Secondary metabolites from *Desmodium ramosissimum* G. Don (Fabaceae) and their radical scavenging properties

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Abstract

This work was designed to carry out the phytochemical study and the radical scavenging screening of the MeOH extract from the whole plant of *Desmodium ramosissimum* used in traditional medicine for the treatment of malaria, fever, diarrhea, lung disorders and venereal diseases. The crude extract was subjected to silica gel open column chromatography (CC) follow by repeated silica gel CC purification and / or Sephadex LH-20 to afford nine compounds, D-pinitol (1), mixture of *β*-sitosterol (2) and stigmasterol (3), *β*-sitosterol-3-*O*-*β*-D-glucopyranoside (4), glyceryl-1-tetracosanoate (5), lutein (6), kaempferol (7), catechin (8) and vitexin (9). The structures of these compounds were identified after analysis of their NMR and MS data and comparison with the literature. The crude extract, fractions III, IV and V displayed constituents possessing radical scavenging activities using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) bioautography while vitexin (9), and catechin (8) showed activity among the isolated compounds.

Keywords: *Desmodium ramosissimum*; constituents; Radical scavenging, Vitexin; Kaempferol

Introduction

The human organism permanently produces reactive oxygen species (ROS) that cause several cellular disorders because of their high reactivity with proteins, DNA and membrane fatty acids resulting to different inflammatory and cardiovascular diseases, rheumatoid arthritis, neurodegenerative diseases and the aging process. The physiological production of ROS is regulated by enzymes and low molecular weight compounds with antioxidant properties (Meyer et al., 1998; Hollman and Katan 1999; Moure et al., 2001; Hunt et al., 2001).

Desmodium ramosissimum (also named as *D. mauritiamun* or *Hedysarum fruticulosum*) a member of Fabaceae family is an herbal or shrub of 10-150 cm in height, mostly found in tropical Africa (Lebrun and Fotius, 1967). It is used in African ethnomedicine for the treatment of several diseases such as malaria (Benin), headache, diarrhea, dysentery, fever, pulmonary disorders, cough, venereal diseases and jaundice (Nigeria) (Yetein et al., 2013, Alli et al., 2011). Plants species and constituents of the genus *Desmodium* has been reported to possess several pharmacological properties such as antibacterial, antidiabetic, anti-inflammatory, cytotoxic, antinephrolithic, nootropic, and lipid peroxidation activities (Ma et al., 2011; Tsafack et al., 2018). Previous phytochemical investigations carried out on the genus *Desmodium* revealed the presence of flavonoids, phenols, anthraquinones, tannins, and triterpene saponins (Alli et al., 2011; Lugudu and Owk, 2016; Thankachan et al., 2017; Tsafack et al., 2017). To the best of our knowledge, no phytochemical study has so far been achieved from this plant species. In the course of our continuing search for bioactive secondary metabolites from medicinal plant species of the family Fabaceae (Awouafack et al., 2015, 2016, 2018; Nguekeu et al., 2017), we investigated the MeOH extract of the whole plant of *D. ramosissimum* to isolate nine compounds namely D-pinitol (1), mixture of *β*-sitosterol (2) and stigmasterol (3), *β*-sitosterol-3-*O*-*β*-D-glucopyranoside (4), glyceryl-1-tetracosanoate (5), lutein (6), kaempferol (7), catechin (8) and vitexin (9). This is the first report on the isolation of secondary metabolites from this plant species.

2. Methods

2.1. General experimental procedures

IR spectra were recorded on a SHIMADZU FT-IR 8400S spectrometer. MS data were measured on SHIMADZU LCMS-IT-TOF spectrometer (Japan). NMR spectra were recorded on 500 MHz (^{1}H) and 125 MHz (^{13}C) on JEOL 500 Spectrometer with TMS as internal reference. Column chromatography was performed on silica gel MERCK 60 F_{254} [(0.2–0.5 mm)] 70–230 and 230–400 mesh (Germany) and Sephadex LH-20. Pre-coated silica gel 60 F_{254} thin layer chromatography (TLC) plates (Germany) were used for monitoring fractions and spots were detected with UV lights (254 and 366 nm) and further sprayed with 50% H_2SO_4 or vanillin-H_2SO_4 reagent followed by heating at 150°C. Solvents used for chromatography were distilled prior to use.

2.2. Plant Materials

The whole plant of *D. ramosissimum* were collected in Dschang, West Region of Cameroon, on October 2017 and were identified by Mr. Victor Nana, a botanist at the Cameroon National Herbarium in Yaoundé where our sample was compared to the available specimen having a voucher number 40528/HNC.

2.3. Extraction and isolation

The dried and powdered plant of *D. ramosissimum* (3.42 kg) was extracted by maceration for three days in methanol (13 L x 3 times) to give the crude extract (287 g) after filtration and removal of the solvent using rotary evaporator. Parts of the extract (150 g and 136 g) were separately subjected to similar column chromatographies (CC) over silica gel (SiO_2) using solvent systems made of *n*-Hex/EtOAc, and EtOAc/MeOH, in gradient polarities to afford 65 fractions of 600 mL each that were pooled into six main fractions (Fr.I-VI) after TLC monitoring. The combined fraction $Fr.V_{51-53}$ [26 g, EtOAc/MeOH (8:2)] formed a white powder in methanol to afford 1 (300 mg) after filtration. A part of Fraction II [6 g, *n*-Hex/EtOAc (4.5:5.5)] was subjected to SiO_2 CC using *n*-Hex/EtOAc, and EtOAc/MeOH, in gradient polarities to afford 35 sub-fractions of 200 mL each. Combined sub-fractions $Fr.II_{14-15}$ formed a white powder in *n*-hexane to give a mixture of 2 and 3 (90 mg) after filtration while combined sub-fractions $Fr.II_{32-35}$ was purified by further SiO_2 CC using isocratic solvent system made of *n*-Hex/AcOEt (8.5:1.5) to afford 7 (0.7 mg). The two major compounds [5 (28 mg) and 6 (17 mg)] of the combined sub-fractions $Fr.II_{23-31}$ were separated and purified by successive SiO_2 CC using isocratic solvent system [*n*-Hex/AcOEt (8.5:1.5)] follow by Sephadex LH-20 with MeOH as mobile phase. A part of fraction III [6.5 g, *n*-Hex/EtOAc (4:6)] was subjected to SiO_2 CC using *n*-Hex/EtOAc, and EtOAc/MeOH, in gradient polarities to afford 162 sub-fractions of 75 mL each. The combined sub-fractions $Fr.III_{91-127}$ [2 g, EtOAc/MeOH (8.5:1.5)] formed a white powder in EtOAc to afford 4 (60 mg) after filtration whereas the combined sub-fractions $Fr.III_{77-90}$ [0.8.g, EtOAc/MeOH (9.5:0.5)] was subjected to SiO_2 CC eluting with an isocratic solvent system [*n*-Hex/AcOEt (7.5:2.5)] to give 60 fractions of 50 mL each. The combined sub-fractions 30–39 from the CC of sub-fractions $Fr.III_{77-90}$ was purified by Sephadex LH-20 using methanol to afford 8 (19 mg). A part of fraction IV [59 g, EtOAc/MeOH (8.5:1.5)] was subjected to SiO_2 CC using *n*-Hex/EtOAc, and EtOAc/MeOH, in gradient polarities to afford 238 fractions of 75 mL each. The combined sub-fractions $Fr.IV_{172-185}$ formed a yellow powder in a mixture of acetone/methanol (9.5:0.5) to afford 9 (38.5 mg) after filtration.

2.4. D-Pinitol (1) : white powder in MeOH; IR (KBr) cm^{-1}: O-H (3502 - 3207) and C-O (1280 – 1080); ^1H NMR (500 MHz, DMSO-d_6): δ_H 3.63 (br.s, H-1) 3.50 (m, H-2), 3.00 (t, J = 9.0 Hz, H-3), 3.34 (m, H-4), 3.44 (m, H-5), 3.59 (br.s, H-6) 3.39 (s, 3-OCH_3), 4.61 (br.s, 1-HO), 4.45 (d, J = 6.0 Hz, 2-OH), 4.50 (d, J = 3.5 Hz, 4-OH), 4.32 (d, J = 5.0 Hz, 5-OH), 4.70 (br.s, 6-OH) and ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 83.8 (C-3), 72.6 (C-4), 72.4 (C-1), 72.0 (C-5), 71.9 (C-6), 70.1 (C-2), 59.7 (3-OCH_3); HR-ESIMS (+): *m/z* 217.0631 ($[M+Na]^+$), (calcd for $C_7H_{14}O_6Na$ 217.0688).

2.5.β-Sitosterol (2) and stigmasterol (3): white powder in *n*-Hex; IR (KBr) cm^{-1} O-H (3473 - 3325), C-H (2974 - 2835) and C-O (1058).

2.6. β-Sitosterol-3-O-β-D-Glucopyranoside (4): white powder in EtOAc; IR (KBr) cm^{-1} : O-H (3400), C-H (3000-2800) and C-O (1107).

2.7.Glyceryl-1-tetracosanoate (5): white powder in *n*-Hex/EtOAc (8.5:1.5); IR (KBr) cm^{-1} O-H (3253), C-H (2956-2848), C=O (1731) and C-O (1180); ^1H NMR (500 MHz, $CDCl_3$): δ_H 4.65 (dd, J = 5.0; 11.5 Hz, H-1), 4.58 (dd, J = 6.5; 11.5 Hz, H-1), 4.36 (m, H-2), 4.06 (t, J = 5.0 Hz, H-3), 2.29 (t, J = 7.5 Hz, H-2′), 1.58 (m, H-3′), 1.23-1.17 (br.s, H-4' – H-22'), 1.16 (m, H-23), 0.79 (t, J = 6.5 Hz, H-24′) and ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 173.8 (C-1′), 70.8 (C-2), 66.6 (C-1), 64.2 (C-3), 34.2 (C-2′), 25.1 (C-3′), 29.1-31.9 (C-4′ - C-22′), 22.8 (C-23′), 14.1 (C-24′); HR-ESIMS (+): *m/z* 465.3846 ($[M+Na]^+$) (calcd for $C_{27}H_{54}O_4Na$ 465.3846).

2.8. Lutein (6): orange powder in *n*-Hex/EtOAc (8.5:1.5); IR (KBr) cm^{-1} O-H (3440-3469), C-H (2918), C=C (1666) and C-O (1365); ^1H NMR (500 MHz, $CDCl_3$): 1.74 (m, H-2), 1.46 (m, H-2), 4.16 (ddd, J = 4.5; 9.5; 11.5 Hz, H-3), 2.34 (m, H-4), 6.09 (d, J =13.2 Hz, H-7), 6.11 (d, J =13.2 Hz, H-8), 6.24 (m, H-10), 6.56-6.65 (m, H-11), 6.35 (m, H-12), 6.24 (m, H-14), 6.56-6.65 (m, H-15), 1.05 (s, H-16), 1.05 (s, H-17), 1.72 (s, H-18), 1.89 (s, H-19), 1.95 (br.s, H-20), 1.34 (m, H-2′), 1.81(m, H-2′), 4.24 (br.s, H-3′), 5.52 (s, H-4′), 2. 40 (br.s, H-6′), 5.41 (dd, J =10.0; 16.0 Hz, H-7′), 6.13 (m, H-8′), 6.13 (m, H-10′), 6.56-6.65 (m, H-11′), 6.34 (m, H-12′), 6. 24 (m, H-14′), 6.56-6.65 (m, H-15′), 0.98 (s, H-16′), 0.83 (s, H-17′), 1.60 (s, H-18′), 1.95 (br.s, H-19′), 1.95 (br.s, H-20′) and ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 37.3 (C-1), 48.5 (C-2), 65.1(C-3), 42.6 (C-4), 126.2 (C-5), 137.8 (C-6), 125.6 (C-7), 138.6 (C-8), 135.8 (C-9), 131.4 (C-10), 124.9 (C-11), 137.6 (C-12), 136.5 (C-13), 132.6 (C-14), 130.3 (C-15), 28.8 (C-16), 30.3 (C-17), 21.7 (C-18), 12.9 (C-19), 12.8 (C-20), 34.1 (C-1′), 44.7 (C-2′), 65.9 (C-3′), 124.7 (C-4′), 138.0 (C-5′), 55.0 (C-6′), 128.9 (C-7′), 137.9 (C-8′), 135.1 (C-9′), 130.9 (C-10′), 124.9 (C-11′), 137.6 (C-12′), 136.5 (C-13′), 132.6 (C-14′), 130.3 (C-15′), 24.3 (C-16′), 29.7 (C-17′), 22.9 (C-18′), 13.2 (C-19′), 12.8 (C-20′).

2.9. Kaempferol (7): yellow powder in EtOAc; IR (KBr) cm^{-1} O-H (3213), C=O (1664) and aromatic ring (1571); ^1H NMR (500 MHz, $CDCl_3$): δ_H 12.14 (1H, s, 5-OH), 6.24 (1H, d, J = 1.5 Hz, H-6), 6.52 (1H, d, J = 1.5 Hz, H-8), 8.12 (2H, d, J = 8.5 Hz, H-2′/H-6′), 6.99 (2H, d, J = 8.5 Hz, H-3′/H-5′); HR-ESIMS (+): *m/z* 287.0540 ($[M+H]^+$) (calcd for $C_{15}H_{11}O_6$ 287.0556).

2.10. Catechin (8): white powder in methanol; IR (KBr) cm^{-1} O-H (3602), aromatic ring (1625), C-O (1147-1081) and C-H (3049); ^1H NMR (500 MHz, DMSO-d_6): δ_H 4.43 (d, J = 7,5 Hz, H-2), 3.77 (m, H-3), 2.60 (dd, J = 5.0; 16.0 Hz, H-4), 2.30 (dd, J = 7.5; 16.0 Hz, H-4), 5.84 (d, J = 2.0 Hz, H-6), 5.64 (d, J = 2.0 Hz, H-8), 6.64 (d, J = 1.5 Hz, H-2′), 6.68 (d, J = 8.0 Hz, H-3′/ H-5′), 6.55 (dd, J = 8.0; 1.5 Hz, H-6′), 4.84 (d, J =5.0 Hz, 3-OH), 9.16 (s, 5-OH), 8.92 (s, 7-OH), 8.84 (s, 3′-OH), 8.79 (s, 4′-OH). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 81.3 (C-2), 66.8 (C-3), 156.6 (C-5), 95.6 (C-6), 156.9 (C-7),

94.4 (C-8), 155.8 (C-9), 99.6 (C-10), 131.1 (C-1′), 114.9 (C-2′), 145.4 (C-3′/C-4′), 115.6 (C-5′), 118.9 (C-6′); *m/z* 289.0722 ([M-H]$^-$) (calcd for $C_{15}H_{13}O_6$ 289.0712).

2.11.Vitexin (10): yellow powder in acetone/methanol (95:5); IR (KBr) cm^{-1} O-H (3238-3390), C=O (1708) et aromatic ring (1662); ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 13.13 (s, 5-OH), 6.75 (s, H-3), 6. 24 (s, H-6), 7.99 (d, *J* = 8.5 Hz, H-2′/ H-6′), 6.87 (d, *J* = 8.5 Hz, H-3′/ H-5′), 4.66 (d, *J* = 10.0 Hz, H-1″), 3.80 (t, *J* = 9.0 Hz, H-2″), 3.22 (m, H-3″), 3.73 (m, H-4″), 3.13 (m, H-5″), 3.75 (dd, *J* = 7.0; 11.0 Hz, H-6″), 3.51 (d, *J* =12.0 Hz, H-6″), and ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 164.5 (C-2), 103.0 (C-3), 182.6 (C-4), 160.9 (C-5), 98.6 (C-6), 163.1 (C-7), 105.1 (C-8), 156.5 (C-9), 104.6 (C-10), 122.1 (C-1′), 129.5 (C-2′/C-6′), 116.4 (C-3′/C-5′), 161.1 (C-4′), 73.9 (C-1″), 71.3 (C-2″), 79.0 (C-3″), 70.8 (C-4″), 82.1 (C-5″), 61.5 (C-6″); HR-ESIMS (+): *m/z* 433.1223 ([M+H]$^+$), HR-ESIMS (-): *m/z* 431.1009 ([M-H]$^-$), 863.2097 ([2M-H]$^-$).

2.12. Radical scavenging screening

The radical scavenging screening was evaluated using the pre-colated TLC silica gel 60 F_{254} (Merck, Germany), with mobile phases made of *n*-hexane, EtOAc and MeOH, while a methanolic solution of (0.2%) DPPH (Wako, Japan) was used as spraying reagent to reveal the activity. Briefly, 10 µL of each sample dissolved in MeOH at concentrations of 10 mg/mL (crude extract and fractions), or 1mg/mL [pure compounds and the standard reference, catechin (wako, Japan)] were spotted on TLC plates and eluted with three mobile phases [*n*-Hex/EtOAc (7.5/1.5), EtOAc/MeOH (9.8:0.2), and EtOAc/MeOH (9.2:0.8)]. Thereafter, the plates were dried and sprayed with the DPPH solution and the appearance of yellow spots as well as the intensity of the color materialized the presence, the number of constituents (for extract and fractions) and the importance of the radical scavenging activity of the sample (Takao *et al*., 1994).

3. Results and Discussion

The MeOH extract of *D. ramosissimum* was subjected to column chromatography techniques to afford nine compounds that were identified as D-pinitol (1) (Raya-Gonzalez et al., 2008), mixture of *β*-sitosterol (2) and stigmasterol (3), *β*-sitosterol-3-*O*-*β*-D-glucopyranoside (4), glyceryl-1-tetracosanoate (5) (Sultana et al., 1999), lutein (6) (Kengne et al., 2016), kaempferol (7) (Xiao et al., 2006), catechin (8) (Almahy and Alhassan, 2011), and vitexin (9) (Cuong et al., 2015) (Figure 1). Their structures were identified by comparison of their spectroscopic data mainly NMR and MS to those reported in the literature.

The radical scavenging activity of the crude extract (CE), some of main fractions (F_{II}-F_V), and some isolated compounds (2, 5, 6, 8, 9) was determined using the free radical DPPH. The crude extract, fractions F_{III}-F_V displayed spots indicating the presence of constituents with good radical scavenging activity while fraction F_{II} was weakly active (Figure 2). Among the isolated compounds, vitexin (9), and catechin (8) had the highest radical scavenging activity and could be appreciated by the intensity of their spots on TLC after spraying with DPPH (Figure 2).

The potential of compounds 8 and 9 for scavenging free radicals is owed to their flavonoid nature. Several mechanisms have been reported on such class of compounds to elucidate how their radical scavenging activity occurred. The highest activity of catechin (8) and vitexin (9) could be justified by the *o*-disubstitution hydroxyl groups in their B-ring (Amić et al., 2003; Hassan et al., 2014; Grzesik et al., 2018).

Flavonoids have been reported as one of the most important class of secondary metabolites in the genus *Desmodium*, thus our results on the isolation and identification of flavonoids such as kaempferol (7), catechin (8) and vitexin (9) in *D. ramosissimum* have chemotaxonomic significance at the genus level.

4. Conclusion

The phytochemical study of the MeOH extract of *D. ramosissimum* was carried out to identify nine secondary metabolites including D-pinitol (1), mixture of *β*-sitosterol (2) and stigmasterol (3), *β*-sitosterol-3-*O*-*β*-D-glucopyranoside (4), glyceryl-1-tetracosanoate (5), lutein (6), kaempferol (7), catechin (8) and vitexin (9). The good activity of catechin (8) and vitexin (9) could justified the used of this plant species as good antioxidant agent in the treatment of some diseases and further study as cytotoxicity should be conducted in view to associate such plant in food supplement development.

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Author contributions

R.A.D.D., and D.H.S.F. carried out the experimental part with the help of Y.M.M.N., while P.T., H.M., and M.D.A. supervised the work. M.D.A. also carried out the NMR and MS analyses and edited the final version of the manuscript.

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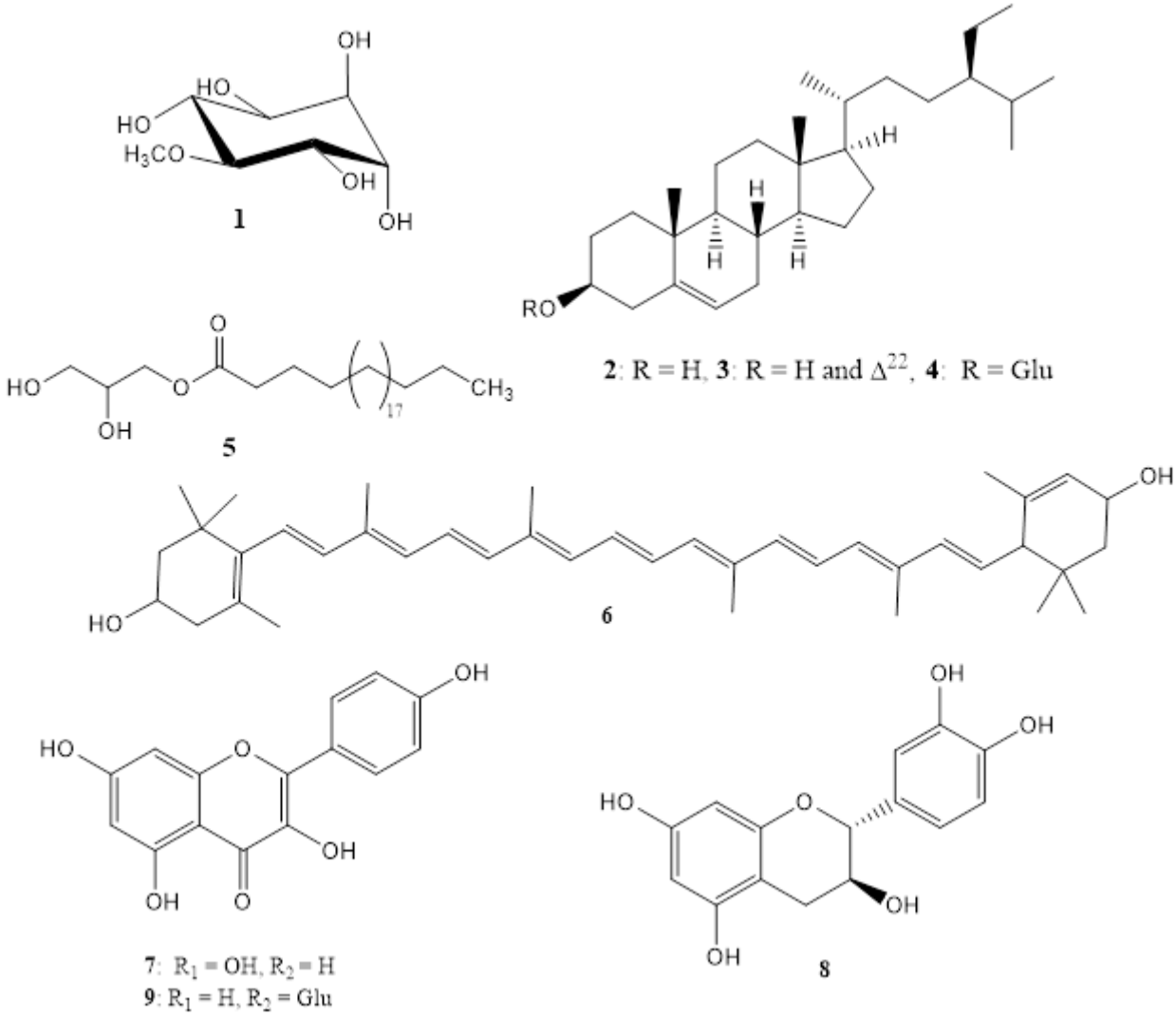
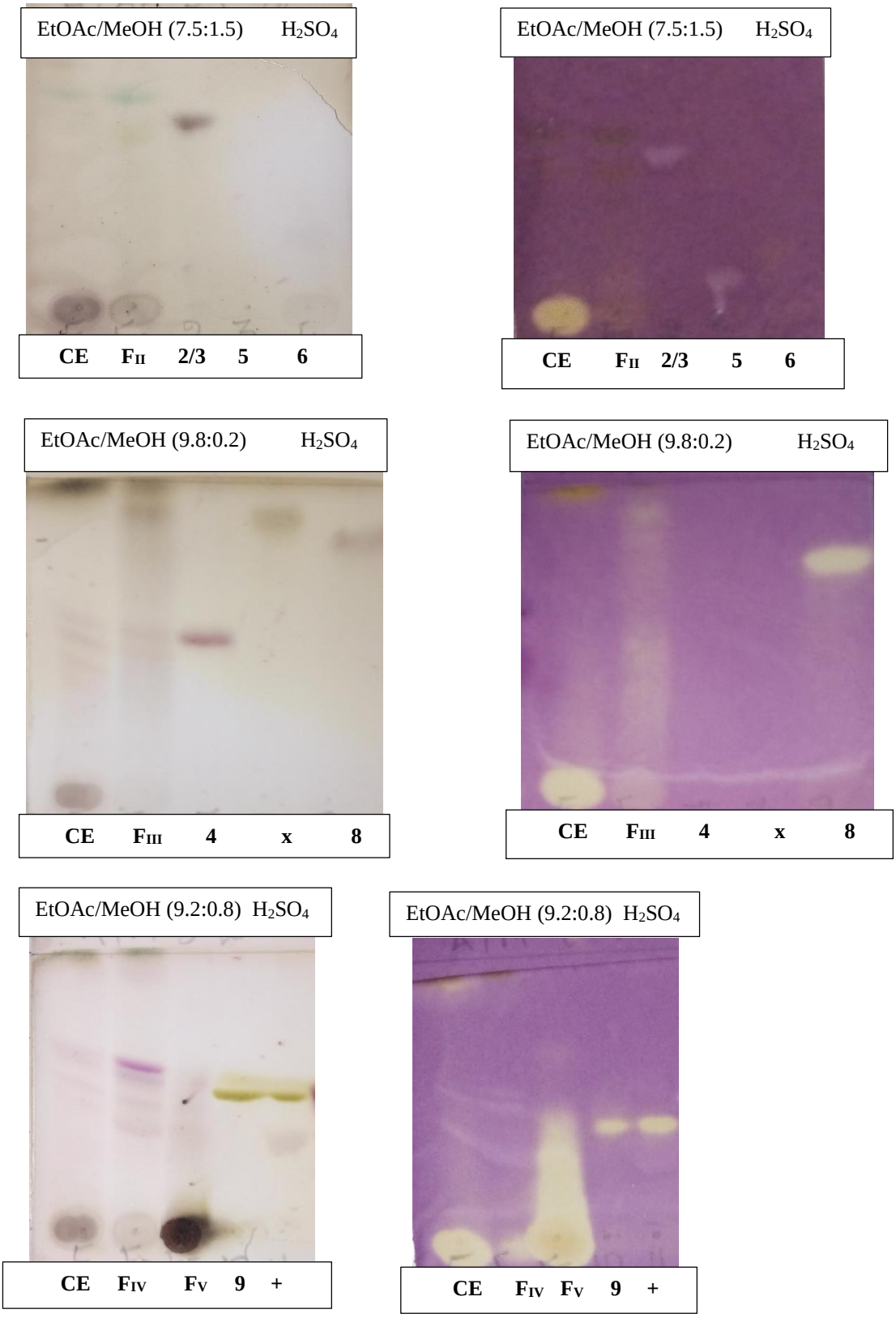
Figure legends



Glu = D-glucose

Figure 1: Chemical structures of compounds (1-9) isolated from D. ramasissimum



X : negative control, +: positive control, CE: crude extract,

Figure 2: Radical scavenging activity of the crude extract, main fractions and some compounds isolated from D. ramasissimum