OPEN ACCESS Pharmacologia

ISSN 2044-4648
DOI: 10.5567/pharmacologia.2016.103.113

Research Article
Antioxidant Activities and Chemical Constituents of Extracts from *Cordyline fruticosa* (L.) A. Chev. (Agavaceae) and *Eriobotrya japonica* (Thunb) Lindl, (Rosaceae)

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Abstract

Background and Objective: *Cordyline fruticosa* (Agavaceae) and *Eriobotrya japonica* (Rosaceae) are two medicinal plants used for the treatment of various diseases such as infections of mammary glands, sore throat and neck pain for the first plant, diabetes, cough, ulcers, protection against oxidative stress and cognitive deficits for the latter. The present study was designed to evaluate the antioxidant activity of the different extracts of these two plants as well as to isolate and identify their chemical constituents. **Materials and Methods:** The plant extract was prepared by maceration in methanol, compounds were isolated from EtOAc and n-BuOH extracts of the two plants using column chromatography and their structures were determined by means of NMR and MS analysis as well as in comparison with published data. Antioxidant tests (DPPH, ferric reduction antioxidant power and anti-hemolytic) were performed over the MeOH, EtOAc and n-BuOH extracts of the plants. **Results:** The antioxidant-guided phytochemical investigation of the MeOH extracts of the two plants led to the isolation of twelve compounds identified as: Farrerol 1, quercetin helichrysoside 2, apigenin 8-C-β-D-glucopyranoside 3, isoquercitrin 4 and rutin 5 from *C. fruticosa*, β-sitosterol 6, catechin 7, oleanolic acid 8, lyoniresinol 9, cinchonain IIb 10, lyoniresinol 2-a-O-β-D-xylopyranoside 11 and β-sitosterol-3-O-β-D-glucopyranoside 12 from *E. japonica*. Amongst the isolated compounds, the most important antioxidant ones were identified as helichrysoside and rutin from *C. fruticosa*, catechin, cinchonain IIb, lyoniresinol 2-a-O-β-D-xylopyranoside from *E. japonica* with EC_{50} of 8.73, 9.91, 4.11, 3.14 and 10.61 μg mL^{-1}, respectively. **Conclusion:** Based on the obtained results, it can be concluded that the high ability to scavenge free radicals, reducing power of Fe^{3+} and hemolysis activity exerted by extracts of *C. fruticosa* and *E. japonica* were due to their high content of phenolic compounds, thus the structure-activity relationships of the isolated flavonoids were discussed. The results of this study suggest that the extracts from these two plants could serve as potential source of antioxidant compounds.

Key words: Medicinal plant, extracts, phenolic compounds, antioxidant activity

Received: December 14, 2015 Accepted: March 01, 2016 Published: March 15, 2016

Citation: Romuald Tematio Fouedjou, Elvine Pami Nguelefack-Mbuyo, Beaudelaire Kemvoufo Ponou, Telesphore Benoit Nguelefack, Luciano Barboni and Léon Azefack Tapondjou, 2016. Antioxidant activities and chemical constituents of extracts from *Cordyline fruticosa* (L.) A. Chev. (Agavaceae) and *Eriobotrya japonica* (Thunb) Lindl, (Rosaceae). Pharmacologia, 7: 103-113.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The presence of active Reactive Oxygen Species (ROS) in excess of the available antioxidant buffering capacity defines the oxidative stress[1]. The oxidation of biological molecules such as DNA, lipids and proteins can be cause by excessive amount of ROS. Therefore, ROS are highly involved in cellular damages and in several human diseases such as cancer, inflammation, atherosclerosis, diabetes, hypertension, neurodegenerative diseases, acute respiratory and pulmonary oedema and cataracts. As such, much attention is being paid to antioxidant substances because it is believed that they can prevent and cure diseases or their subsequent complications related to oxidative stress. Besides, the antioxidant capacity is widely used as a parameter to characterize food, medicinal plants and their bioactive components[2].

The genus Cordyline with approximately 20 species having a Southern hemisphere distribution with the greatest diversity concentrated in Australia and New Zealand. *Cordyline fruticosa* is a woody plants dont la graine n'a qu'un seul cotylédon[3]. The plant is traditionally used for the treatment of various diseases; the leaves are used as haemostatic[4] and to induce abortion[5] and the roots are used for toothache, laryngitis and mammary glands infections[6]. *Cordyline fruticosa* is used in Malaysia to cure cough, bloody cough, dysentery, fever, headache, inflammation of the digestive tract, kidney diseases, bloody urine and also difficulties to urinate[7].

The genus Eriobotrya includes about 26 species[8,9], distributed in tropical and sub-tropical Eastern and Southern Asia. The *E. japonica* is a well-known medicinal plant in Japan and China and its astringent leaves have been used since long time to treat chronic bronchitis, cough, fever and gastroenteric disorders. A decoction of the leaves has also been applied locally to wounds, ulcers and cancers[10]. Phytochemical studies have shown that the flowers of *E. japonica* are rich sources of phenolic compounds and flavonoids[11,12].

Currently a variety of synthetic antioxidant supplements are marketed to balance oxidants/antioxidants but unfortunately these substances have suspected toxic effects. Some synthetic antioxidant such as butylated hydroxyanisole, gallic acid esters and butylated hydroxytoluene are suspected to have negative health effects[13]. Consequently, strict restrictions on the use of these substances have been implemented in several countries[14]. For these reasons, researchers are currently focusing their attention on natural antioxidants, particularly those obtainable from plants. According to Cook and Samman[15], the antioxidant activity of plants is mainly due to the presence of secondary metabolites from the polyphenol class. In the continuous search of potential antioxidant substances from Cameroonian medicinal plants[16-18], a bio-guided investigation was conducted for two medicinal plants growing in the Western region of Cameroon, namely *Cordyline fruticosa* and *Eriobotrya japonica*.

MATERIALS AND METHODS

Plant materials: The leaves of *Cordyline fruticosa* (L.) A. Chev. (Agavaceae) were collected in Dschang city (west region of Cameroon) in December, 2011. The plant was identified by Mr. J.P. Dondjang, botanist at the Department of Forestry, University of Dschang, under the voucher specimen (Ref: LACAPE 0001) kept in our Laboratory.

The stem barks of *Eriobotrya japonica* (Thunb) Lindl (Rosaceae) were collected in Bafou village near Dschang (west region of Cameroon) in March, 2013. The plant was identified at the Cameroon National Herbarium, Yaoundé, Cameroon by Mr. Tadjouteu Fulbert where a voucher specimen was deposited under the reference number 44164/HCN.

Extraction and isolation

Preparation of *Cordyline fruticosa* extracts: The dried and pulverized leaves of *C. fruticosa* (3 kg) were extracted three times (each time for 24 h) with MeOH. The combined filtrate was concentrated under reduced pressure to give a dark residue (503 g) which was suspended in distilled water and partitioned successively with n-hexane, EtOAc and n-BuOH, yielding 58, 69 and 60 g of dry extracts, respectively. Part of the EtOAc extract (59 g) was fractionated by silica gel column chromatography using a gradient of EtOAc in n-hexane and then a gradient of MeOH in EtOAc to give ten main fractions (A-J). Part of the n-BuOH extract was also fractionated prior to the purification of different fractions using silica gel column chromatography.

Isolation of compounds from *Cordyline fruticosa*: Fraction C (0.5 g) (eluted with Hexane-EtOAc (6-4)) was chromatographed on a silica gel column using hexane-EtOAc (70-30) as eluent to mainly yield compound 1 (27 mg). Fraction F (7.12 g) (eluted with EtOAc) was submitted to silica gel CC eluted with EtOAc-MeOH (98:2) and on sephadex LH-20 CC using MeOH as eluent to give compound 2 (7 mg). Compound 3 (57 mg) was obtained upon recrystallization from fraction G (2.6 g) and the resulting filtrate was chromatographed on silica gel column eluted with EtOAc-MeOH-H_2O (97:3:1) and then on sephadex LH-20 column (eluted with MeOH) to afford compound 4 (12 mg).

Part of the n-BuOH extract (50 g) was fractionated by column chromatography eluted with EtOAc containing increased amounts of MeOH. The fraction obtained by eluting with EtOAc-MeOH (75:25) (9.04 g) was rechromatographed on a silica gel column using EtOAc-MeOH (90:5) as eluent to afford six sub-fractions. One of these sub-fractions (157 mg) was purified on a sephadex LH-20 column eluted with MeOH to yield compound 5 (57 mg).

Preparation of *Eriobotrya japonica* extract: The dried and ground stem bark of *E. japonica* (3 kg) was extracted three times (each time for 24 h) with MeOH. The combined filtrate was concentrated under reduced pressure to give a dark residue (368 g), after removal of the solvent by evaporation, part of this residue (358 g) was suspended in distilled water and partitioned successively with n-hexane, EtOAc and n-BuOH yielding 35.0, 38.5 and 46.5 g of dry extracts, respectively. Part of the EtOAc extract (35 g) was absorbed with silica gel and then fractionated on a silica gel column using a gradient of EtOAc in n-hexane and then a gradient of MeOH in EtOAc, affording twelve fractions (A-L).

Isolation of compounds from *Eriobotrya japonica*: Compound 6 (11 mg) was obtained upon recrystallization of fraction C (1.4 g) (eluted with hexane-EtOAc (90:10)). Fraction I (9.7 g) (eluted with EtOAc-MeOH (95:5) was applied on silica gel column with EtOAc as eluent to give 3 sub-fractions (I_1, I_2 and I_3). Sub-fraction I_1 (3.2 g) was purified on a silica gel column with hexane-EtOAc (70:30) as eluent to yield compounds 7 (21 mg) and 8 (51 mg). Similarly, sub-fraction I_2 (3.2 g) was subjected to silica gel column eluted with hexane-EtOAc (80:20) to yield compound 9 (6 mg) and sub-fraction I_3 (2.3 g) was purified on a silica gel column using hexane-EtOAc (70:30) as eluent to yield compound 10 (22 mg). Fraction J (6 g) eluted with EtOAc-MeOH (90:10) was subjected to silica gel CC eluting with EtOAc to give compounds 11 (18 mg) and 12 (27 mg).

Structure elucidation and identification of the isolated compounds: Samples for NMR experiments were dissolved in deteurated solvents (CD_3OD and DMSO-d_6) and analyzed on a varian mercury plus spectrometer (400 MHz for ^1H and 100 MHz for ^{13}C). The ESI-MS were carried out on an agilent Technologie LC/MSD Trap SL (G2445D SL). The CC was performed on silica gel 60 (0.040-0.063 or 0.063-0.200 mm) and sephadex gel LH-20. Fractions were monitored by TLC using Merck pre-coated silica gel sheets (60 F_{254}) and spots were visualized under UV light (254 and 365 nm) and by spraying with 50% H_2SO_4 and heating at 110°C.

Farrerol 1: Yellow powder, ^{13}C NMR (CD_3OD, 100 MHz) δ 78.6 (C-2), 42.7 (C-3), 196.9 (C-4), 157.9 (C-5), 102.5 (C-6), 162.7 (C-7), 101.8 (C-8), 103.3 (C-9), 158.8 (C-10), 130.0 (C-1′), 127.4 (C-2′), 114.8 (C-3′), 157.4 (C-4′), 114.8 (C-5′), 127.4 (C-6′), 6.7 (6-Me), 5.9 (8-Me). The ^1H NMR (CD_3OD, 400 MHz) δ 5.30 (1H, dd, J = 12.9, 3.1 Hz, H-2), 3.05 (1H, dd, J = 17.1, 12.9 Hz, H-3b), 2.73 (1H, dd, J = 17.1, 3.1 Hz, H-3a) 7.32 (2H, d, J = 8.3 Hz, H-2′, 6′), 6.81 (2H, d, J = 8.8 Hz, H-3′, 5′), 1.99 (3H, s, 6-Me), 2.00 (3H, s, 8-Me).

Quercetin 3-O-[6″trans-p-coumaroyl-β-D-glucopyranoside] (Helichrysoside) 2: Yellow powder, ^{13}C NMR (CD_3OD, 100 MHz) δ 158.5 (C-2), 135.3 (C-3), 179.6 (C-4), 161.3 (C-5), 100.1 (C-6), 166.1 (C-7), 94.9 (C-8), 159.3 (C-9), 105.7 (C-10), 123.5 (C-1′), 114.9 (C-2′), 146.1 (C-3′), 149.9 (C-4′), 117.0 (C-5′), 123.2 (C-6′) for aglycone; 104.0 (C-1″), 75.9 (C-2″), 78.2 (C-3″), 71.9 (C-4″), 76.0 (C-5″), 64.5 (C-6″), 127.3 (C-1‴), 131.3 (C-2‴), 116.1 (C-3‴), 163.1 (C-4‴), 116.1 (C-5‴), 131.3 (C-6‴), 146.6 (C-7‴), 117.4 (C-8‴), 169.0 (C-9‴) for sugar moiety. The ^1H NMR (CD_3OD, 400 MHz) δ 6.12 (1H, d, J = 1.9 Hz, H-6), 6.30 (1H, d, J = 1.9 Hz, H-8), 7.58 (1H, d, J = 8.3 Hz, H-2′), 6.80 (1H, dd, J = 2.4 Hz, 8.3 Hz, H-5′), 7.56 (1H, d, J = 2.4 Hz, H-6′) for aglycone. About 5.26 (1H, d, J = 7.6 Hz, H-1″), 3.46 (1H, m, H-2″), 3.44 (1H, m, H-3″), 3.32 (1H, m, H-4″), 3.42 (1H, m, H-5″), 4.29 (1H, dd, J = 1.8, 2.3 Hz, H-6a), 4.18 (1H, dd, J = 11.8, 6.7 Hz, H-6b), 7.30 (2H, d, J = 8.8 Hz, H-2‴, H-6‴) for sugar moiety, 6.06 (2H, d, J = 8.8 Hz, H-3‴, H-5‴), 7.38 (1H, d, J = 16.1 Hz, H-7‴), 6.10 (1H, d, J = 16.1 Hz, H-8‴).

Apigenin 8-C-glucoside 3: Yellow powder, ^{13}C NMR (DMSO-d_6, 100 MHz) δ 164.3 (C-2), 102.8(C-3), 182.5 (C-4), 161.5 (C-5), 98.5 (C-6), 163.0 (C-7), 105.0 (C-8), 156.0 (C-9), 104.2 (C-10), 122.0 (C-1′), 129.4 (C-2′), 116.2(C-3′), 160.8 (C-4′), 116.2 (C-5′), 160.8(C-6′) for aglycone; 73.8 (C-1″), 71.2 (C-2″), 79.0 (C-3″), 70.9 (C-4″), 82.2 (C-5″), 61.6 (C-6″) for sugar moiety. The ^1H NMR (DMSO-d_6, 400 MHz) δ 6.79 (1H, s, H-3) 6.27 (1H, s, H-6), 8.03 (2H, d, J = 8.3 Hz, H-2′, 6′), 6.89 (2H, d, J = 8.6 Hz, H-3′, 5′), for aglycone; 4.69 (1H, d, J = 10.0 Hz, H-1″), 3.84 (1H, overlapped, H-2″), 3.26 (1H, overlapped, H-3″), 3.37 (1H, overlapped, H-4″), 3.24 (1H, m, H-5″), 3.75 (1H, m, H-6a″), 3.52 (1H, m, H-6b″) for sugar moiety.

Quercetin 3-O-β-D-glucopyranoside 4: Yellow powder, ^{13}C NMR (CD_3OD, 100 MHz) δ 157.6 (C-2), 134.2 (C-3), 178.1 (C-4), 161.1 (C-5), 98.5 (C-6), 164.7 (C-7), 93.3 (C-8), 157.6 (C-9), 104.2 (C-10), 121.7 (C-1′), 116.1 (C-2′), 144.5 (C-3′), 148.4 (C-4′), 114.6 (C-5′), 121.6 (C-6′) for aglycone; 102.8 (C-1′), 76.7 (C-2′), 77.0 (C-3′), 69.8 (C-4′), 74.3 (C-5′), 61.1(C-6′) for sugar moiety. The ^1H NMR (CD_3OD, 400 MHz) δ 6.20 (1H, d, J = 2.1 Hz, H-6), 6.39 (1H, d, J = 2.1 Hz, H-8), 7.70 (1H, d, J = 2.2 Hz, H-2′), 6.86 (1H, d, J = 8.5 Hz, H-5′), 7.58 (1H, dd,

J = 2.2, 8.5 Hz, H-6′) for aglycone; 5.26 (1H, d, J = 7.5 Hz, H-1′′), 3.42 (1H, overlapped, H-2′′), 3.21 (1H, overlapped, H-3′′), 3.34 (1H, overlapped, H-4′′), 3.47 (1H, overlapped, H-5′′), 3.70 (1H, d, J = 11.9, 2.4 Hz, H-6′′a), 3.57 (1H, d, J = 11.9, 5.3 Hz, H-6′′b) for sugar moiety.

Quercetin 3-O-α-l-rhamnopyranosyl-(1→6)-β-d-glucopyranoside (rutin) 5: Yellow powder, ^{13}C NMR (CD_3OD, 100 MHz) δ 158.5 (C-2), 135.6 (C-3), 179.4 (C-4), 162.9 (C-5), 99.9 (C-6), 166.0 (C-7), 94.8 (C-8), 159.3 (C-9), 105.7 (C-10), 123.5 (C-1′), 116.0 (C-2′), 145.8 (C-3′), 149.8 (C-4′), 117.6 (C-5′), 123.1 (C-6′) for aglycone 104.7 (C-1′′), 75.7 (C-2′′), 78.1 (C-3′′), 71.3 (C-4′′), 77.2 (C-5′′), 68.5 (C-6′′), 102.4 (C-1′′′), 72.1 (C-2′′′), 72.2 (C-3′′′), 73.9 (C-4′′′), 69.7 (C-5′′′), 17.8 (C-6′′′) for sugar moiety. The ^{1}H NMR (CD_3OD, 400 MHz) δ 6.25 (1H, d, J = 2.1 Hz, H-6), 6.35 (1H, d, J = 2.1 Hz, H-8), 6.80 (1H, d, J = 8.5 Hz, H-2′), 7.70 (1nonoH, d, J = 2.2 Hz, H-5′), 7.65 (1H, dd, J = 8.5, 2.2 Hz, H-6′) for aglycone. 5.15 (1H, d, J = 7.6 Hz, H-1′′), 3.45 (1H, m, H-2′′), 3.42 (1H, m, H-3′′), 3.27 (1H, m, H-4′′), 3.30 (1H, m, H-5′′), 3.40 (1H, broad single, H-6a′′), 3.80 (1H, m, H-6b′′), 4.40 (1H, d, J = 1.6 Hz, H-1′′′), 3.62 (1H, m, H-2′′′), 3.52 (1H, m, H-3′′′), 3.28 (1H, m, H-4′′′), 3.45 (1H, m, H-5′′′), 1.10 (3H, d, J = 6.2 Hz, H-6′′′) for sugar moiety.

β-Sitosterol 6: White powder, ^{13}C (CD_3OD, 100 MHz) δ 37.3 (C-1), 31.6 (C-2), 71.8 (C-3), 42.2 (C-4), 140.8 (C-5), 121.7 (C-6), 31.9 (C-7), 31.9 (C-8), 51.2 (C-9), 36.5 (C-10), 21.1 (C-11), 39.8 (C-12), 42.3 (C-13), 56.8 (C-14), 24.3 (C-15), 28.3 (C-16), 56.0 (C-17), 11.9 (C-18), 19.4 (C-19), 36.2 (C-20), 18.8 (C-21), 33.9 (C-22), 26.1 (C-23), 45.9 (C-24), 29.2 (C-25), 19.8 (C-26), 19.3 (C-27), 23.1 (C-28), 12.2 (C-29). The ^{1}H (CD_3OD, 400 MHz) δ 3.52 (1H, m, H-3), 5.35 (1H, brs, H-6), 0.92 (3H, d, J = 6.4 Hz, H-21), 0.81 (3H, d, J = 6.5 Hz, H-26), 0.83 (3H, d, J = 6.5 Hz), 0.85 (3H, t, H-29).

Catechin 7: Yellow powder, ^{13}C NMR (CD_3OD, 100 MHz) δ 80.0 (C-2), 67.6 (C-3), 29.4 (C-4), 157.8 (C-5), 96.5 (C-6), 158.2 (C-7), 96.0 (C-8), 146.0 (C-9), 100.2 (C-10), 132.4 (C-1′), 115.4 (C-2′), 149.5 (C-3′), 157.5 (C-4′), 116.0 (C-5′), 119.5 (C-6′). The ^{1}H NMR (CD_3OD, 400 MHz): δ 4.80 (1H, br*s*, H-2), 4.18 (1H, m, H-3), 2.74 (1H, dd, J = 16.8, 2.8 Hz, H-4a), 2.86 (1H, dd, J = 16.8, 4.6 Hz, H-4b), 5.95 (1H, d, J = 2.3 Hz, H-6), 5.90 (1H, d, J = 2.3 Hz, H-8), 6.97 (1H, d, J = 2.0 Hz, H-2′), 6.76 (1H, d, J = 8.6 Hz, H-2′), 6.78 (1H, dd, J = 8.6, 2.0 Hz, H-6′).

Oleanolic acid 8: White powder, ^{13}C NMR (CD_3OD, 100 MHz) δ 38.5 (C-1), 27.4 (C-2), 78.7 (C-3), 38.7 (C-4), 55.2 (C-5), 18.3 (C-6), 36.6 (C-7), 39.8 (C-8), 47.6 (C-9), 37.0 (C-10), 23.1 (C-11), 122.1 (C-12), 143.4 (C-13), 41.6 (C-14), 27.7 (C-15), 23.4 (C-16), 46.6 (C-17), 41.3 (C-18), 45.8 (C-19), 30.6 (C-20), 33.8 (C-21), 32.3 (C-22), 28.1 (C-23), 15.6 (C-24), 15.3 (C-25), 16.8 (C-26), 26.0 (C-27), 181.0 (C-28), 33.1 (C-29), 23.6 (C-30).

Lyoniresinol 9: White powder, ^{13}C NMR (CD_3OD, 100 MHz) δ 33.7 (C-1), 40.9 (C-2), 66.9 (C-2a), 47.0 (C-3), 64.2 (C-3a), 42.5 (C-4), 147.7 (C-5), 139.0 (C-6), 148.7 (C-7), 107.8 (C-8), 130.3 (C-9), 126.4 (C-10), 139.4 (C-1′), 106.9 (C-2′), 149.1 (C-3′), 134.5 (C-4′), 106.9 (C-5′), 60.3 (C-6′), 56.6 (5-OMe), 56.7 (7-OMe), 56.7 (3′, 5′-OMe). The ^{1}H NMR (CD_3OD, 400 MHz): δ 2.70 (1H, dd, J = 15.2, 4.9 Hz, H-1a), 2.59 (1H, J = dd, 15.2, 11.4 Hz, H-1b), 1.62 (1H, m, H-2), 3.49 (1H, m, Ha-2a), 4.59 (1H, m, Hb-2a), 1.95 (1H, m, H-3), 3.49 (2H, m, H-3), 4.30 (1H, d, J = 5.7 Hz, H-4), 6.58 (1H, s, H-8), 6.38 (2H, s, H-2′, 6′), 3.36 (3H, s, 5-OMe), 3.85 (3H, s, 7-OMe), 3.73 (6H, s, 3′, 5′-OMe).

Cinchonain IIb 10: Yellow powder, ^{13}C NMR (CD_3OD, 100 MHz) δ 77.1 (C-2), 73.2 (C-3), 37.1 (C-4), 156.4 (C-5), 95.5 (C-6), 151.1 (C-7), 105.5 (C-8), 153.8 (C-9), 109.1 (C-10), 133.0 (C-1′), 115.6 (C-2′), 145.8 (C-3′), 145.2 (C-4′), 116.4 (C-5′), 119.5 (C-6′), 80.5 (C-2′′), 67.6 (C-3′′), 30.1 (C-4′′), 156.2 (C-5′′), 96.5 (C-6′′), 155.7 (C-7′′), 108.9 (C-8′′), 146.2 (C-9′′), 100.2 (C-10′′), 132.8 (C-1′′′), 116.4 (C-2′′′), 145.6 (C-3′′′), 145.0 (C-4′′′), 116.0 (C-5′′′), 119.7 (C-6′′′), 136.0 (C-1′′′′), 119.5 (C-2′′′′), 145.5 (C-3′′′′), 144.3 (C-4′′′′), 115.2 (C-5′′′′), 119.8 (C-6′′′′), 37.8 (C-α), 35.3 (C-β), 171.8 (C=O). The ^{1}H NMR (CD_3OD, 400 MHz) δ 5.64 (1H, H-2), 3.99 (1H, s, H-3), 4.65 (1H, s, H-4), 5.94 (1H, s, H-6), 6.73 (1H, d, J = 1.9 Hz, H-2′), 6.68 (1H, d, m, H-5′), 6.52 (1H, dd, J = 8.2, 2.0 Hz, H-6′), 4.33 (1H, s, H-2′′), 3.81 (1H, s, H-3′′), 2.82 (1H, brs, H-4′′), 6.12 (1H, brs, H-6′′), 6.68 (1H, m, H-2′′′), 6.70 (1H, m, H-5′′′), 6.62 (1H, dd, J = 8.21, 2.2Hz, H-6′′′), 6.69 (1H, m, H-2′′′′), 6.85 (1H, brs, H-5′′′′),6.70 (1H, m, H-6′′′), 2.00 (1H, dd, J = 6.6, 15.6, H-$α_1$), 2.56 (1H, dd, J = 6.6, 15.6, H-$α_2$), 4.12 (1H, dd, J = 6.6, 15.6, H-β).

Lyoniresinol 2a-O-β-D-xylopyranoside 11: Pink powder, ^{13}C NMR (CD_3OD, 100 MHz) δ 33.9 (C-1), 40.4 (C-2), 65.9 (C-2a), 43.5 (C-3), 69.2 (C-3a), 41.6 (C-4), 146.2 (C-5), 137.0 (C-6), 147.2 (C-7), 106.3 (C-8), 128.7 (C-9), 125.6 (C-10), 138.9 (C-1′), 105.3 (C-2′, 6′), 147.5 (C-3′, 5′), 132.5 (C-4′), 58.6 (5-OMe), 55.1 (7-OMe), 55.2 (3′, 5′-OMe) for aglycone 103.9 (C-1′′), 73.4 (C-2′′), 76.6 (C-3′′), 69.7 (C-4′′), 65.5 (C-5′′) for sugar moiety. The ^{1}H NMR (CD_3OD, 400 MHz) δ 2.70 (1H, dd, J = 15.2, 4.9 Hz, H-1a), 2.63 (1H, dd, J = 15.2, 11.2 Hz, H-1b), 1.70 (1H, m, H-2), 3.56 (1H, m, Ha-2a), 3.65 (1H, m, Hb-2a), 2.04 (1H, m, H-3), 3.84 (1H, m, Ha-3a), 3.41 (1H, m, Hb-3a), 4.37 (1H, d, J = 6.7 Hz, H-4), 6.56 (1H, s, H-5), 6.42 (2H, s, H-2′, 6′), 3.31 (3H, s, 6-OMe), 3.85 (3H, s, 8-OMe), 3.31 (6H, s, 3′, 5′-OMe) for aglycone 4.20 (1H, d, J = 7.5 Hz, H-1′′), 3.23 (1H, overlapped, H-2′′), 3.31 (1H, overlapped, H-3′′), 3.46 (1H, overlapped, H-4′′), 3.16 (1H, overlapped, H-2′′) for sugar moiety.

β-Sitosterol-3-O-β-D-glucopyranoside 12: White powder, ^{13}C (CD_3OD, 100MHz) δ 37.3 (C-1), 30.2 (C-2), 78.5 (C-3),

39.2 (C-4), 140.8 (C-5), 121.7 (C 6), 31.9 (C-7), 31.9 (C-8), 51.2 (C-9), 36.5 (C-10), 21.1 (C-11), 39.8 (C-12), 42.3 (C-13), 56.8 (C-14), 24.3 (C-15), 28.3 (C-16), 56.0 (C-17), 11.9 (C-18), 19.4 (C-19), 36.2 (C-20), 18.8 (C-21), 33.9 (C-22), 26.1 (C-23), 45.9 (C-24), 29.2 (C-25), 19.8 (C-26), 19.3 (C-27), 23.1 (C-28), 12.2 (C-29) for aglycone, 102.5 (C-1'), 75.3 (C-2'), 78.6 (C-3'), 71.6 (C-4'), 78.1 (C-5'), 62.5 (C-6') for sugar moiety. The ^{1}H (CD_3OD, 400 MHz) δ 0.66-1.25 (three CH_3 groups), 3.50 (1H, m, H-3), 5.35 (1H, m, H-5) for aglycone; 5.02 (1H, d, J = 7.5 Hz, H-1') representing the anomeric proton of the sugar.

Antioxidant activity

DPPH radical scavenging test: The antioxidant activity of the extracts was determined in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH (2, 2-diphenyl-1-picrylhydrazyl), according to the procedure described by Nguelefack-Mbuyo *et al.*[18].

Ferric Reduction Antioxidant Power (FRAP): This experiment was conducted as previously described by Athukorala *et al.*[19].

AAPH-induced Red Blood Cells (RBC) hemolysis assay: A modified method of Zhang *et al.*[20] was used to assess the antihemolytic effect of extracts, fractions and compounds. Blood samples were collected from healthy male wistar rats through abdominal aorta into tubes containing EGTA (Ethylene Glycol tetra acetic). The RBCs were separated from plasma by centrifugation at 1500 rpm for10 min. The RBCs were then washed three times with five volumes phosphate buffer saline (PBS, pH 7.4). Thereafter, the packed RBCs were suspended into PBS solution to obtain a 5% cell suspension. 100 µL of RBCs suspension were mixed with 100 µL solution of extracts, fractions, isolated compounds or ascorbic acid. 100 µL of 200 mM 2', 2'-Azobis (2-Methylpropionamidine) dihydrochloride (AAPH) were then added to the mixture and incubated at 37°C for 3 h with gentle shaking. At the end of the incubation period, 4 mL of PBS were added into the reaction milieu followed by centrifugation at 3500 rpm for 10 min. The absorbance of the supernatant was read at 540 nm. To yield a complete hemolysis, an aliquot of RBC suspension was treated with equal volume of ice-cold distilled water. The percentage inhibition was calculated as follow:

$$I\,(\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Statistical analysis: All the pharmacological test data are expressed as the Mean±Standard Error of the Mean (SEM). About 50% Effective Concentrations (EC_{50}) were determined after logarithmic transformation of the concentration-response curve using GraphPad Prism 5.0 software. The Efficiency Index (EI) was expressed as EC_{50}/E_{max}.

RESULTS AND DISCUSSION

Phytochemical analysis: When the methanol extract of *C. fruticosa* and *E. japonica* were developed on the silica gel TLC, the spots showed not only the UV absorbance at 254 or 365 nm but also a yellow colorization by spraying 10% H_2SO_4 solution and then heating the TLC plate, indicating the presence of flavonoids in the extracts.

The MeOH extracts of the leaves of *C. fruticosa* and the stem bark of *E. japonica* were suspended into distilled H_2O and then partitioned successively with hexane, EtOAc and n-BuOH to yield the hexane, EtOAc and n-BuOH extracts, respectively. Fractionation and purification of the above EtOAc and n-BuOH extracts by column chromatography led to the isolation of twelve compounds. The structural identification of these compounds was carried out by interpretation of their spectral data especially, NMR spectra in conjunction with 2D experiments (^{1}H-^{1}H COSY, HSQC, HMBC), mass spectrometry in comparison with published information and also by co-TLC comparison with some authentic samples previously obtained in our research group for some cases.

The isolated compounds (Fig. 1) were identified as: farrerol 1[21], quercetin 3-O-[6-trans-p-coumaroyl]-β-D-glucopyranoside 2 (helichrysoside)[22], apigenin 8-C-β-D-glucopyranoside 3[23], quercetin 3-O-β-D glucopyranoside (isoquercitrin) 4[24] and quercetin 3-O-α-l-rhamnopyranosyl-(1→6)-ß-d-glucopyranoside 5[25] from C. fruticosa. β-sitosterol 6[26], catechin 7[14], oleanolic acid 8[27], Lyoniresinol 9[28], cinchonain IIb 10[29], Lyoniresinol 2-a-O-β-D-xylopyranoside 11[30] and β-sitosterol-3-O-β-D-glucopyranoside 12[26] from *E. japonica*.

Antioxidant activity

DPPH radical scavenging test: As depicted in Fig. 2a-d crude extracts (MeOH extracts) and their sub-sequent extracts (n-BuOH and EtOAc extracts) and isolated compounds from *C. fruticosa* and *E. japonica* exhibited a good radical scavenging activity on DPPH. It can be noticed that the activity increases with the fractionation of crude extracts. *Eriobotrya japonica* appeared to be more potent than *C. fruticosa* and compound 4 was the less active of all the substances tested. More importantly compounds 7 and 10 isolated from *E. japonica* acted similarly to ascorbic acid used as the reference drug. Indeed these compounds were as powerful as ascorbic acid with respective efficiency index (EI) of 0.04, 0.03 and 0.03 (Table 1).

Table 1: Radical scavenging activity of extracts, fractions and isolated compounds from *C. fruticosa* and *E. japonica* on DPPH

	Test substances	EC_{50} (μg mL^{-1})	E_{max} (%)	EI
Cordyline fruticosa	MeOH extract	181.30	61.86	2.93
	n-BuOH extract	49.34	90.69	0.54
	EtOAc extract	50.68	97.99	0.52
	1	11.28	96.23	0.12
	2	8.73	94.70	0.09
	3	45.22	93.45	0.48
	4	ND	63.83	ND
	5	9.91	93.49	0.11
Eriobotrya japonica	MeOH extract	16.55	93.59	0.18
	EtOAc extract	7.19	94.56	0.08
	7	4.11	95.25	0.04
	10	3.14	94.61	0.03
	11	10.61	95.11	0.11
	Ascorbic acid	3.11	96.51	0.03

ND: Not determined

The antioxidant activity of phenolic compounds is due to their ability to scavenge free radicals, donate hydrogen atoms or electron or chelate metal cations. The structure of phenolic compounds is a key determinant of their radical scavenging and metal chelating activity and this is referred to as structure-activity relationships (SAR)[31].

Comparing the structures (Fig. 1) and activities of compounds 2, 4 and 5 (Fig. 2c), the main differences are the substituents of the C-3 glucose moiety at position C-6''. It is inferred that the presence of an additional p-coumaroyl moiety (compound 2) or sugar group (compound 5) at position C-6'' seems to have some little influence on antioxidant activity compare to compound 4. By comparing the activity of compounds 3 and 4, it is inferred that the

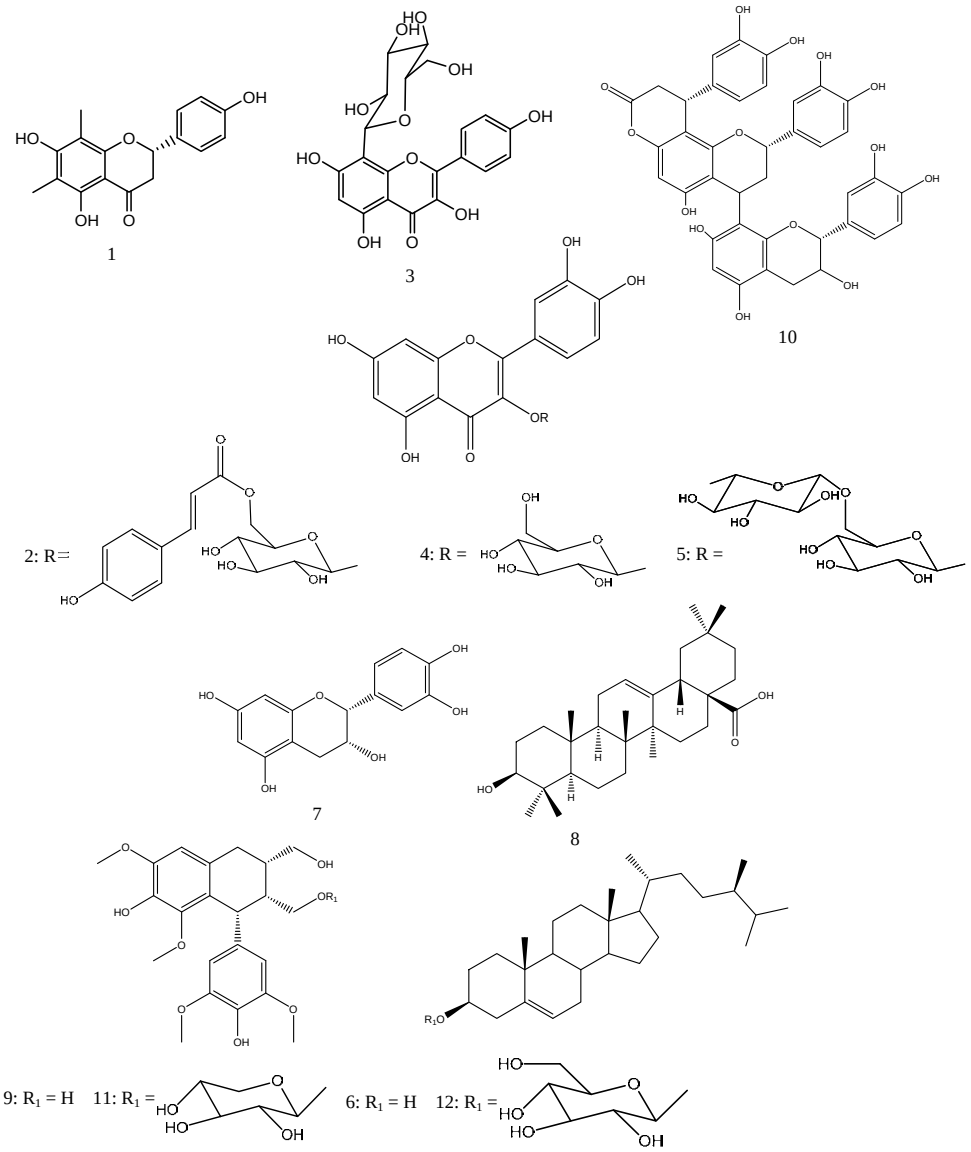

Fig. 1: Chemical structures of compounds isolated from the leaves of *Cordyline fruticosa* and stem bark of *Eriobotrya japonica*

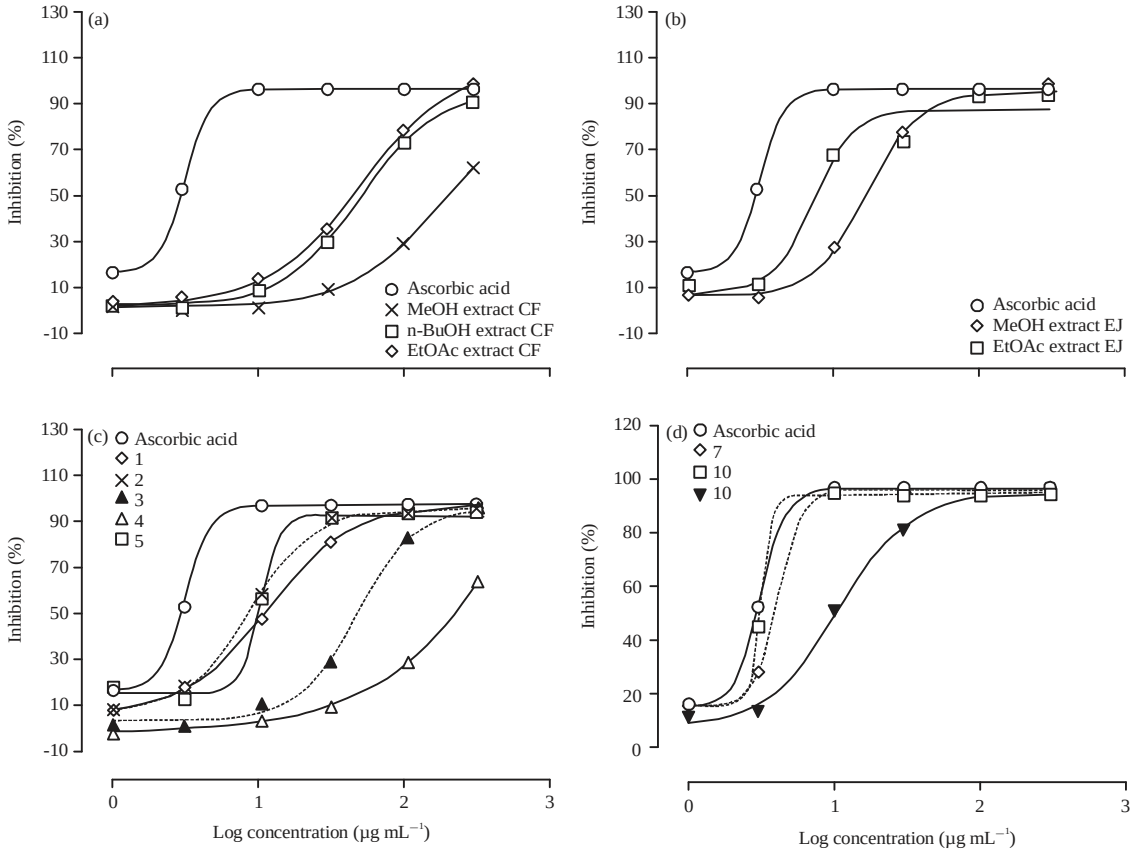

Fig. 2(a-d): DPPH scavenging activity of, (a) *Cordyline fruticosa* extracts, (b) *Eriobotrya japonica* extracts, (c) Some compounds isolated from *C. fruticosa* and (d) Some compounds isolated from *E. japonica* and reference antioxidants (Ascorbic acid)

C-glycosylation (compound 3) or O-glycosylation and catechol group (compound 4) are the key factor that affects the activities, Although, compound 4 bears a catechol group in B-ring, which is very important for radical scavenging activity in flavonoides[32] compare to compound 3, the later was more active than the former owing to the free hydroxyl residue at C-3 position of C-ring in compound 3 which is also a key factor for radical scavenging activity in flavonol derivatives[33-34]. Thus, the high activity of compound 10 could be attributed to its greater number of catechol and free hydroxyl groups.

Ferric Reduction Antioxidant Power (FRAP): The ability of plant extracts and isolated compounds to induce the reduction of ferric cyanide complex to the ferrous form is associated with increase optical density. Figure 3a-d shows that crude and sub-sequent extracts, pure isolated compounds induced a concentration-dependent antioxidant activity. As observed with DPPH, *E. japonica* was more active than *C. fruticosa* in this assay. Interestingly, the reducing power of the EtOAc extract of *E. japonica* was higher than that of ascorbic acid.

In both methods, compounds 2, 7 and 10 showed potent activity, while compound 3 showed very weak antioxidant activity, which is consistent with the reported results. Furthermore, for compound 3 and 4 quantitative structure-activity relationship analysis also suggested that compound 3 would not show effective activity because of the absence of a catechol residue in the B ring and less free-OH groups in its structure, which are required for high antioxidant activity. Comparing the structures and activities of compounds 1 and 7, it is inferred that the presence of methyl group at C-6 and C-8 seems to have little influence on the activity.

AAPH-induced Red Blood Cells (RBC) hemolysis assay: All extracts and isolated compounds potentiated the effect of AAPH at lower concentrations. A weak antioxidant activity was observed at a higher concentration (300 µg mL^{-1}) with n-BuOH, EtOAc extracts and compound 5 derived from *C. fruticosa* (Fig. 4a, c). The *E. japonica* failed to inhibit AAPH-induced hemolysis (Fig. 4b, d).

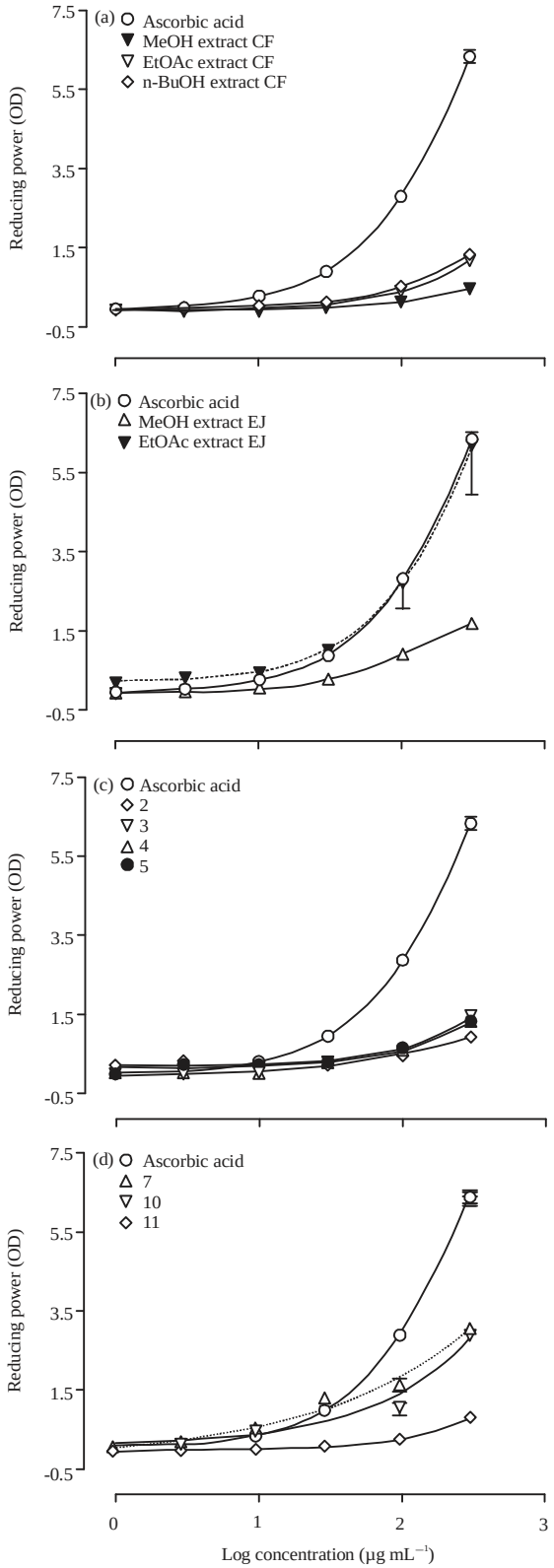

Fig. 3(a-d): Ferric Reducing Ability Power (FRAP) at various concentrations of ascorbic acid, (a) *Cordyline fruticosa* extracts, (b) *Eriobotrya japonica* extracts, (c) Some compounds isolated from *C. fruticosa* and (d) Some compounds isolated from *E. japonica*

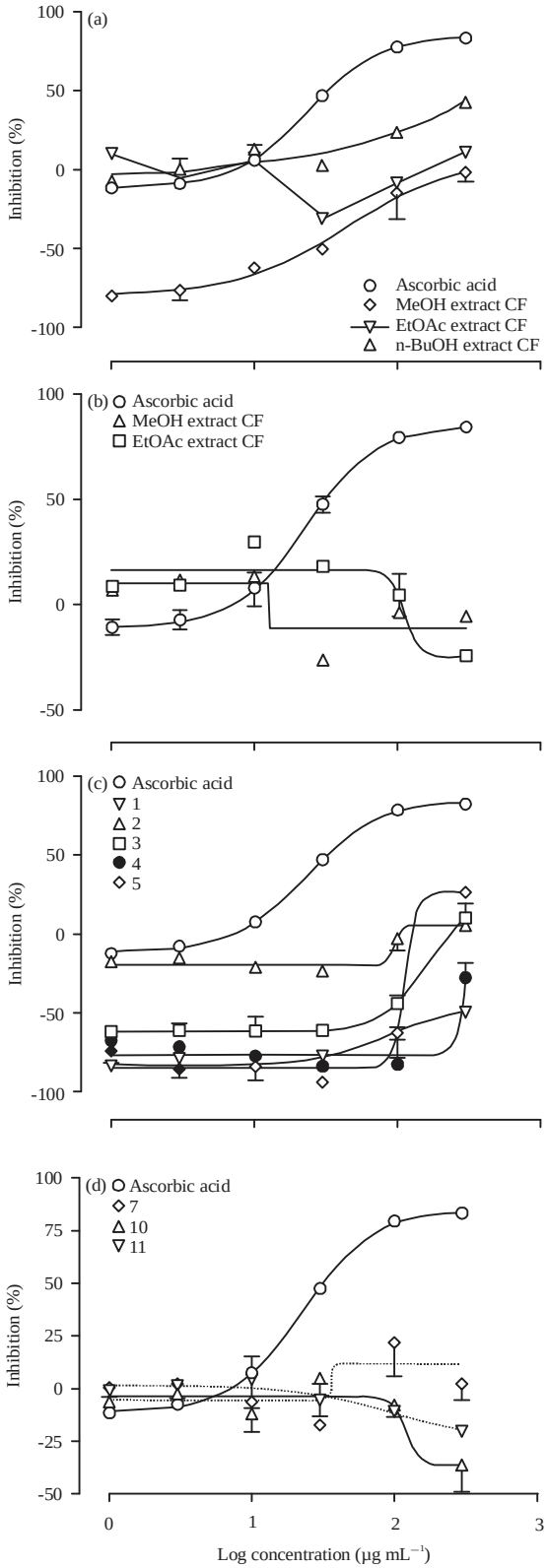

Fig. 4(a-d): Effects of, (a) *Cordyline fruticosa* extracts, (b) *Eriobotrya japonica* extracts, (c) Some compounds isolated from *C. fruticosa* and (d) Some compounds isolated from *E. japonica* and reference antioxidants (Ascorbic acid) on AAPH-induced hemolysis

The SAR of flavonoids is generally more complicated due to the relative complexity of the flavonoid molecules. Some of the structural features and nature of substitutions on rings B and C which determine the antioxidant activity of flavonoids include the following:

- The degree of hydroxylation and the positions of the -OH groups in the B ring, in particular an ortho-dihydroxyl structure of ring B (catechol group) results in higher activity as it confers higher stability to the aroxyl radical by electron delocalization[32] or acts as the preferred binding site for trace metals[35]
- A double bond between C-2 and C-3 conjugated with the 4-oxo group in C-ring enhances the radical scavenging capacity of flavonoids[35]
- A double bond between C-2 and C-3, combined with a 3-OH in ring C, also enhances the active radical scavenging capacity of flavonoids, as seen in the case of kaempferol[32]. Substitution of the 3-OH results in increase in torsion angle and loss of coplanarity and subsequently reduced antioxidant activity[36]

CONCLUSION

At the end of this study, it is considered that the bio-guided purification of *C. fruticosa* and *E. japonica* revealed six compounds with excellent proven antioxidant and scavenging activities. The results indicated that *C. fruticosa* and *E. japonica* fractions exhibit excellent radical scavenging ability in all assays employed and the EtOAc fraction of *E. japonica* was the most active fraction among them. Phytochemical investigation of the fractions led to the isolation of twelve compounds and the radical scavenging assays indicated that all compounds have stronger antioxidant capacity but less than the positive control ascorbic acid. Overall, *C. fruticosa* and *E. japonica* are promising sources of natural antioxidants ingredients.

It is well established that the efficacy of flavonoids as antioxidants depend on the number and position of the hydroxyl substitutions on the basic structure; an increase in number of hydroxyl groups is directly correlated with increasing activity and the 3, 4-dihydroxy substitution is significant.

ACKNOWLEDGMENTS

The authors are grateful to the Italian Ministry of Education (MIUR) for supporting this research through the COOPERLINK 2011 (Prot.CII113PPUC "Tesi di Dottoratosullo studio di molecule biologicamenteattiveestratte da piantedellamedicina tradizionale del Camerun". Project Manager L. Barboni) and the IFS (International Foundation for Science, Stockholm, Sweden) program through grant to Prof. A. Léon TAPONDJOU (RGA No.F/3976-3F).

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