27, s, 3H); 0.88(H-24, s, 3H); 0.85 (H-23, s, 3H). ^{13}C NMR ($CDCl_3$, 125.78 MHz) δ(ppm):183.9(C-28); 171.3(C-1′); 160.5(C-14); 116.7(C-15); 80.6(C-3); 55.7(C-5); 51.7(C-17); 49.2(C-9); 41.5 (C-18); 40.9 (C-7); 39.3 (C-8); 38.1 (C-10); 37.7 (C-4); 37.5 (C-13); 37.4 (C-1); 35.3 (C-19); 33.7 (C-21); 33.5 (C-12); 31.9 (C-29); 31.5 (C-16); 30.9 (C-22); 29.9 (C-20); 28.6 (C-30); 28.1 (C-23); 26.3 (C-26); 23.7 (C-2); 22.6(C-27); 21.4 (C-2′); 18.8(C-6); 17.5(C-11); 16.8 (C-24); 15.8 (C-25).

Product 8: white amorphous powder

δ (ppm): ^{1}H NMR (CD_3COCD_3, 600 MHz) δ(ppm): 4.72 (1H,s,H-29); 4.58 (1H, s,H-29); 3.34 (1H,t,H-2); 3.15 (1H,d,H-3); 2.34 (1H, m,H-19); 1.71 (1H,s,H-30); 1.01 (3H,s,H-24); 1.08 (3H,s,H-26); 0.98 (3H,s,H-27); 0.88 (3H,s,H-25); 0.82 (3H,s,H-23); 0.79 (3H,s,H-28).

Product 9: white amorphous powder

δ (ppm): ^{1}H NMR ($CDCl_3$, 600 MHz) δ(ppm): δ ppm : 5.65 (1H,dd,H-4); 4.93 (1H,dd,H-23); 4.90 (1H, dd,H-23); 2.35((2H, t,H-2); 1.57 (2H,t,H-16); 1.56 (1H, t,H-18); 1.50 (3H,t,H-22β, H-21); 1.44 (2H, t,H-6); 1.43 (1H,t,H-1 α); 1.41 (1H,m,H-19β); 1.37(2H,m,H-10); 1.35 (1H,m, H-8); 1.32 (2H,s,H-11); 1.30 (2H,s,H-15); 1.22(1H,m,H-19α); 1.19(3H,s,H-28); 1.043(3H,s,H-27); 1.017 (3H,s,H-30); 1.016 (3H,s,H-26); 1.011 (3H,s,H-24); 0.97 (3H,s,H-29); 0.95(1H,s,H-22α); 0.92 (1H,t,H-10); 0.90 (3H,s,H-25). ^{13}C NMR(125.78 MHz, $CDCl_3$) δ ppm (DEPT) : 177.7 (C-3); 151.2 (C-4); 110.9 (C-23); 58.3 (C-10); 52.8 (C-8); 42.8 (C-18); 42.1 (C-5); 41.5 (C-6); 39.6 (C-13); 39.3 (C-22); 38.7(C-9); 38.3 (C-14); 36.7 (C-2); 36.1 (C-16); 35.3 (C-11); 35.2 (C-19); 34.9 (C-29); 32.8 (C-21); 32.2 (C-15); 32.1 (C-28); 31.8 (C-30); 30.2 (C-12); 29.9 (C-17); 28.2 (C-20); 21.0 (C-21); 20.1 (C-26); 18.9 (C-27); 18.2 (C-24); 18.0(C-25); 17.9(C-7).

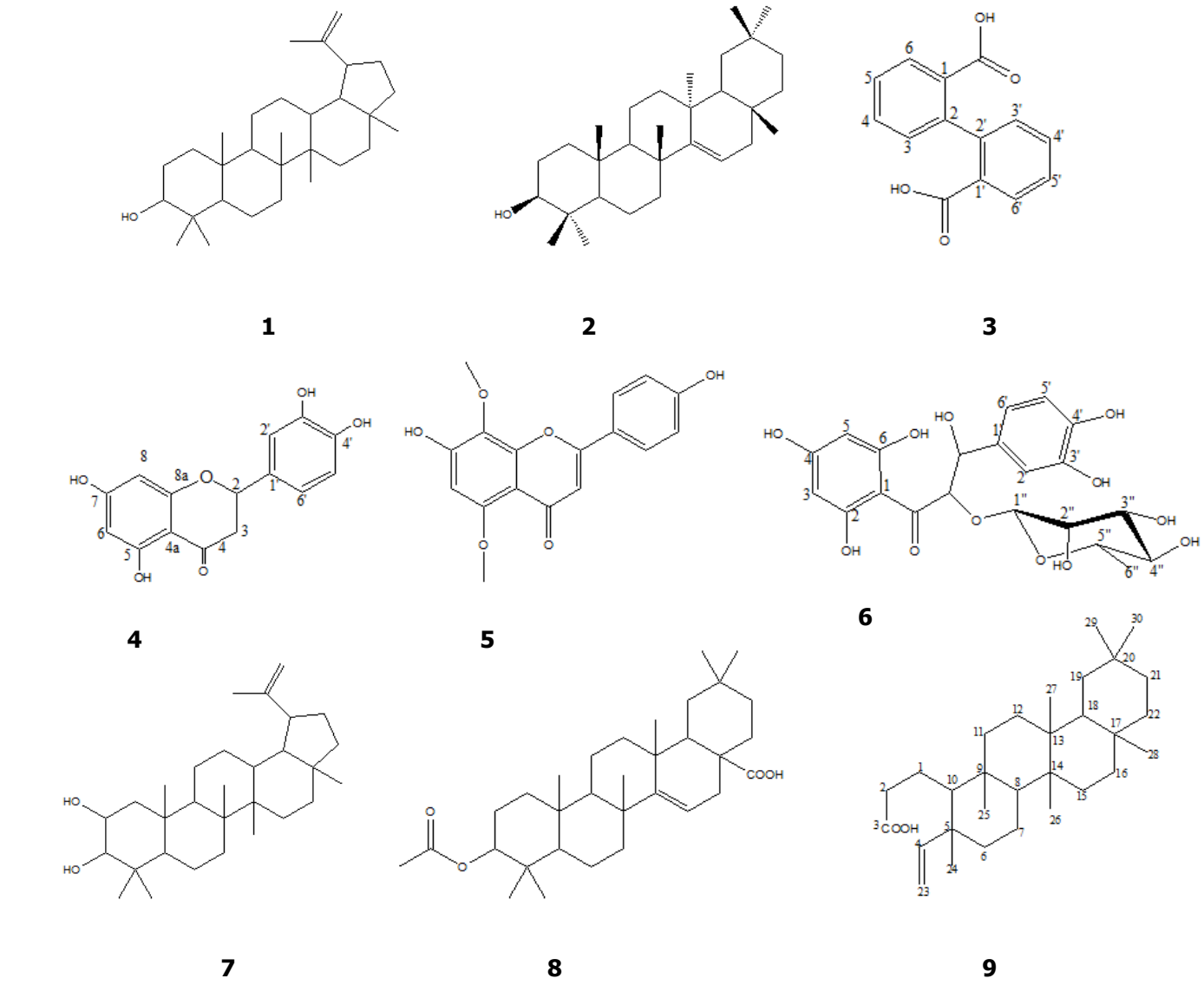


Figure 3: Chemical structures of compounds 1 to 9 isolated from *Psorospermum cerasifolium* leaves.

Product 3: white amorphous powder

δ (ppm) ^{1}H NMR (600MHz, $CDCl_3$): 10.31 (2H, s, -OH); 7.87 (2H, d, H-3, H-3′); 7.46 (2H, t, H-5,H-5′); 6.95 (2H, d, H-6, H-6′); 6.87 (2H, t, H-4,H-4′). δ (ppm) ^{13}C NMR(125.78 MHz, $CDCl_3$): 174.8 (COOH); 162.4 (C-1,C-1′); 137.2 (C-5,C-5′); 131.1 (C-3, C-3′); 119.6 (C-4, C-4′); 117.7 (C-6, C-6′); 111.3 (C-2, C-2′).

The ^{1}H NMR spectrum of compound 3 showed signals consistent with the presence of a chelated hydroxyl group (δ 10.31, s) and four aromatic protons (δ 7.87, d, H-3, H-3′; 7.46, d, H-5, H-5′; 6.95, d, H-6, H-6′; 6.87, d, H-4, H-4′). The ^{13}C NMR spectrum of 3 disclosed 7 carbon resonances consistent with a ketone carbonyl (δ 174.8, C-4), two substituted sp2 hybridized carbons (δ 162.4, C-1, C-1′, 111.3, C-2, C-2′) and four aromatic methines (δ 137.2, C-5, C-5′; δ 131.1, C-3, C-3′; δ 119.6, C-4, C-4′; δ 117.7, C-6, C-6′). Taking into account, the ^{1}H and ^{13}C NMR spectra, and the HMBC spectrum, we characterized compound 3 as a 2,2′-di(benzenecarboxylic).
The aromatic methine protons at H-3, H-3′ and at C-5, C-5′ were assigned as follow. In the HMBC spectrum, the H-3 proton signal at δ 7.87 (H-3′ proton signal at δ 7.87) exhibited strong 4J coupling with carbon signals at δ 162.4 (C-1,C-1′), 137.2 (C-5/C-5′) and 174.8 (COOH). Similarly, the H-5 proton signal at δ 7.46 (H-5′ proton signal at δ 7.46) exhibited strong 4J coupling with carbon signals at δ 131.1 (C-3,C-3′), 162.4 (C-1/C-1′). The H-5 proton signal at δ 6.95 (H-5′ proton signal at δ 6.95) exhibited strong 4J coupling with carbon signals at δ 174.8 (COOH), 119.6 (C-4/C-4′) and 111.3 (C-2,C-2′). Similarly, the H-4 proton signal at δ 6.87 (H-4′ proton signal at δ 7.46) exhibited strong 4J coupling with carbon signals at δ 117.7 (C-6,C-6′), 111.3 (C-2/C-2′). From these spectroscopic a disubstituted benzene was established for 3. The attachment of the carboxyl group at C-1 was corroborated by the observation of HMBC cross-peaks between the chelated hydroxyl group at δ 10.31 with 162.4 (C-1), 117.7 (C-6), 111.3 (C-2). Key correlations observed in the HMBC spectrum leading to the structure of 3 are shown in figure 4. The existence of C-4 and C-4′ concluded that compound **3** is a bimolecular. Accordingly, compound **3** was concluded to be 2,2′-di(benzenecarboxylic).

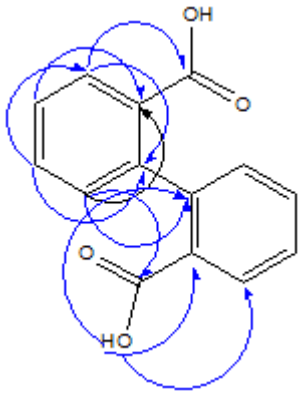


Figure 4: Key HMBC correlations observed in **3**

4. CONCLUSION

The results presented here constitute the first information on the chemistry study and the antioxidant activity of *Psorospermum cerasifolium* leaves. This study reveals that the crude ethanolic extract of *Psorospermum cerasifolium* leaves showed the strongest DPPH scavenging activity. Its antioxidant activity is focalised in ethyl acetate extract. Via this research, one new bisphenol 2,2′-di(benzenecarboxylic) and eight known compounds lupeol, taraxerol, 2-hydroxylupeol, acetyl aleuritolic acid, putranjivic acid, eriodictyol, 4′,7-dihydroxy-5,8-dimethoxyflavone and β,2,3′,4,4′,6-hexahydroxy-α-(α-L-rhamnopyranosyl)dihydrochalcone , were isolated from *Psorospermum cerasifolium* leaves for the first time.

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