Antioxidant activity, total phenolic and flavonoid contents of *Anthocleista djalonensis* root extracts

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ABSTRACT

Background: *Anthocleista djalonensis* (Chev.), a plant native to tropical West Africa, is used ethnomedicinally in the management of several diseases.

Objectives: This study seeks to determine the free radical scavenging ability of the methanol, ethyl acetate and aqueous root extracts of *A. djalonensis* on 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and hydrogen peroxide radicals and to determine the total phenolic and flavonoid contents of the extracts.

Method: The antioxidant effect of *A. djalonensis* root extracts was compared with that of ascorbic acid using DPPH (2, 2- diphenyl-2- picrylhydrazyl) assay and hydrogen peroxide scavenging assay methods. Percentage antioxidant activity (AA %), IC_{50}, total phenolic content and total flavonoid of extracts were determined.

Result: The total phenolic content of the extracts ranges from 132.73 ± 20.25 to 207.93 ± 11.7 mgGAE/g of dry plant extract while the total flavonoid content of the extracts ranges from 78.2 ± 0.12 to 100.6 ± 2.08 mg QE/g dry plant extract. The extracts showed a good but lower DPPH radical scavenging activity compared to ascorbic acid. The methanol extract showed a maximum percentage radical scavenging of 94.76 ± 0.05% (IC_{50} = 148.54 µ/ml) on DPPH radicals at concentration of 1000 µg/ml among the three extracts. The extracts showed a non-significant ($P > 0.05$) radical scavenging activity on hydrogen peroxide compared to ascorbic acid (74.14 ± 0.05 %). The methanol extract have the highest hydrogen peroxide scavenging activity (79.24 ± 0.07 %).

Conclusion: This result indicate that the root extracts of *Anthocleista djalonensis* possesses antioxidant phytochemicals and can be a good source of antioxidants to ameliorate conditions that are caused by oxidative effects of free radicals.

Key words: *Anthocleista djalonensis*, DPPH, free radicals, oxidative stress

Activité antioxydante, teneurs totales en phénoliques et flavonoïdes des extraits de racine *Anthocleista djalonensis*

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RESUME

Contexte : *Anthocleista djalonensis* (Chev.), une plante originaire de l'Afrique de l'Ouest tropicale, est utilisée de manière ethno-médicale dans le traitement de plusieurs maladies.

Objectifs : Cette étude cherche à déterminer la capacité de piégeage des radicaux libres des extraits de méthanol, d'acétate d'éthyle et de racines aqueuses d'*A. Djalonensis* sur les radicaux 2, 2-diphényl-1-picrylhydrazyle (DPPH) et de peroxyde d'hydrogène, ainsi que tous les contenus phénolique et flavonoïde des extraits.

Méthode : L'effet antioxydant des extraits de racine de *A. djalonensis* a été comparé à celui de l'acide ascorbique à l'aide des méthodes de dosage DPPH (2, 2-diphényl-2-picrylhydrazyle) et de purification au peroxyde d'hydrogène. Le pourcentage d'activité antioxydante (AA%), la IC_{50}, la teneur en composés phénoliques totaux et le flavonoïde total des extraits ont été déterminés.

Résultat : la teneur totale en phénol des extraits varie de 132,73 ± 20,25 à 207,93 ± 11,7 mgGAE/g d'extrait sec de plante tandis que la teneur totale en flavonoïdes des extraits varie de 78,2 ± 0,12 à 100,6 ± 2,08 mg d'EQ/g d'extrait sec de plante. Les extraits ont montré une activité de piégeage des radicaux de DPPH bonne mais inférieure à celle de l'acide ascorbique. L'extrait au méthanol a montré un pourcentage maximum de balayage de radical de 94,76 ± 0,05% (IC50 = 148,54 µ/ml) sur les radicaux DPPH à une concentration de 1000 µg/ml parmi les trois extraits. Les extraits ont montré une activité de piégeage des radicaux non significative ($P > 0,05$) sur le peroxyde d'hydrogène par rapport à l'acide ascorbique (74,14 ± 0,05%). Les extraits au méthanol ont la plus grande activité d'élimination du peroxyde d'hydrogène (79,24 ± 0,07%).

Conclusion : ce résultat indique que les extraits de racine de *Anthocleista djalonensis* possèdent des composés phytochimiques antioxydants et peuvent constituer une bonne source d'antioxydants pour améliorer les conditions provoquées par les effets oxydants des radicaux libres.

Mots-clés : *Anthocleista djalonensis*, DPPH, radicaux libres, stress oxydatif

INTRODUCTION

Plants contain phytochemicals (phenolics, phenolic acids, flavones, isoflavones, flavonols and tannins) with antioxidant properties in varying degrees.[1] Antioxidants are free radical scavengers which protect the human body against the deleterious effects of free radicals. [2] The antioxidants present in plants are capable of neutralizing these free radicals by donating required number of electrons to stabilize them.[3,4] Free radicals have been linked to various pathological conditions such as diabetes mellitus, arthritis, Parkinson's disease, Alzheimer's disease, male infertility and cancer.[5] They are naturally produced in the body as a result of chemical reactions during cellular and biochemical processes. Physiologically, the oxygenated free radicals are among the most important radical species. A number of chemical and physical phenomena can initiate oxidation, which proceeds continuously in the presence of substrate until a blocking defense mechanism occurs.[6] Target substances for oxidative stress include polyunsaturated fatty acids, phospholipids, cholesterol, oxygen and DNA.

In recent years, there has been a tremendous surge in interest in antioxidants of plant origin.[7] Many reported scientific studies on *in-vitro* antioxidant activities of medicinal plants have supported the idea that plant constituents with antioxidant activity have the ability of providing protection against oxidative stress in humans.[8]

Anthocleista djalonensis belongs to the family Loganiaceae. It have been used traditionally as anti-infectives, anti-inflammatory, anti-protozoal, anti-spasmodic, fertility enhancer.[9,10] Different phytochemicals from *A. djalonensis* have been isolated and reported.[11] The antioxidant activity of the root extract of the plant has not been reported. Since oxidative stress is reported to be a hallmark in the pathogenesis of various disease conditions. Hence the objective of the present study was to evaluate the *in-vitro* antioxidant activity and to determine quantitatively the total phenolics and flavonoid contents. This will provide a scientific basis for its use in ethnomedicine as a source of antioxidants for treating disease conditions linked to oxidative stress caused by free radicals.

METHODS

Chemicals and Standards

l-ascorbic acid, gallic acid, quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, hydrogen peroxide, sodium bicarbonate, aluminium chloride, methanol, distilled water. All the chemicals were of analytical grade and procured locally.

Collection and preparation of plant material

The root of *Anthocleista djalonensis* was collected from a bush in Ikpoba Hill area of Benin City in June 2017. The plant was identified and authenticated by Pharm. H. Uwumarongie of the Department of Pharmacognosy, University of Benin, Benin City, where a voucher specimen was deposited and assigned number UBN/PCG/1036. The roots were washed, cut into bit, air-dried at room temperature and pulverized to fine powder. The powdered sample (1000 g) was defatted using n-hexane and then extracted successively by maceration with ethyl acetate, methanol and water for 72 hr. The extracts were filtered and concentrated using a rotary evaporator, air-dried and then stored in a refridgerator at 4°C until used.

Qualitative phytochemical analysis

Test for alkaloid, phenolics, tannins, flavonoids, terpenoids, saponins, steroids, glycosides, proteins and carbohydrates were carried out using standard methods by.[12-13]

In *vitro* anti-oxidant assay

DPPH Radical scavenging assay

The free radical scavenging activities of the ethyl acetate, methanol and aqueous root extracts of *A. djalonensis* were determined using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay method as described by.[14] Graded concentrations (62.5, 125, 250, 500, 1000 µg/ml) of the extracts and L-ascorbic acid (standard) were prepared in distilled water. To 2 ml of the different extract solutions and standard was added 0.5 ml of freshly prepared DPPH solution (0.4 mM). The mixtures was shaken and kept in a dark cupboard at room temperature for 30 min. The absorbance of the solutions was measured spectrophotometrically at 518 nm against a blank containing methanol. DPPH and methanol without the extract and standard was used as negative control. The experiment was done in triplicates. The percentage (%) radical scavenging activity was calculated using the formula:

$$\% \text{ scavenging activity} = \frac{A_o - As}{A_o}$$

Where A_o is the absorbance of the control and A_s is the

absorbance of the sample. The IC_{50} of the different extracts was interpolated from their absorbance-concentration curve.

Hydrogen peroxide scavenging assay

The ability of *A. djalonensis* root extracts to scavenge hydrogen peroxide was determined using the method described by Ruch.[15] The extracts and standard were prepared in distilled water at different concentrations (62.5, 125, 250, 500, 1000 µg/ml). The extracts (4 ml) were mixed with 0.6 ml of hydrogen peroxide solution (40 mM) prepared in phosphate buffer (0.1 M P^H 7.4). The absorbance of the solutions was taken after 10 min at 230 nm against a blank containing phosphate buffer without hydrogen peroxide and extract. The experiment was done in triplicates. The percentage scavenging activity of the extracts and standard were calculated using the equation:

$$\% \text{ scavenging activity} = \frac{A_o - As}{A_o}$$

Where A_o is the absorbance of the control and A_s is the absorbance of the sample. The IC_{50} of the different extracts was interpolated from their absorbance-concentration curve.

Determination of total phenolic content

The total phenolic content of the ethyl acetate, methanol and aqueous root extracts of *A. djalonensis* were determined using Folin-Ciocalteu reagent (FCR) as described by Singleton[16] Gallic acid was used as the standard. Graded concentrations of gallic acid and 1 mg/ml of the extract were prepared. To 0.5 ml of the extract and standard was added 2 ml of Folin-Ciocalteu reagent (diluted 10 times with water) and allowed to stand for 3 min. Then 2.5 ml of 7.5 % sodium carbonate was added to the mixture. The mixture was shaken and kept in the dark at room temperature for 45 minutes. The absorbance was taken at 760 nm against a blank containing all the reagents except the standard and extract. A standard gallic acid calibration curve was constructed. The total phenolic content of the extracts was calculated as gallic acid equivalent (mgGAE/ g dry sample). The tests were done in triplicates. Values where expressed as mean ± standard error of mean (SEM).

Determination of total flavonoid content

The total flavonoid content in the extracts of *A. djalonensis* root was determined using the aluminium chloride complex forming assay as described by Mervat *et al.*[17] Quercetin was used as the standard. Graded concentration of quercetin was prepared in methanol. To 1 ml of the standard and extract (1 mg/ml) were added 0.1 ml of 10 % aluminium chloride and 0.1 ml of 1M potassium acetate prepared in methanol. The mixture was shaken to mix thoroughly and then kept in the dark for 30 min to complete the reaction. The absorbance of the solution was taken at 420 nm using a spectrophotometer, against a blank. The test was done in triplicates. The total flavonoid content of the extracts was estimated as milligram of quercetin equivalent (QE) per gram of dry sample (mgQE/g dry sample). Values where expressed as mean ± standard error of mean (SEM).

Statistical analysis

The results were analyzed using the Graph Pad Prism 6.01 for Windows. Results were analyzed using students t-test and ANOVA and were expressed as mean ± standard error of mean (SEM). The level of significance was accepted at $P < 0.05$

RESULTS

In this study, the in *vitro* antioxidant activity of *A. djalonensis* root extracts using DPPH and hydrogen peroxide scavenging assay methods was compared with L-ascorbic acid. The total phenolics and flavonoid contents of the extracts were estimated.

Qualitative phytochemical analysis

Preliminary phytochemical test of the ethylacetate, methanol and aqueous root extracts of *A. djalonensis* revealed the presence of alkaloids, saponins, tannins, phenolics, flavonoids and terpenoids in all the extracts.

DPPH radical scavenging assay

The radical scavenging activity of ethyl acetate, methanol and aqueous root extracts of *A. djalonensis* on DPPH is shown in figure 1, table 1. The extracts showed a significantly lower ($P < 0.05$) antioxidant activity compared to L-ascorbic acid. The IC_{50} of the ethyl acetate, methanol and aqueous extracts are 511.67 µg/mL, 148.54 µg/mL and 580.01 µg/mL respectively. The methanol extract showed the highest percentage scavenging activity of 85.22 ± 0.05% at concentration of 1000 µg/ml compared to the ethyl acetate (59.72 ± 0.09 %) and aqueous (66.34 ± 0.04 %) extracts (table 1). The IC_{50} value is used to determine the concentration of extracts at which they scavenge 50 % of the DPPH radical.

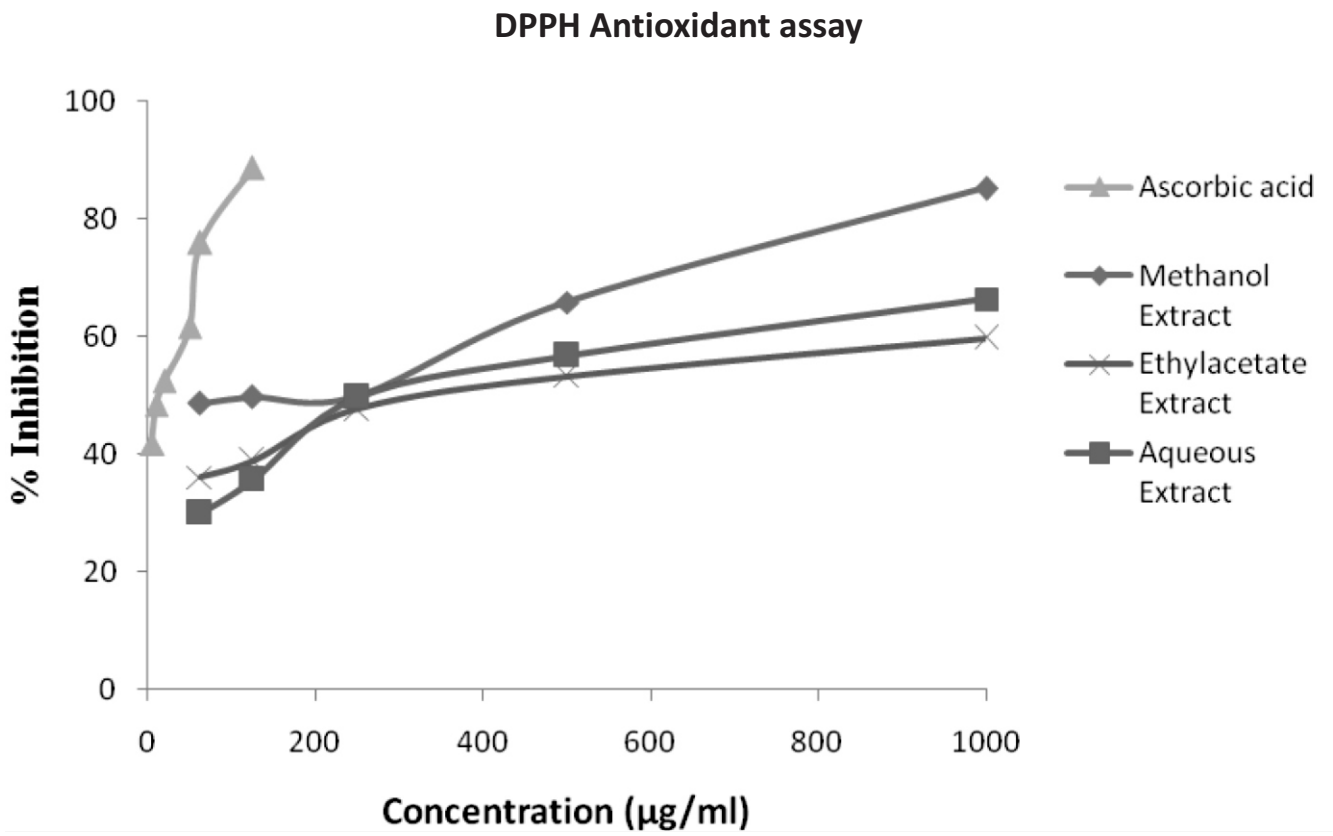

Figure 1: Percentage inhibition on DPPH radical by *A. djalonensis* root extracts compared to ascorbic acid.

Hydrogen peroxide scavenging activity

As shown in figure 2, the ethyl acetate, methanol and aqueous root extracts of *A. djalonensis* showed a dose-dependent non-significant ($P > 0.05$) hydrogen peroxide scavenging activity compared to ascorbic acid. The methanol extract had the best H_2O_2 scavenging activity with IC_{50} = 34.19 µg/ml compared to the ethyl acetate (IC_{50} = 259.5 µg/ml and aqueous (IC_{50} = 348.26 µg/ml) extracts. Maximum percentage inhibition of hydrogen peroxide at concentration of 1000 µg/ml was seen in the methanol extract (79.24 ± 0.07 %) compared to the ethyl acetate (61.13 ± 0.02 %), aqueous (62.43 0.01%) extracts and ascorbic acid (70.83 ± 0.05 %). The result clearly shows that the methanol extract had more hydrogen peroxide scavenging capacity than the ethyl acetate, aqueous extracts and ascorbic acid.

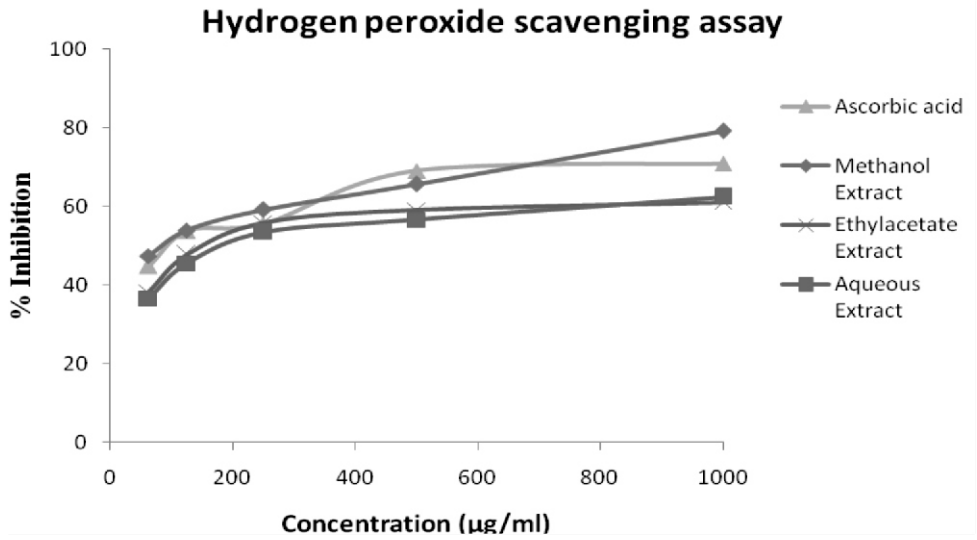

Figure 2: Percentage inhibition on hydrogen peroxide radical by *A. djalonensis* root extracts compared to ascorbic acid.

Total Phenolics (TPC) and Total flavonoid Contents (TFC)
The ethyl acetate, methanol and aqueous root extracts of *A. djalonensis* contained important phytochemicals which include phenolics, flavonoids and tannins. The total phenolic content of the ethyl acetate, methanol and aqueous root extracts *A. djalonensis* are 137.47 ± 35.15, 207.93 ± 11.75 and 132.73 ± 20.25 mg gallic acid equivalernts (GAE)/g dry weight sample respectively. Maximum total phenolic content was seen in the methanol extract with TPC of 207.93 mgGAE/mg dry weight sample.

The total flavonoids content of the extracts was estimated to be 78.2 ± 0.12, 100.6 ± 2.08 and 93.6 ± 2.08 mg quercetin equivalents (QE)/mg dry weight sample, of the ethyl acetate, methanol and aqueous extracts respectively (table 4). The methanol extract had a maximum TFC of 100.6 ± 2.08 compared to the other extracts.

Table 1: Total phenolic and flavonoid contents of the root extracts of *A. djalonensis*

Constituents	Ethylacetate extract	Methanol extract	Aqueous extract
Total phenolics ‡	137.47 ± 35.15	207.93 ± 11.75	132.73 ± 20.25
Total flavonoid *	78.2 ± 0.12	100.6 ± 2.08	93.6 ± 2.08

Data represented as Mean ± SD (n = 3).

‡ Expressed as mg gallic acid equivalents / g dry weight extract.

* Expressed as mg quercetin equivalents / g dry weight extract.

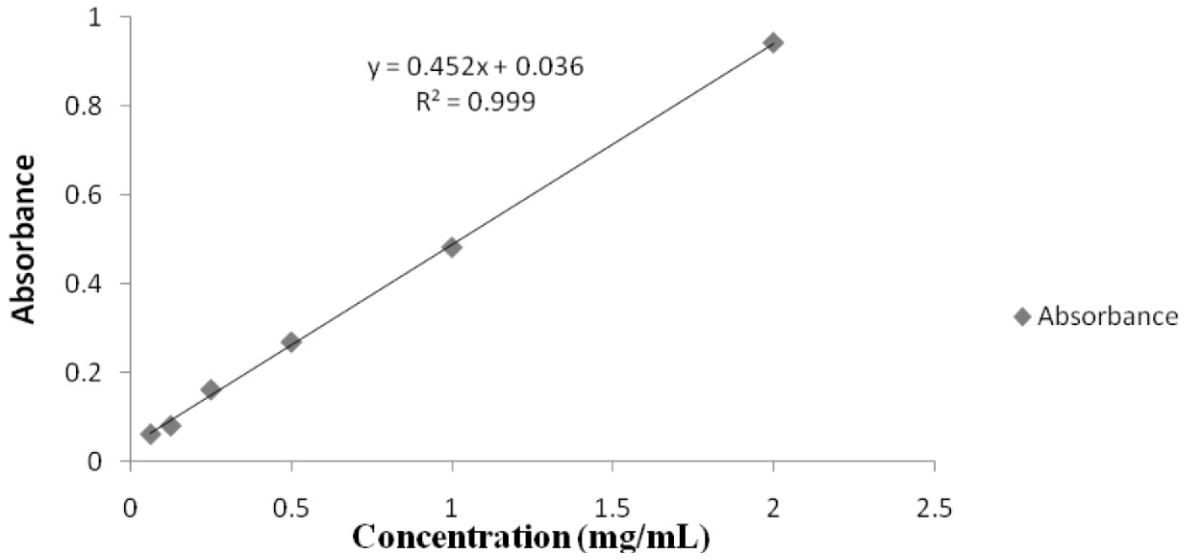

Figure 3: Standard gallic acid calibration curve

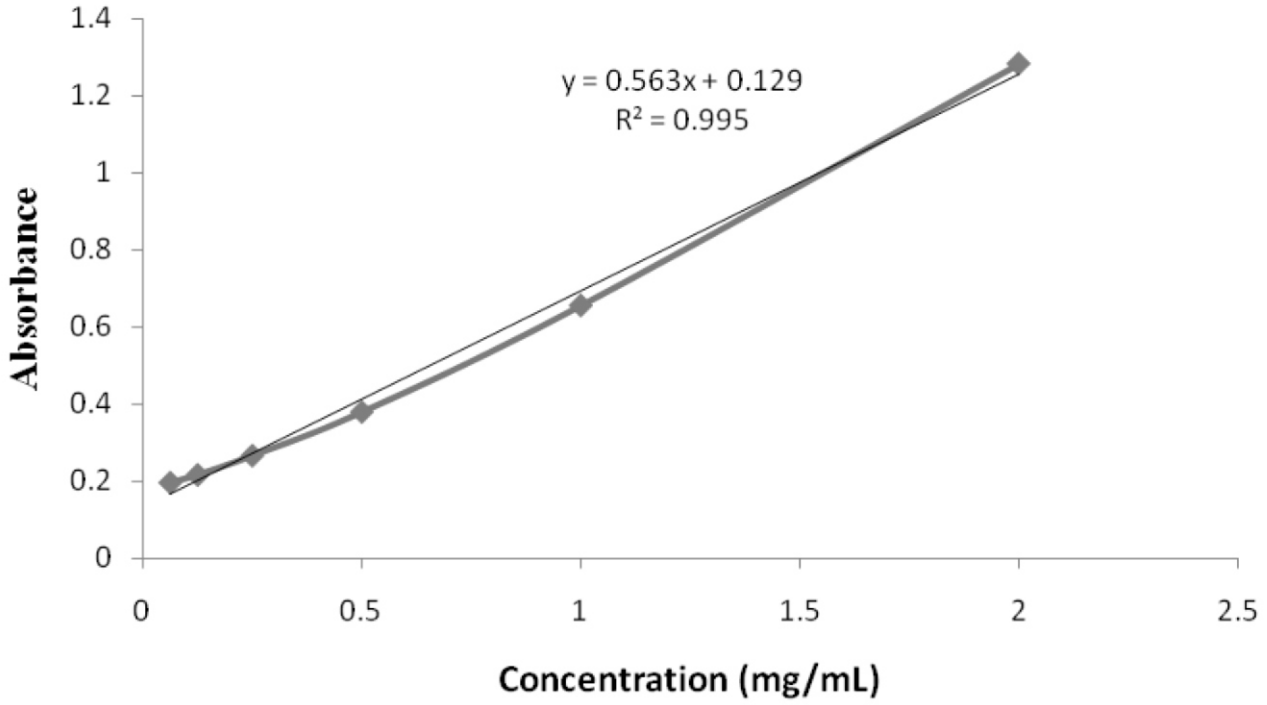

Figure 4: Standard quercetin calibration curve

DISCUSSION

The use of medicinal plants as antioxidants in their crude or herbal formulations has gained high popularity all over the world in recent years. These plants possesses the ability to scavenge free radicals and protect the body against the development of diseases caused by oxidative stress, such as cancer, coronary heart disease, type 2 diabetes, arthritis, male infertility.[18] In humans, reactive oxygen species are continuously generated and cause oxidative damage to cellular components in the presence of depleted antioxidant defense mechanism in cells.[19]

For the DPPH assay, the extracts showed a good antioxidant scavenging activity by causing a change of the DPPH solution from purple to yellow. Mosquera *et al*.[20] observed that a change of the DPPH colour from purple to yellow signifies that its radical is being scavenged by an electron donating substrate. The methanol extracts having the highest percentage scavenging activity on DPPH radical suggests that its strong radicals scavenging power could be closely related to the high levels of phenolic compounds (which have the ability to donate hydrogen ion to stabilize the radical ion) as observed in the total phenolic and flavonoid contents (table 3) of the extract.

Hydrogen peroxide is one of the sources of reactive oxygen species (ROS) in human body.[21] It generates hydroxyl radicals inside cells, thereby causing lipid peroxidation, DNA damage and resultant cell death.[22] The root extracts of *A. djalonensis* was observed to possess a high capacity to scavenge hydrogen peroxide radicals, thus terminating the chain reaction that causes cell damage. Figure 2 showed that the extracts possessed substantial dose-dependent antioxidant activity with the methanol extract with IC_{50} = 70.56 µg/ml, having the highest percentage scavenging activity (79.99 %) compared to the ethyl acetate (68.3 %) and aqueous (64.42 %) extracts. The scavenging potential of the extracts may possibly be dependent on the phenolic compounds present.

Antioxidant capacity of plant extracts depend on factors such as the composition, chemical structures of the constituents and conditions of the test used.[23] These results indicates that the phytochemical (s) present in *A. djalonensis* root extracts performed as good electron or electron donors and therefore should be able to terminate radical chain reaction by converting free radicals to more stable products.

The nature and quantity of phytochemicals present in a plant is dependent on the area where they were collected. The plant was collected from a location in Benin City and the environmental condition of the area might affect the quantity of the determined parameters.

CONCLUSION

This study shows that the root extracts of *A. djalonensis*

exhibited high antioxidant activity. The antioxidant property indicates that the plant is a rich source of natural antioxidants, which is invariably attributed to the phenolic and flavonoid contents in the plant. Thus, this plant can be utilized as an alternative source of useful drug for people seeking herbal antioxidant formulation.

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