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Antioxidant Capacity of Fractionated Extract and Structural Elucidation of Isolated Compounds from Leaf Extract of *Hydrocotyl bonariensis* Comm. Exlam

Emmanuel O Ajani [1]*, Saheed Sabiu [1], Mariam Zakari [1], Bolatito E Olanipekun [2], Jacob K. Akintunde [3] and Fisayo A Bamisaye [1]

[1] Phytomedicine Toxicology and Drug Development Laboratory, Department of Biosciences and Biotechnology, Kwara State University, Malete, Nigeria
[2] Chemistry Department, College of Pure and applied Sciences, Kwara state University, Malete Nigeria.
[3] Department of Public Health, College of Medicine, University Of Ibadan, Nigeria

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Abstract: *Hydrocotyl bonariensis* is a plant which anticataractogenic potentials have been reported. In the present study an attempt was made to evaluate the *in vitro* antioxidant activity of the fractionates of the leaves extract and also characterize some of its chemical constituents. DPPH, H_2O_2, OH and NO free radical scavenging, metal chelating and reducing power activity was used to evaluate the antioxidant activity of the crude extract fractionates. Fresh leaves of *H. bonariensis* leaves were extracted in 70 % methanol. The extract was partitioned with different solvent system of increasing polarity (n-hexane, chloroform, ethyl acetate methanol and water). Compounds were isolated from the aqueous practitionate using accelerated gradient chromatography, vacuum liquid chromatography, preparative TLC and conventional column chromatography. The presence of the chemical groups was established with HPLC and Fourier Transform Infra Red. The structures of isolated compounds were elucidated by spectroscopic study and chemical shifts. Data from the study indicates that all the fractionates contain compounds with free radical scavenging activity. This activity was more pronounced in the aqueous fractionate (DPPH $IC_{50,}$ 0025 ± 0.011 mg/ml, metal chelating capacity 27.5 %, OH^- scavenging IC_{50}, 0.846 ± 0.037 mg/ml, H_2O_2 scavenging IC_{50} 0.521 ± 0.015 mg/ml, reducing power IC_{50} 0.248 ± 0.025 mg/ml and NO scavenging IC_{50} 0.537 ± 0.038 mg/ml). Two compounds were isolated and when compared with data from the literature; the structures were suggestive of polyphenolic flavonoid, quercetin and 3-*O*-β-D-glucopyranosyl-sitosterol. The result indicates that *H. bonariensis* leaves contain bioactive compounds with antioxidant activity.

Key words: Antioxidant, anticataract, free radical scavenging activity, flavonoids, fractionates *Hydrocotyl bonariensis.*

Introduction

Hydrocotyl bonariensis Comm. Ex Lam (Apiacae) commonly called Large Leaf Pennyworth is a herbaceous, hairless, perennial plant which is prostrate with creeping lateral stem. It is found in the tropical and subtropical regions of the world. It is a rhizomatous perennial herb common to the barrier islands of Southern United states. The plant is important in agriculture and in its usefulness in land conservation. It is valued for treating various kinds of diseases such as tuberculosis, relieving the pain of rheumatism and arthritis, to increase brain capacity and for longevity [1]. The leaf of the plant has also been reported

*Corresponding author (Emmanuel O Ajani)
E-mail: < emmanuel.ajani@kwasu.edu.ng; ajaniimman@gmail.com >

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to be useful as emetic, diuretic and laxative [2]. While Ajani *et al.*[3] have scientifically reported the *in vivo* anticataractogenic effect of the aqueous leaves extract of the plant. its leaf has also been demonstrated to be useful as emetic, diuretic and laxative [2].

Free radicals and reactive oxygen species (ROS) have been proposed as causative factors in the toxicity of numerous chemicals and in the pathogenesis of many diseases, such as cardiovascular disease [4], certain cancers [5], diabetes [6], rheumatoid arthritis and cataracts [7]. They are capable of attacking the healthy cells of the body, causing them to lose structure and function. Antioxidants are able to prevent the adverse effects of reactive oxygen by helping to capture and neutralize free radicals, thereby eliminating free radical damage to the human body. Nitric oxide (NO) along with superoxide (O^{2-}) and the products of their interaction initiate a wide range of toxic oxidative reactions causing tissue injury [8].

In a previous study, we reported that administration of aqueous extract of *Hydrocotyl bonariensis* boost the level of endogenous antioxidants and reduce the level of malondialdehyde [9]. The study also reported on some of the secondary metabolites present in the leaves of the plant. However, no active or inactive principle(s), to the best of our knowledge, has/have been isolated and identified from *H. bonariensis* leaves. In the present study, the work has been extended to investigate the free radical scavenging activities of successive fractions of the extract of *H. bonariensis* leaves and to analyze the phytochemical patterns of the plant by extracting and isolating compounds from this medicinally important plant

Materials and methods

Chemicals and reagents

Quercetin, 2,2-diphenyl-1-picryl hydrazine (DPPH), trichloroacetic acid (TCA), glacial acetic acid, nicotinamide adenine dinucleotide phosphate (NADPH), ferrozine, napthylenediamine, dihydrochloride, 1,10-phenanthroline, sulfanilic acid, rutin and 2- mercaptoethanol were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). All other solvents and chemicals used were of analytical grade and were obtained from commercial sources.

Plant material

Whole plant of *Hydrocotyl bonariensis* was collected from a botanical garden in Ago-Iwoye, Ogun State, Nigeria in August 2015. The plant was identified at the herbarium of Plant Biology, Kwara State University, Malete, Nigeria, where a voucher specimen (KS/PLH/HB04/011) was deposited.

Preparation of extract and fractions

Fresh leaves of *Hydrocotyl bonariensis* collected were cut into small pieces and air dried for several days. The dried leaves were then pulverized with a local kitchen blender. Exactly 100 g of the pulverized leaves were macerated in 500 ml of methanol for 7 days in air tight clean flat bottomed container at room temperature with occasional shaking and stirring. The extract was then filtered first through a fresh cotton plug and then with a Whatman filter paper. The extract was again macerated in another 500 ml of methanol for another 7 days and then filtered as above. The filtrate was combined and concentrated using a rotary evaporator at low temperature (40^{o}C) and pressure. The concentrated product was further dried over a water bath at 40°C. The yield was 9.8 %. The dried extract was then fractionated successively by solvent-solvent partitioning. This was done by first suspending it in distilled water. Hexane was added to the suspension in ratio of 1:2, shaken well and allowed to stand for about 15 min until two layers were formed. The hexane layer was removed and more hexane was added to the aqueous layer. The process was repeated once, and then a colorless hexane layer was seen. The two hexane layers were combined and dried to obtain the hexane fraction. The procedure was repeated with the aqueous layer using chloroform and ethyl acetate respectively. Each fraction obtained, including the aqueous fraction, was then collected and dried. The aqueous layer was dried by lyophilization. The weights of the dried fractions were calculated and each sample was then kept air-tight for further use.

Assay for antioxidant activities

DPPH radical scavenging assay

The DPPH (1, 1-diphenyl-2-picryl hydrazyl) radical scavenging assay was carried out accord-

ing to the method of Hemalatha et al. (2010). DPPH (0.1 mM) was prepared in methanol and 1.0 ml of its solution was mixed with 1.0 ml of extract/fractions prepared in methanol at different concentrations (20, 40, 60, 80 and 100 µg/ml). The mixture was shaken well and incubated at room temperature for 30 min and absorbance was measured at 517 nm using a UV spectrophotometer. All the experiments were performed in triplicate and the mean was taken. Scavenging activity was calculated using the equation:

DPPH - Scavenging capacity (%) = (A^0control -A^0sample/A^0control) × 100

Ascorbic acid was used as positive control. The IC_{50} values (concentration of extract/fraction required to reduce 50 % of DPPH radical) were estimated from the graph and compared.

Metal chelating activity

The method described by Decker and Welch [10] was used to estimate the metal chelating activity. The extract/fraction (0.5 g) was mixed with $FeCl_3$ (2 mM) and ferrozine (0.2 ml) in a test tube, and the total volume was diluted with methanol (2 ml). The mixture was vigorously shaken and left standing for 10 min at room temperature. The absorbance of the solution was measured spectrophotometrically at 562 nm after the mixture had reached equilibrium. EDTA was used as positive control and the percent inhibition of ferrozine-Fe^{2+} complex was calculated using the formula:

Percent scavenging = [(A^0control - A^0sample)/A^0control] × 100

Where: A^0control = absorbance of ferrozine - Fe^{2+} complex; A^0 sample = absorbance of test compound.

Hydroxyl radical scavenging activity

The activity was carried out according to the method described by Yu *et al*.[11]. The reaction medium was made up of 60 µl of 1 mM, $Fecl_3$, 90 µl of 1 mM 1,10-phenanthroline, 2.4 ml of 0.2 M phosphate buffer (pH 7.8), 150 µl of 0.17 M H_2O_2 and 1.5 ml of various concentration of each fractions/methanol extract. Reaction mixture was kept at room temperature for 5 min. incubation and absorbance was then measured at 560 nm using spectrophotometer. The concentration of the indi-vidual sample required to neutralize 50 % hydroxyl radicals were considered as IC_{50} values.

Nitric oxide radical scavenging activity

The method described by Garrat [12] was used for estimating nitric oxide radical scavenging activity. The reaction mixture containing 2 ml of 10 mM sodium nitroprusside, 0.5 ml of phosphate buffer saline (pH 7.4) and 0.5 ml of plant extract/fraction was incubated at 25°C for 2 h 30 min. After incubation time, 0.5 ml of reaction mixture was mixed with 1 ml of sulfanilic acid reagent (33 % in 20 % glacial acetic acid) and incubated for 5 min. This was followed by addition of 1 ml napthylenediamine dihydrochloride (0.1 % w/v). The mixture was incubated at room temperature for 30 min. The absorbance was measured at 560 nm using UV-VIS spectrophotometer. The amount of sample required to scavenge 50 % nitric oxide radicals generated in the control set were calculated as $IC_{50.}$

Hydrogen peroxide scavenging activity

The method of Ruch *et al*.[13] was used to determine the ability of plant extracts/fraction to scavenge hydrogen peroxide radical. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). The extract/fractions prepared in distilled water were mixed with 0.6 ml of hydrogen peroxide solution (40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing plant extract/fraction in phosphate buffer without hydrogen peroxide. The absorbance of hydrogen peroxide (40 mM) without plant extract was considered as control (100 %). The concentration of plant extract/fraction required to scavenge 50 % hydrogen peroxide was calculated as IC_{50}.

Reducing power assay

This was determined by the previously described method of Oyaizu [14]. The reaction mixture containing 0.75 ml of various concentrations of plant extract/fraction, 0.75 ml of phosphate buffer

(0.2 N, pH 6.6) and 0.75 ml of potassium hexacyanoferrate ($K_3Fe(CN)_6$) (1 % w/v) was incubated at 50°C in water bath for 20 min. The reaction was stopped by the addition of 0.75 ml trichloroacetic acid (10 %) and then centrifuged for 10 min at 800 rpm. The supernatant (1.5 ml) of the individual reaction mixture was collected in different clean tubes and was mixed with 1.5 ml of distilled water followed by addition of 0.1 ml ferric chloride (0.1 % w/v) and kept for 10 min. The absorbance of reaction mixture was measured at 700 nm as the reducing power. The absorbance of control was considered as 100 % of Fe^{3+} ions and IC_{50} values were determined as the concentration of plant extract/fraction required to inhibit 50 % reduction of Fe^{3+} ions.

Isolation and characterization of active compounds

The isolation and purification of the secondary metabolites of the leaf aqueous extract was carried out by chromatographic techniques. The different chromatographic techniques used include:

Accelerated gradient chromatography (AGC)

The extract (30 g) was pre-adsorbed on silica gel by mixing and allowed to dry. The dried sample was loaded on the accelerated gradient chromatography column (75 cm x 2.0 cm) previously packed with 50 g silica gel. The elution solvent (ethylacetate: methanol mixtures) was prepared in increasing polarity and used in the gradient elution**.** A total of 75 fractions were collected. The fractions were concentrated *in vacuo* using rotary evaporator. The progress of elution was monitored with analytical TLC. On the basis of similarity in TLC pattern, the test tubes were combined into four (4), labeled A-D.

Vacuum liquid chromatography

The column was sucked dry after each fraction was collected. Each tube content was concentrated at room temperature. TLC analysis was then carried out on fractions collected. Fractions with similar R_f values were combined. Combined fractions were concentrated immediately using a rotary evaporator. Combined fractions designated as B (tube 6-10) and E (tube 22-25) showed one major spot each on TLC with some impurities at the base point and crystallized. The two eluants were evaporated to get the two components. Other components did not crystalline and were of insufficient quantities therefore they were not considered for further investigation.. The samples were subjected to further purification.

Preparative TLC (fraction E)

Concentrated fractions E was applied on the plate and developed in a chromatography jar containing hexane: ethylacetate (4:1). Developed plate was air dried, visualized under UV light and then placed in an iodine tank. The single band florescent spot that was stained under iodine was marked. The marked spot was scraped and collected along with silica gel. It was then eluted into a clean sample rube with ethanol. The eluted sample, concentrated at room temperature was greenish yellow amorphous solid and was labeled X. The compound was then analyzed using spectroscopy technique.

Conventional column chromatography (fraction B)

The stationary phase was a finely divided silica gel packed in a glass column in a form of slurry in hexane. The eluants obtained were monitored by analytical TLC. The compound showed a single spot on TLC examination indicating that it contained a major compound that was labeled Y. This compound was obtained in a very small quantity and hence in order to facilitate its structural elucidation, the compound was acetylated as described below. About 15 mg of Y was dissolved in minimum dried pyridine (1 ml) and acetic acid anhydride (3 ml) was added. This was stirred overnight on a magnetic stirrer. The resulting mixture was added dropwisely into ice and water (15 ml) with stirring. The solution was extracted with $CHCl_3$ (3 x 20 ml). The $CHCl_3$ extracts were combined, washed with dilute HCl (3 x 30 ml), with water (3 x 30 ml), dried over anhydrous $MgSO_4$ and then evaporated to dryness. The resulting colourless solid was crystallized from methanol to give crystalline solid. The structure of the acetylated compound was then elucidated using spectroscopy technique.

Spectroscopic analyses

Infrared spectroscopy

The infrared spectroscopic analyses of the isolates were carried out so as to know the different functional groups present in the isolated compounds. The infrared spectra were recorded on Shimadzu 8400s (Schimadzu Corporation, Kyoto Japan) Fourier Transform-Infrared (FT-IR) Spectrophotometry using KBr pellet in the range of 400-4000/cm. The mass spectrum of the complex was obtained using Jeol Gcmate.

Nuclear magnetic resonance spectroscopy (NMR)

^{1}H-NMR and ^{13}C-NMR spectra were acquired using a Bruker AC 300, varian G-600 instruments. Carbon multiplicities were monitored by the Distortionless Enhancement by Polarization Transfer (DEPT) and Attached Proton Test (APT) experiments. The DEPT data was recorded on the WM-360 spectrometer. The Heteronuclear Multiple Quantum Correlation (HMQC) and Heteronuclear Multiple Bond Correlation (HMBC) were acquired on DMX 600 instrument (600 MH_z). All the NMR spectra were recorded in deuteriochloroform (CDCl), deuteriopyridine (C_6D_5N), DMSO or D_2O and chemical shifts were reported in ppm downfield or tetramethylsilane (TMS). The residual solvent signals at 7.24 ppm for $CDCl_3$ and 8.6ppm/7.5ppm/7.15ppm for C_6D_5N were used as internal reference standards.

Data analysis

Data are expressed as means ± standard error of mean. Statistical significance was determined by one way analysis of variance. Duncan's multiple range test was used to detect differences between groups. A $p < 0.05$ was considered significant

Results

Result of the free radical scavenging activity **(Table 1)** showed that the highest DPPH scavenging activity was obtained with methanol and aqueous fraction with IC_{50} of 0.026 ± 0.011 and 0.026 ± 0.011 mg/ml respectively while the hexane fraction showed the least DPPH scavenging activity with IC_{50} of 0.096 ± 0.012 mg/ml. The

Table 1. Antioxidant capacity of *Hydrocotyl bonariensis* leaves

Extract/ Fractions	DPPH IC_{50} (mg/ml)	Metal chelating activity (%)	OH IC_{50} (mg/ml)	H_2O_2 IC_{50} (mg/ml)	Reducing power IC_{50} (mg/ml)	NO IC_{50} (mg/ml)
Methanol	0.026±0.011^{a}	28.3	0.846±0.014^{a}	0.868±0.015^{a}	0.521±0.012^{a}	0.755±0.018^{a}
Aqueous	0.025±0.011^{a}	35.5	0. 652±0.037^{b}	0.521±0.015^{b}	0.248±0.025^{b}	0.537±0.038^{b}
Ethylacetate	0.065±0.017^{b}	21.3	0.758±0.025^{a}	1.018±0.035^{c}	0.603±0.014^{a}	0.781±0.031^{a}
Chloroform	0.058±0.021^{a}	20.2	0.857±0.041^{a}	0.863±0.009^{a}	0.674±0.026^{a}	0.979±0.028^{a}
Hexane	0.096±0.012^{c}	6.6	2.504± 0.018^{c}	1.361±0.037^{c}	0.688 ±0.024^{c}	0.969±0.017^{a}

All values are expressed as mean±SD, n=3

Values in the same column with similar superscripts are not significantly different from each other

metal chelating activity (35.5 %), OH (IC_{50}, 0.652 ± 0.037) mg/ml, H_2O_2 (IC_{50}, 0.521 ± 0.015 mg/ml) NO (0.537 ± 0.038 mg/ml) free radical scavenging activity and reducing power activity (IC_{50} 0.248 ± 0.025) was highest with the aqueous fraction. This was closely followed with the methanolic fraction. The hexane fraction showed the least scavenging activity

The results of the FTIR spectroscopy (Figure 1) data indicates the following peaks (cm^{-1}); 3333 (O-H), 1555 (C=C bending variation), 1409 (C-H bending), 1049 (C-O) and 884 (=C-H).

The compounds X and Y as mentioned in the experimental section, were subjected to spectroscopic analysis for identification. The Techniques used were UV, IR, ^{1}HNMR and ^{13}CNMR spectroscopy. Figure 2 is the infrared absorption spectrum of compound X. While Figures 3 and 4 were

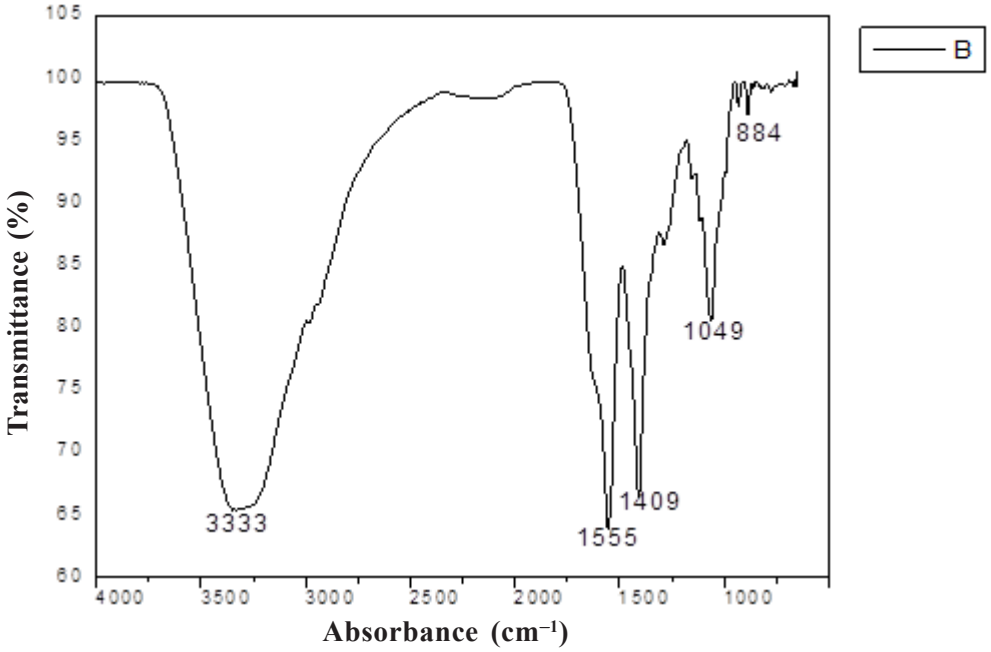


Figure 1. Fourier Transform-Infrared (FT-IR) spectra of isolates

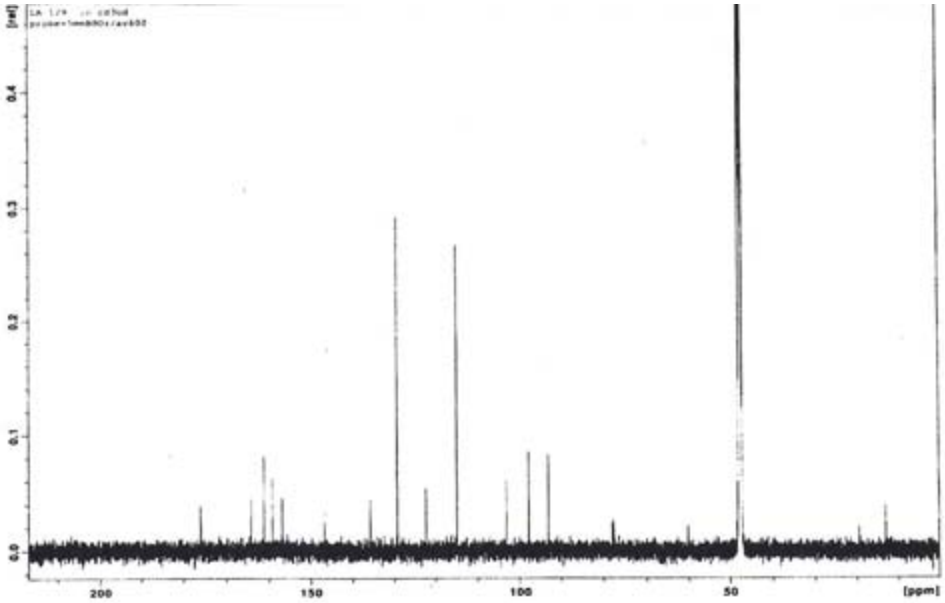


Figure 2. ^{13}C Nuclear magnetic Resonance of compound X

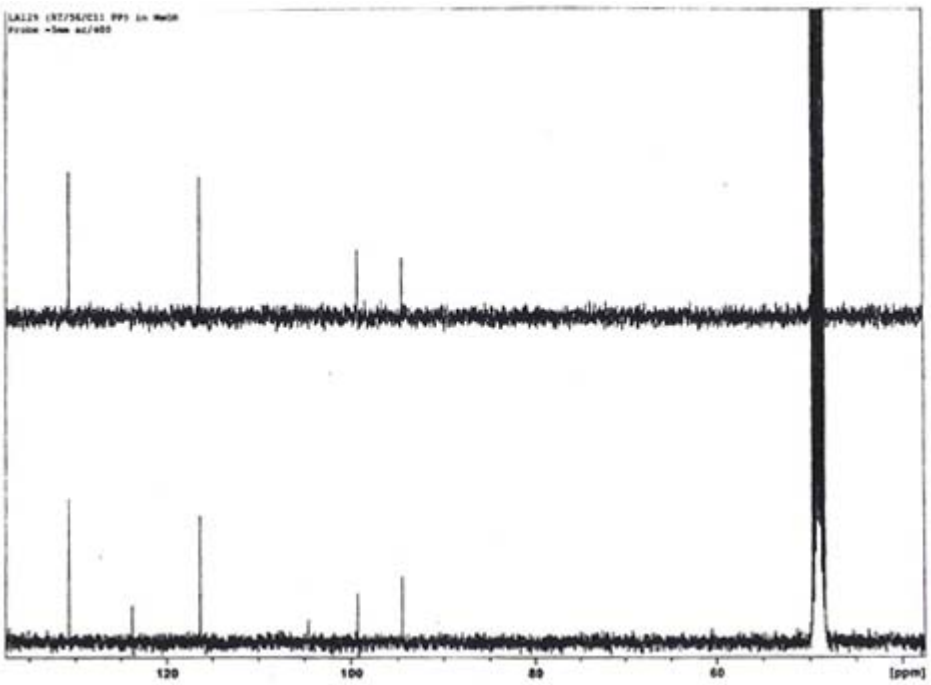


Figure 3. DEPT spectrum of compound X

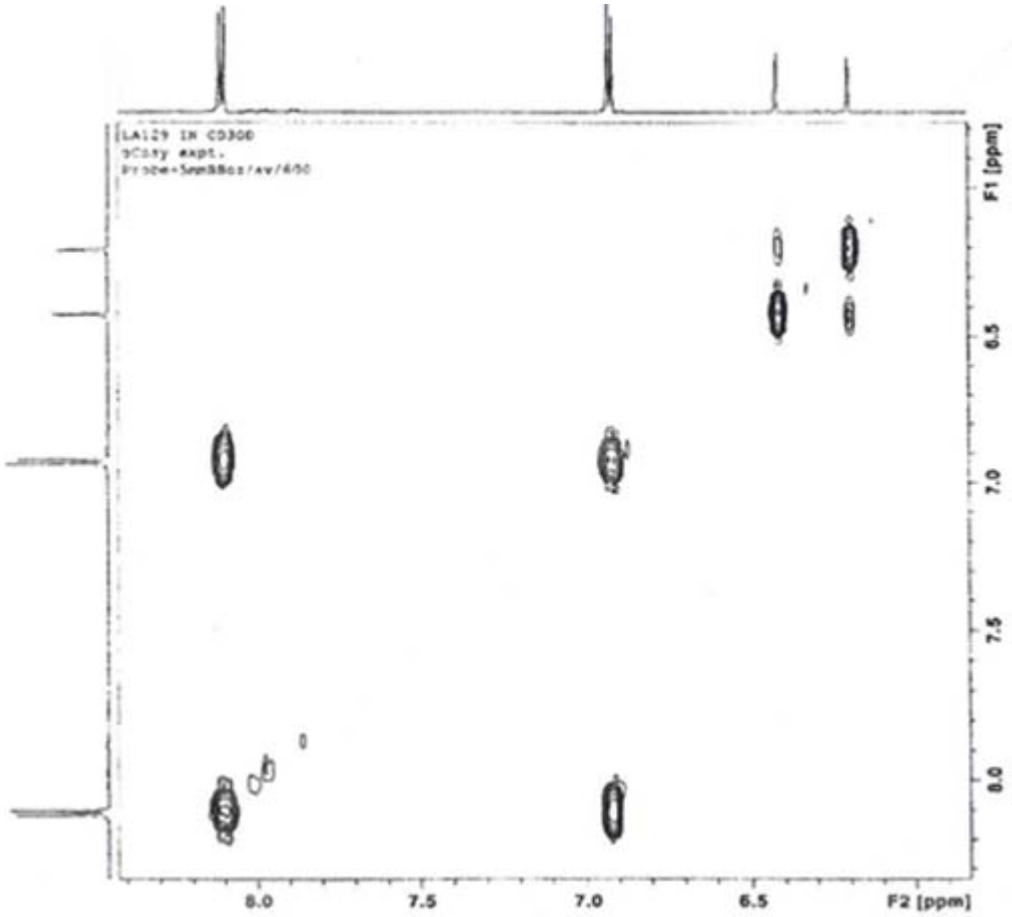


Figure 4. HH COSY spectrum of compound X

its DEPT and HH COSY spectrum respectively. The hydrogen-hydrogen correlation analysis of LOSP/CF-1 was presented as Figure 5. The details of the spectral peaks were noted as follows: ^{13}C NMR (500 MHz, DMSO-d6) δ: 146.71 (C-2), 153.73 (C-3), 176.00 (C-4), 126.87 (C-5), 97.87 (CH-6), 164.17 (C-7), 93.07 (CH-8), 161.10 (C-9), 103.15 (C-10), 122.33 (C-1'), 129.29 (CH-2', 6'), 114.92 (CH-3', 5'), 159.4 (C-4'). ^{1}H NMR spectra (500 MHz, DMSO-d6) δ: 6.20 (1H,d,j=2.0Hz), 6.44 (1H,d,j=2.0Hz), 8.12 (2H,d,j=8.8Hz), 6.93 (2H,d,j=8Hz). Correlating with data from the literature, the structure of compound X is shown in Figure 6.

The NMR data obtained for the compound Y was ^{13}C NMR (500 MHz, DMSO-d6) δ: 38.9 (CH_2-1), 28.2 (CH_2-2), 8.0 (CH-3), 39.7 (CH_2-4), 40.3 (C-5), 122.2 (CH-6), 31.9 (CH_2-7), 31.9 (CH-8), 50.1 (CH-9), 37.3 (CH-10), 21.6 (CH_2-12), 42.3 (CH-13), 56.7 (CH-14), 24.3 (CH_2-15), 29.4 (CH_2-16), 56.0 (CH-17), 11.9 (CH_3-18), 19.4 (CH_3-19), 36.2 (CH-20), 19.3 (CH_3-21), 33.9 (CH_2-22), 29.7 (CH_2-23), 45.8 (CH-24), 29.1 (CH-25), 19.8 (CH_3-26), 19.4 (CH-27), 21.0 (CH_2-

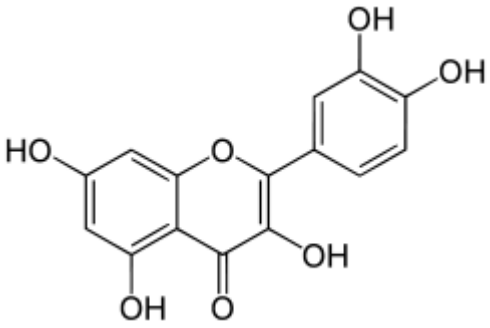


Figure 5. Quercetin

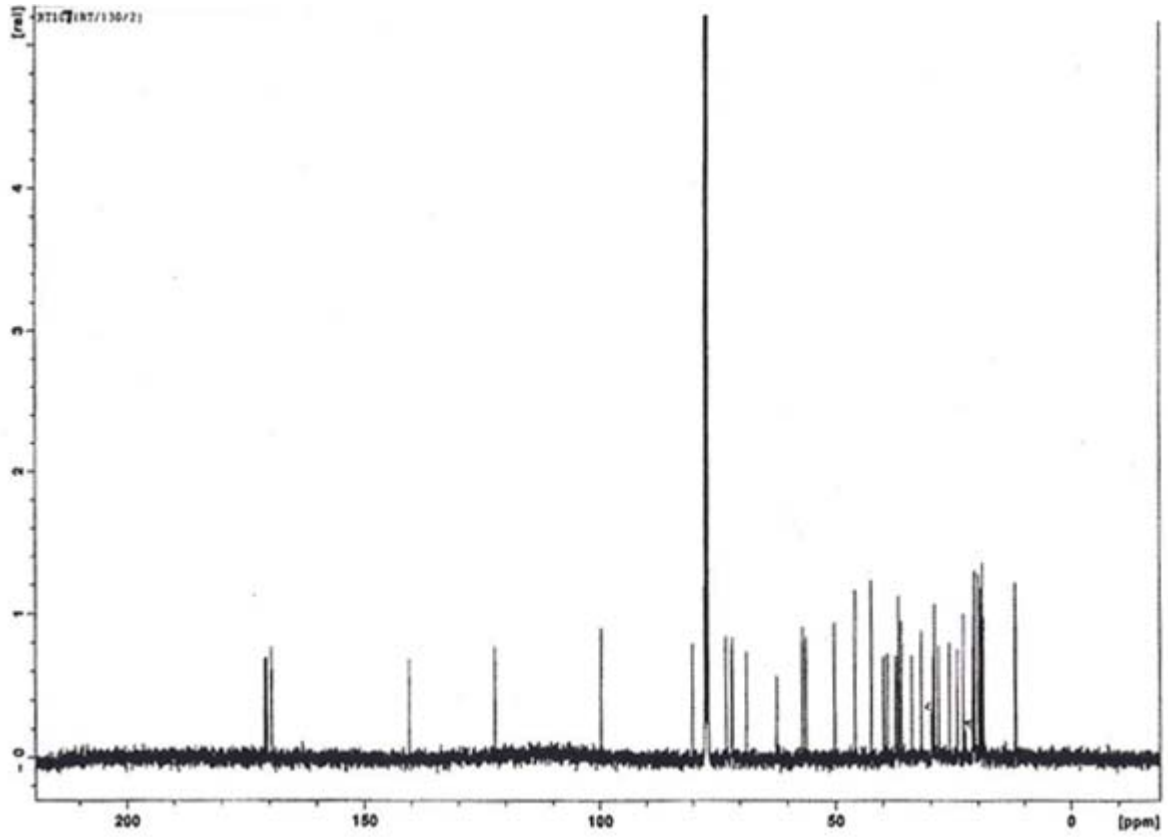


Figure 6. ^{13}C NMR spectrum of compound Y

28), 12.0 (CH_3-29), 99.6 (C-1'), 71.5 (CH-2'), 72.9 (CH-3'), 68.5 (CH-4', 71.7 (CH-5'), 62.1 (CH_2-6'), 170.8 (CO-2'), 170.4 (CO-3'), 169.3 (CO-4'), 169.4 (CO-6'), 20.8 (CH_3-2'), 20.7 (CH_3-3'), 20.6 (CH_3-4'), 20.6 (CH_3-6') (Figure 7) 1H NMR spectra (500 MHz, DMSO-d6) δ: 3.52(1H, m), 5.38 (1H,m), 0.67 (3H, s), 0.96 (3H, s), 1.04 (3H, d, j=6.4Hz), 0.80 (3H, d, j=6.8Hz), 0.84 (3H, d, j=6.9Hz), 0.82 (3H, t, j=7.3Hz), 4.55 (1H, j=8.0Hz), 4.85 (1H, t, j=7.9Hz), 5.25 (1H, t, j=9.0Hz), 5.18 (1H, t, j=9.0Hz), 3.65 (1H, m), 4.20 (1H, dd), J=11.6Hz, 2.1Hz), 4.10 (1H, dd, j=11.7 Hz, 5.2Hz), 1.87 (3H, s), 1.95 (3H, s), 2.03 (3H, s), 2.05 (3H, S) (Figure 7). The DEPT diagram and proposed structure for the acetylated compound was shown in Figure 8 and 9 respectively.

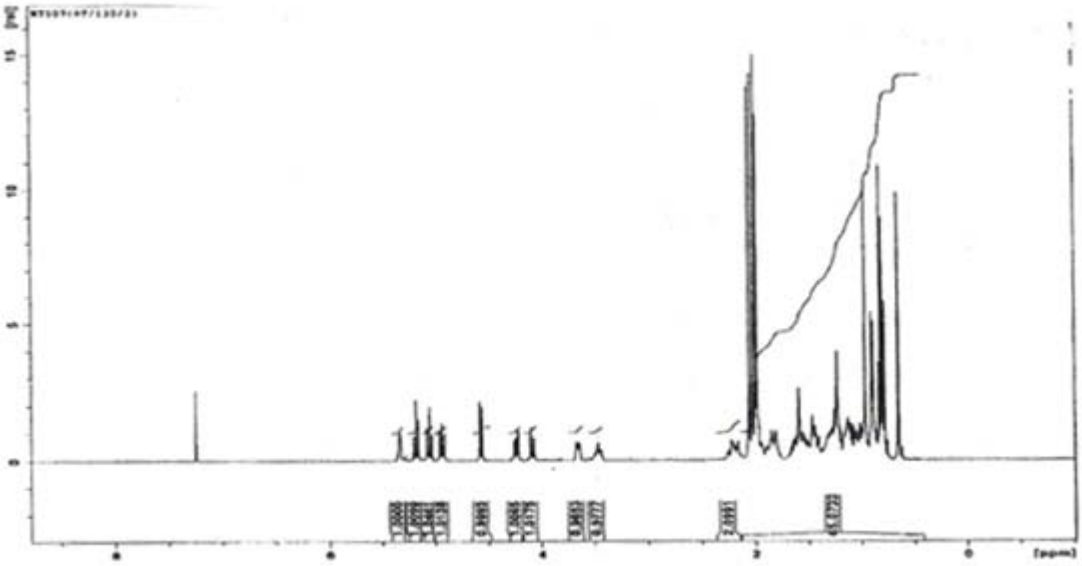


Figure 7. [1]H NMR spectrum of compound Y

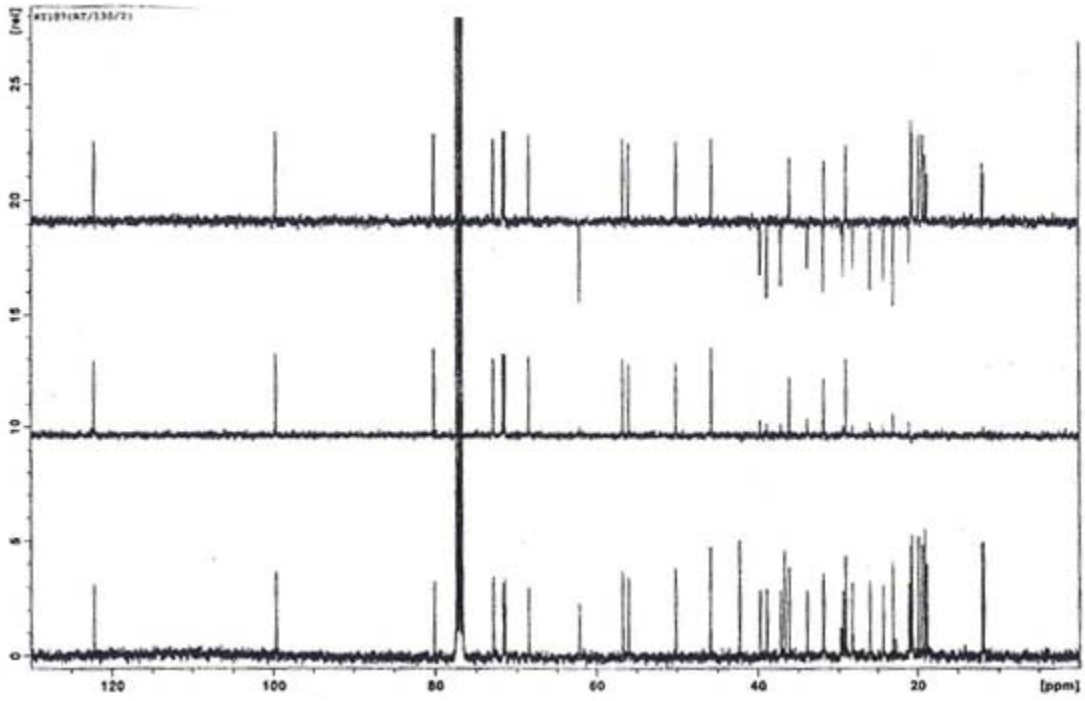


Figure 8. DEPT spectrum of compound Y

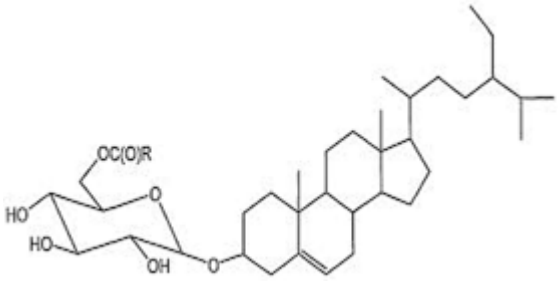


Figure 9. Proposed structure of acetylated compound Y

Discussion

In a previous study, we reported the aldose reductase activity of crude extract of *Hydrocotyl bonariensis* leaves and its potentials as an anticataractogenic agent [3]. In the present study, we have evaluated the free radical scavenging activities of successive fractions of the extract so as to provide explanation for the mechanism of action of the anticataractogenic compound and also isolate natural compounds from the leaves in its pure form. Data from the study indicates that all the fractions showed free radical scavenging activity. The study further showed that the aqueous fraction has the best antioxidant properties while hexane fraction has the least. ROS are known to damage cellular membranes by inducing lipid peroxidation. They can also damage cellular macromolecules including DNA, proteins, lipids and chlorophyll [15]. The most popular ROS are $\cdot O^{2-}$ (superoxide radical), H_2O_2 (hydrogen peroxide) and ·OH (hydroxyl radical) originating from one, two or three electron transfers to dioxygen (O_2). Under physiological conditions $\cdot O^{2-}$ is not very reactive against the biomolecules of the cell and in aqueous solutions at neutral or slightly acidic pH disproportionates to H_2O_2 and $\cdot O^{2-}$ H_2O_2 is relatively stable and not very reactive, electrically neutral ROS, but is very dangerous because it can pass through cellular membranes and reaches cell compartments far from the site of its formation [16]. Free radicals are very dangerous because they can initiate the oxidation of biomolecules, such as protein, lipid, amino acids and DNA which will lead to cell injury and can induce numerous diseases. Our study indicates that *Hydrocotyl bonariensis* leaves contain bioactive compounds with antioxidant potentials. Data from the spectroscopy studies revealed that *H. bonariensis* is rich in flavonoids which are polyphenolic compound. Previous reports have shown that many dietary polyphenolic constituents derived from plants are more effective antioxidants *in vitro* than vitamins E or C and thus might contribute significantly to protective effect *in vivo* [17]. The antioxidant activity of polyphenols has been attributed to their ability to scavenge free radicals (which allow them to act as reducing agents and hydrogen or electron donor) or chelate metal cations [18-20]. Data obtained in this study is in agreement with this report. Also, Zhang *et al*.[20] reported that the presence of electron-donating and electron-withdrawing substituent in the ring structure of phenolics as well as the number and arrangement of the hydroxyl groups determines their antioxidant potential. We have also observed based on this study that the free radical scavenging capacities of the *H. bonariensis* extract is correlated with the solvent employed in the fractionation. It was shown to be reduced with decrease in the polarity of the solvent. This observation agrees with the report of Moure *et al*.[21].

Compound X was obtained as a greenish yellow amorphous solid. The UV in MeOH gave absorption maxima at 254 and 365 nm identical with literature [22]. The ^{13}C NMR spectrum of compound showed fifteen carbon signals which were resolved into six methines and nine quaternary carbon atoms through the DEPT experiments (Figure 3). The ^{1}H NMR of the compound indicated a two doublet signal integrating for two protons in the aromatic region at d8.2 (d, J = 8.8Hz), ascribable to H-2' and H-6'. An identical doublet signal was also observed at d5.93 (d, J = 8.8Hz) ascribable to H-3' and H-5' with the aid of HMQC. The COSY-45^{o} spectrum showed cross-peaks between the two aromatic signals which confirmed the *ortho*-coupling of the doublets. With the spectroscopy studies and comparative analysis with the literatures [22], the structure of compound X was confirmed to be Quercetin.

The ^{13}C NMR spectrum of compound Y displayed forty-three carbon signals which included eight quaternary carbons, thirteen methines, twelve methylenes and ten methyls resolved by the DEPT experiments. The ^{1}H NMR and the ^{13}C

NMR spectrum characteristic spectra data, when compared with related sterols in literature [23-25], were suggestive of 3-*O*-β-D-glucopyranosyl-sitosterol (β-sitosterol-3-*O*-β-D-glucopyranoside).

Conclusion

The result of the research demonstrated the efficacy of the constituents of *Hydrocotyl bonariensis* as antioxidant and that the extraction of this bioactive compound is dependent on the solvent polarity. This property may be attributed to the presence of flavonoid (polyphenolic compound) present in its leaves. One of these flavonoids that has been isolated in this study is quercetin

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