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# Antioxidant Activity of Leaves' Extracts of Citrus Sinensis: Determination of Radical Scavenging Capacity, Antiradical Power, Total Polyphenols and Flavonoids Content

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## ABSTRACT

*Citrus sinensis* has intensively been used for its nutritional and therapeutic values, usually attributed to their antioxidant properties. Several studies have earlier focused on its fruits, seeds, oils, juice and flowers. This study focuses on leaves and aimed at determining radical scavenging capacity (RSC), antiradical power(ARP), total polyphenols (TPC) and flavonoids (TFC) content of the aqueous (AE), hydroethanolic (HEE) and ethanolic (EE) extracts and their activities on free radicals DPPH•, ABTS+ and NO. Phenolic compounds of all the extracts were measured by Folin-Ciocalteu assays. RSC of 1,1-diphenyl-2-picrylhydrazyl (DPPH•), 2,2'-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS+) and nitric oxide (NO•) radicals were measured. Also, the reducing power of extracts on phosphomolybdenum was evaluated by total antioxidant capacity (TAC) assay, antiradical power and $EC_{50}$ were determined. All the extracts possessed high TPC ranging from 306.67 ± 43.72 to 353.33 ± 30.55 µg of Eq Catechin /mg respectively for EA and EE. EE, EA, EC showed their ability to scavenge all the radicals and an important reducing power. Aqueous extracts presented the lower $EC_{50}$ (mg/ml) 4.60, 4.27, and $4.01x10^{-4}$ with DPPH•, $ABTS^{+}$, NO• respectively and the highest ARP. Many correlations observed between TAC and $ABTS^{+}$-RSC ($r = 0.958$, $p<0.001$), TAC and DPPH•-RSC ($r= 0.934$, $p<0.001$); TPC and DPPH•-RSC ($r=0.738$, $p<0.05$) justified its ability. leaves' Aqueous Extracts possess high radical scavenging activity and total polyphenols, good TAC and ARP. Leaves' extracts can be used as natural antioxidant source and deserve to be further explored for biological activities.

**Keywords:** *Citrus sinensis, L*eaves, Radical scavenging capacity, polyphenols, antioxidants

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## INTRODUCTION

Oxidative stress is involved in the pathomechanism of over 100 diseases such as cancer, malaria, rheumatoid arthritis, atherosclerosis, obesity, diabetes mellitus, hypertension , aging[1,2], Alzheimer's disease[3] , Parkinson's disease[4] and prion disease[5,6], Oxidative stress involves free radicals which are inevitably produced not only by normal physiological processes in biological systems, but also exogenously[7]. They include oxygen radicals and reactive non-radicals which play an important role in cell signaling pathways. Oxidative stress results when there is an insufficient capacity of the biological system to neutralize excess free radicals that have been produced[6], leading to damaged cells. Cell's inability to repair the incurred damage may cause genetically programmed cell's death (apoptosis) or mutations in the DNA, which leads to carcinogenesis or development of many neurodegenerative diseases.[8] Fortunately, substances called antioxidants which are ubiquitous and widespread in the nature, have the ability to suppress, reduce, and delay oxidative stress.[9] They have diverse mechanisms via their interaction with free radicals.

Several studies have reported that secondary metabolites of plant tissues contain polyphenols, such as flavonoids, hydroxycinnamic acids and proanthocyanidins, which act as powerful antioxidants, offering protection against damages[10,11,12]. Polyphenols in food industries, contribute to the color, flavor, odor, bitterness, astringency, and oxidative stability[12,13]. Given that natural antioxidant are safe, effective, cheap compared to synthetic ones,[14,15] plant-based antioxidants and fruits beside their nutritional value, have become the target of a great number of research studies.[16] Orange, fruit from *Citrus sinensis* L. that belongs to the Rutaceae family is for instance a prominent source of phytochemical and secondary metabolites.[17] *Citrus sinensis* is one of the plants most cultivated in the world. It is a source of food and vitamins for humans[18]. It has been used traditionally to treat constipation, cramps, colic, diarrhea, bronchitis, tuberculosis, cough, cold, obesity, menstrual disorder, angina, hypertension, anxiety, depression and stress.[18] Several studies reported on antioxidant activity of different varieties of Citrus[19] and its parts including; oils from the seeds,[20] flesh and peels;[21,22,23,24] juice[25] and development stages of its fruits[26]; its diverse crude extracts[27]. Although more attention has been paid to different edible parts of the plants, Seyed *et al.*,[28] recently reported on flowers hyro alcoholic extracts. However, only few studies have reported on leaves. This study therefore aimed at determining and comparing the *in vitro* antioxidant (TAC), radical scavenging activities (DPPH•, ABTS+,

NO•), antiradical power, total phenols (TPC), flavonoids *(FLC),* of aqueous, ethanolic and hydro-ethanolic extracts of *Citrus sinensis* leaves.

## MATERIALS AND METHOD

### Chemicals

All chemicals used were of analytical grade. They included; Folin- ciocalteu reagent; catechin; Aluminium Trichloride ($AlCl_3$), Potassium acetate; quercetin; Ethanol, 1,1diphenyl-2-picrylhydrazyl (DPPH), 2,2'-Azinobis-(3-ethylbenzothiazoline 6-sulfonic acid) (ABTS), Permanganate potassium ($KMnO_4$); Phosphate buffer, Sodium nitroprusside; Sulfanilic acid reagent; Glacial acetic acid; Naphthylethylenediamine dichloride; Sulphuric acid; Sodium phosphate and ammonium molybdate were purchased from SIGMA (USA).

### Sample Collection

Fresh leaves of *Citrus sinensis* were harvested in June 2016 in Yaoundé, Capital of Cameroon. They were identified in national herbarium according to ethno-botanical characteristics described by *Letouz*ey *et al.*,[29.]

### Preparation of ethanolic, hydroethanolic and aqueous extracts

The leaves were dried at room temperature to constant mass, ground into uniform powder to increase the surface area of the sample with extraction solvents. Solvents used were distilled water, absolute ethanol and a mixture of water-ethanol (1:1 v/v).Plant dried material (100g) was weighed, poured into three sterile glass beakers each containing 800 ml of 95% ethanol, water/ethanol (95 %) in a ratio of 1:1 (v/v) and water respectively for preparation of Ethanolic, hydroethanolic and aqueous extracts. Each beaker was tightly covered, shaken vigorously and kept for 2 days to enhance proper dissolution and extraction of the bioactive compounds in the samples[15]. Each solution was shaken vigorously and filtered with Whatmann filter paper $N^o3$ (Whatmann Int. Ltd., Maidstone, U.K) at room temperature. Filtrates obtained from ethanol and hydro-ethanol were evaporated using a rotary evaporator (BUCHI Rotavapor R-114, Switzerland) at 45 ºC until the extracts became completely dry. Filtrate from water extract was kept in an oven at $50^OC$ until it dried. All the extracts were stored at 4 ºC in a refrigerator or further analyses.

### Quantitative determination bioactive molecules

The quantitative analysis of some bioactive compounds done included total polyphenols (TPC), total flavonoids (TFC)

### Determination of Total Polyphenol content

The total phenolic content of each extract was determined spectrophotometrically, using the Folin-Ciocalteu method as described by Singleton and Rossi[30] .To 30 µL of the extract, 1 mL of the Folin- ciocalteu reagent (diluted 10 times with distilled water) was added. Thirty minutes after incubation at 25 °C, the absorbance was read at 750 nm with a spectrophotometer. Catechin was used as the standard at different concentrations (0 – 1000 µg/mL) for the calibration curve. Analyses were performed in triplicate and the content of phenolic compounds in extracts were expressed as µg equivalence of catechin/mg of extract.

**Determination of flavonoids' content**

The total flavonoid content was evaluated using the method described by Aiyegoro and Okoh[31]. To 1 mL of extract (1 mg/mL) prepared in ethanol solution, 1 mL of aluminium chloride, 1 mL of potassium acetate and 5.6 mL of distilled water were added. The mixture was allowed to stand at 25 °C for 30 minutes. The absorbance of the reaction mixture was measured at 420 nm with a spectrophotometer. Quercetin was used as standard at different concentrations (0 – 1000 µg/mL) for calibration curve. The analyses were performed in triplicate and the amount of flavonoids in the extracts was calculated and expressed as µg equivalence of quercetin/mg of extract obtained from the calibration curve.

**Determination of Radical Scavenging Capacities (RSC)**

The radical scavenging capacities (RSC) of different crude extracts were evaluated against DPPH•, $ABTS^{+}$, NO• radicals.

**DPPH• scavenging assay**

DPPH•-RSC assay was based on measurement of the loss of DPPH• color after reaction with test compounds. The DPPH• scavenging activity was measured according to the procedure reported by Katalinie *et al.*[32] . Fifty microliters (50 µL) of the extract at different concentrations (2.5, 5.0, 7.5 and 10 mg/mL) were introduced in 1.950 mL of an ethanolic solution of DPPH. After 30 minutes of incubation in the dark, the absorbance was measured at 515 nm against the blank consisting of reagent. For each sample three replicates were done. RSC was calculated by the following equation:

$$\text{RSC} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

**ABTS+ scavenging assay**

The ABTS-RSC was measured according to a modified procedure reported by Re *et al.* [33]. One hundred microliters (100 µL) of the extract at different concentrations (2.5, 5, 7.5 and 10 mg/mL) was introduced in test tubes, followed by 1000 µL of $ABTS^{+}$ reagent, then incubated for

30 minutes in the dark. The absorbance was then read at 734 nm against the blank consisting of reagents. For each sample, three replicates were carried out. RSC was calculated by the following formula:

$$\text{RSC} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

**Nitric oxide (NO•) scavenging Assay**

**NO•-RSC** was evaluated by measuring the accumulation of nitrite (formed by the reaction of NO with oxygen).The scavenging activity of extracts on nitric oxide was evaluated using the method of Sreejayan and Rao[34]. Two (2) ml of sodium nitroprusside (10mM) dissolved in phosphate buffer saline (pH 7.4) was mixed with 1mL of extract at various concentrations (0.25-1mg/mL). The mixture was then incubated at 25°C. After 15min incubation, 0.5 mL of the incubated solution was pipetted and mixed with 1mL sulfanilic acid reagent (0.33% in 20% glacial acetic acid) at room temperature for 5 minutes followed by the addition of 1mL of naphthylethylenediamine dichloride (0.1% w/v). The mixture was then incubated at room temperature for 30 min and its absorbance read at 540nm. For each sample, three replicates were carried out. RSC was calculated by the following equation:

$$\text{RSC} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

**Antioxidant activities**

**Total antioxidant capacity (TAC)**

This reduction mechanism was evaluated using the method described by Prieto *et al.* [35].The extract (0.2 mL) prepared in ethanol was mixed with 2 mL of the reagent (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). All the tubes were sealed and incubated at 95 °C for 90 min. After cooling the tubes, the absorbance of the solutions were measured at 695 nm against the blank containing 2mL of the reagent and 0.2 mL of ethanol. The total antioxidant capacity was expressed in milligram equivalents of ascorbic acid per gram of dry material (mg EAA/ g dry material).

**Determination of $EC_{50}$ and Antiradical Power**

The concentration (in the final reaction media in each method) that causes a decrease in the initial absorbance (control) by 50% is defined as $EC_{50}$. The $EC_{50}$ values for all RSC were determined by regression of the inhibition values (RSC). The antioxidant capacity of the extracts was expressed as antiradical power (ARP)[36,37] defined as: ARP= $(1/EC_{50})$ x 100. ARP is used to define antioxidant action of an antioxidant and it is a reciprocal of $EC_{50}$[37,38]

**Statistical analyses**

All data on all bioassays were expressed as the averages of triplicate analyses. Results were recorded as mean ± standard deviation. The Software SPSS 20.0 (Chicago-Illinois Inc.) was used and Tukey's test was performed to compare variable amongst groups. Sperman's correlation test was used to study interactions between variables. All the results with a $p < 0.05$ were considered significant. Using GraphPad Prism 6.0 (GraphPad Prism INC., CA, USA), the logarithm of the extract concentration was plotted against RSC to obtain a nonlinear regression curve –fitting and a variable slope to determine $EC_{50}$.

## RESULTS AND DISUCSSION

**Total polyphenol and flavonoids content**

The quantity of bioactive compounds found in each extract is indicated in Table 1. Polyphenols' content varies from 306.67 to 353.33 µg EC/mg and flavonoids from 0.11 to 12.84 µg EQue/mg). The ethanolic and aqueous extract have the highest amount ($p<0.05$) of polyphenolic compounds compared to hydroethanolic extracts. Correlation test of Spearman between flavonoids and TPC showed a positive and significant correlation with HEE ($r = 0.972$, $p < 0.001$) stronger than EE ($r=0.672$, $p<0.05$) compared to AE ($r= 0.444$, $P = 0.232$)

**Table 1: Total Polyphenols and flavonoids' content of different extracts**

| Extracts / Bioactive molecules | Aqueous | Hydroethanolic | Ethanolic |
|---|---|---|---|
| Polyphenols(µg of EC/mg) | $351.11 \pm 55.91^{a}$ | $306.67 \pm 43.72^{b}$ | $353.33 \pm 30.55^{a}$ |
| Flavonoids (µg d'EQue/mg) | $0.11 \pm 0.01^{a}$ | $1.11 \pm 0.20^{b}$ | $12.84 \pm 0.59^{c}$ |

Values with different letters a, b, c associated in the same line are significantly different $p< 0.05$; EQue: Equivalent quercetin; ECat: equivalence of catechin

**DPPH• Scavenging Capacity (DPPH$^{\cdot}$-RSC)**

All extracts showed scavenging activities on DPPH radicals in a manner proportional to the concentration of the extracts (Figure 1). RSC expressed as percentage of inhibition varies from 15.90% (5 mg/mL) to 65.59 % (20 mg/mL) for EE, from 34.52 (5 mg/mL) to 43.27 % (20 mg/mL) for AE and from 3.87 (10 mg/mL) to 29.46 (20 mg/mL) for HEE. EE have shown a higher scavenging activity on DPPH• radical.

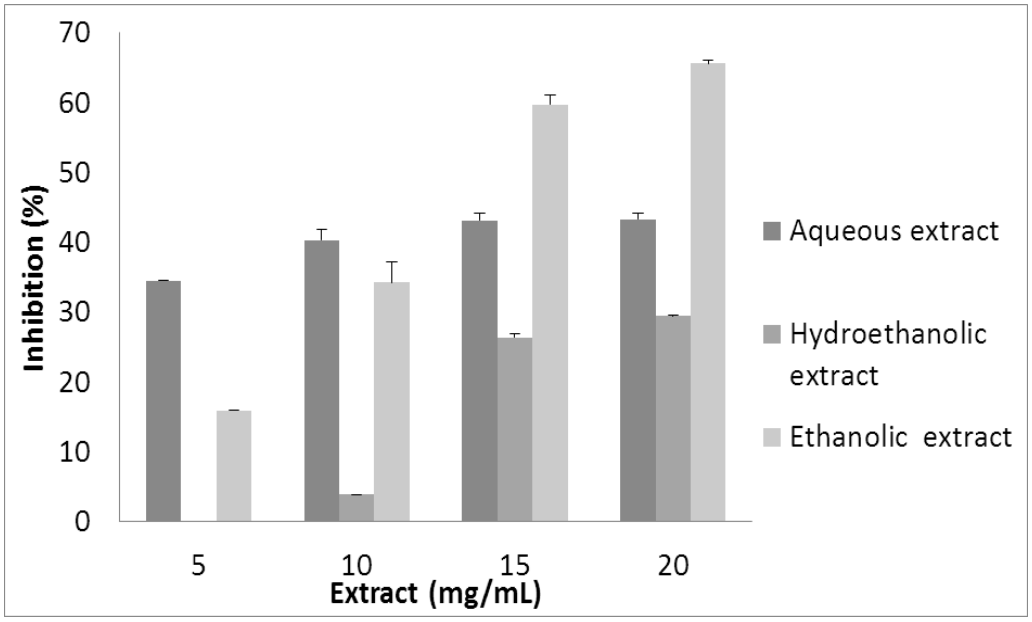


**Figure 1: DPPH• scavenging activity of different concentrations of leaves' extracts of *Citrus sinensis***

**ABTS$^+$ Radical scavenging capacity**

The different extracts have shown scavenging activities on ABTS$^+$ radicals in a manner proportional to the concentration of the extracts. ABTS$^+$ -RSC vary from 78.25% (5 mg/mL) to 92.67 (20 mg/mL) for HEE; 56.79 (5 mg/mL) to 92.06 % (20 mg/mL) for AE and from 68.23% (5 mg/mL) to 89.46 % (20 mg/mL) for EE as shown in Figure 2. HEE has shown a higher scavenging activity on ABTS+ radical.

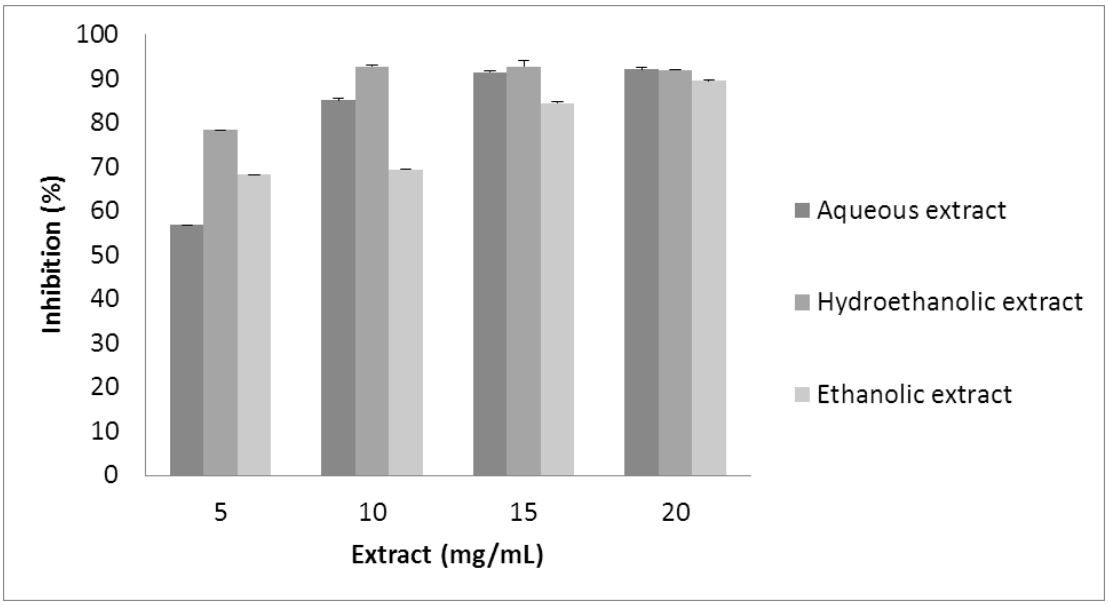


**Figure 2: ABTS$^+$ scavenging activity of different concentrations of leaves' extracts of *Citrus sinensis***

**Nitric oxide (NO•) Scavenging Capacity**

The different extracts have shown scavenging activities on ·NO-RSC in a manner proportional to the concentration of the HEE and inversely proportional to the concentration of the EE and AE. The activity expressed as percentage of inhibition varies from 75.19% (0.25 mg/mL) to 80.48% (1 mg/mL) for HEE; from 61.73% (0.25 mg/mL) to 52.20 % (1 mg/mL) for aqueous extract and 63.98 (0.25 mg/mL) to 0.74 % (1 mg/mL) for EE as shown in Figure 3. HEE have shown a higher scavenging activity on $ABTS^{+}$ radical.

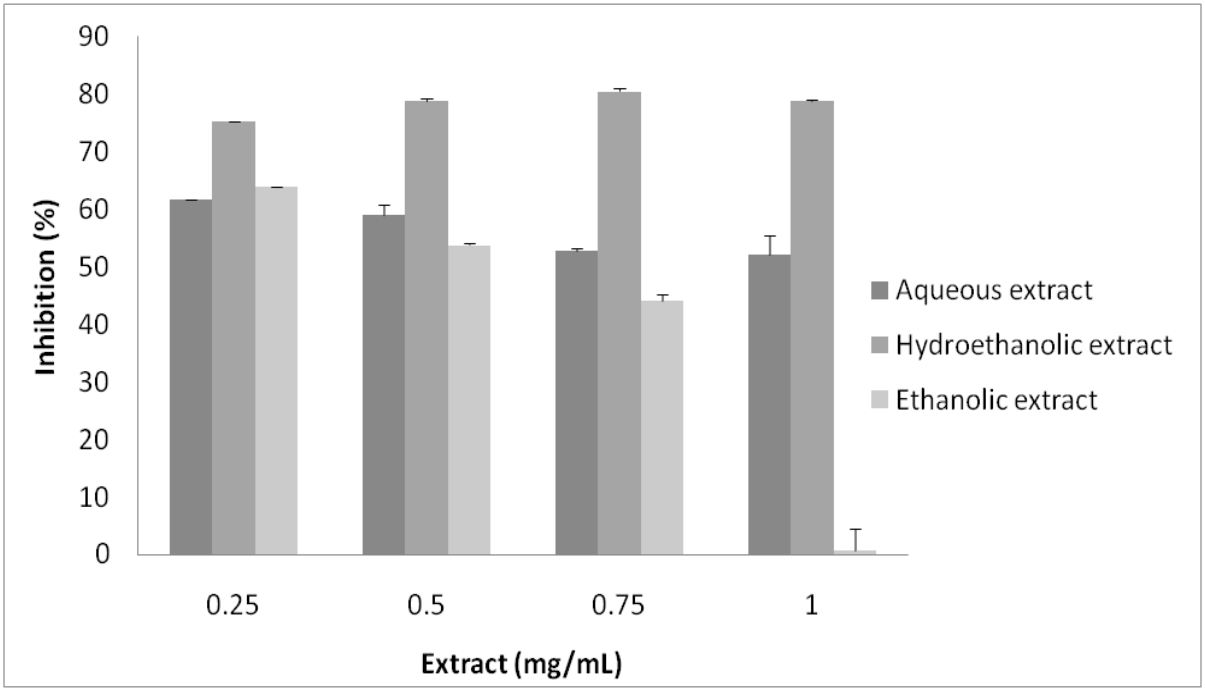


**Figure 1 : NO• scavenging activity of different concentrations of leaves' extracts of *Citrus sinensis***

**Total Antioxidant Capacity**

EA, HEE, EE leaves' extracts of *Citrus sinensis* demonstrated a total antioxidant capacity in a manner proportional to the concentration of the extracts (Figure 4). The results expressed as equivalence of ascorbic acid vary from 0.65 (0.5 mg/mL) to 1.32 µg EAA/mL (1 mg/mL) for EE, 0.47 (0.5 mg/mL) to 1 µg EAA/mL (1 mg/mL) for HEE and 0.32 (0.5 mg/mL) to 0.62 µg EAA/mL (1 mg/mL) for AE.

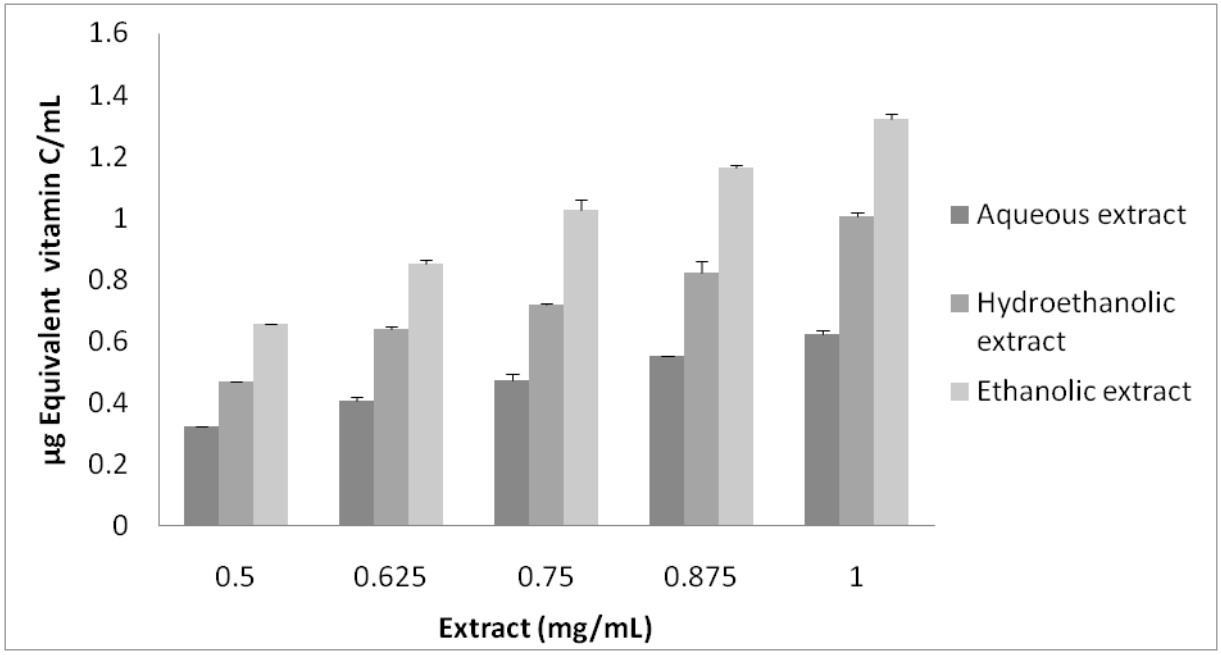


**Figure 4: Total Antioxidant Capacity of different concentrations leaves' extracts of *Citrus sinensis***

**Determination of $EC_{50}$ and antiradical power (ARP)**

It is observed from Table 2, that AE has the lowest $EC_{50}$ (4.604 mg/ml) for DPPH•, and ABTS+ (4.273 mg/ml) as well as NO• radicals (0.000401 mg/ml). Obviously, AE has the highest ARP against free radicals studied.

**Table 2: Determination of $EC_{50}$ and antiradical power (ARP)**

| Radicals / Extracts | DPPH• | | ABTS+ | | NO• | |
|---|---|---|---|---|---|---|
| | $EC_{50}$ | ARP | $EC_{50}$ | ARP | $EC_{50}$ | ARP |
| EE | 11.99 | 8.34 | 7.11 | 14.06 | 7.082 | 14.12 |
| AE | 4.604 | 21.72 | 4.27 | 23.40 | 0.000401 | $24.9x.10^4$ |
| HEE | 12.05 | 8.0 | 4.54 | 22.02 | 0.47 | 210.52 |

*EE: ethanolic extract; AE: Aqueous Extract; HEE: hydroethanolic extract, ARP: antiradical power*

**Study of Spearman's Correlation with different extracts**

**Aqueous extract**

Strong positive correlations were found between TAC and ABTS+-RSC ($r = 0.958$, $p<0.001$), TAC and DPPH•-RSC ($r = 0.934$, $p<0.001$). TPC and DPPH• ($r = 0.738$, $p<0.05$)

**Ethanolic extracts**

Strong positive correlations were found between TPC and TAC ($r = 0.988$, $p<0.001$), TPC and ABTS+ ($r = 0.796$, $p<0.05$). Also, a correlation between TAC and TFC ($r = 0.997$, $p<0.001$) and TAC and ABTS+-RSC (0.880).

**Hydroethanolic Extracts**

Strong positive correlations were found between TAC and ABTS+-RSC ($r = 0.948$, $p < 0.001$), TAC and NO•-RSC ($r=1$, $p<0.001$) but weak between TAC and DPPH•-RSC ($r = 0.381$, $p > 0.05$)

## DISCUSSION

Antioxidants are becoming key molecules in food, pharmaceutical and cosmetic industries.[39] The search of natural antioxidant sources remain a challenge for researchers. *C. sinensis* represents the largest citrus cultivar groups grown around the world, accounting for about 70% of the total annual production of Citrus species[40]. This study contributes in the exploration of leaves, an inedible part. Results demonstrated that ethanolic extracts contain higher amount of Polyphenols (TPC) and flavonoids (TFC) than the aqueous extracts (AE) and hydroethanolic (HEE) (Table 1). These results are in accordance with those obtained by of Omodamiro and Umekwe[42], which showed that ethanolic extracts of *Citrus sinensis* leaves collected from Umuahia, Nigeria, possess flavonoids. Aseel and Muazaz[16] also noted the content of total phenols and flavonoids of ethanolic extracts of *Citrus sinensis* leaves collected from Baghdad, Iraq. In fact, the higher content of polyphenols and flavonoids in EE compared to AE an HEE could be due to the polarity and degree of solubility of the bioactive compounds found in the leaves[15]. However, the amount of TPC found in all the extracts was higher than in *Irvingia wombulu* peel kernels[43] and TFC of ethanolic extracts of (12.05 Eque mg/g DM) was higher (Table 1) compared to TFC of root extracts of *M. arboreus* and more interestingly higher than in Oligopin®, a synthetic antioxidant[2]. Results obtained also present a strong correlation between, TPC and TFC ($r = 0.972$, $p<0.001$) mainly with EE, which can justify their antiradical power.

All the extracts presented $EC_{50s}$ ranging from 4.06 -12.05 mg/mL for DPPH• and from 0.000401 to 7.08mg/mL for $ABTS^{+}$. AE presented the lowest $EC_{50s}$ against all the radicals tested and also the highest ARP (Table 2). The scavenging activities of all samples with both DPPH• (Figure 1) and ABTS+ (Figure 2) methods showed that percentage inhibition increased with concentrations which is consistent with the trapping of free radicals due to an increase of the number of antiradical species. This result is in accordance with Djouonzo *et al.*,[44] findings on stem bark of *Pterocarpus erinaceus* extracts. The highest ARP and the lower

$EC_{50}$ can be justified by the positive correlation found between TPC and DPPH• (r=0.738, p<0.05) in AE**,** in accordance with Wenzel *et al.*[45] on *Juglans nigra* extracts. In fact, polyphenols contained in the extracts act by donating electrons and reacting with free radicals to convert them into more stable products and terminate free radical chain reactions[46]. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, by donating hydrogens or electrons or to react with certain precursors of peroxides, to prevent peroxide formation[47,48]. Interestingly, Ethanolic Extracts showed a significant correlation between all phenolic classes and TAC in accordance with kasangana *et al.*[2]

The scavenging capacity $EC_{50}$ (Table 2) of the leaves' extracts with all extracts was higher than $E_{50}$s of aqueous, methanolic, and acetone extracts of different citrus fruit juices assessed on the basis of their ability to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH•) as reported by Duzzioni *et al.*,[25]. In addition, this result is in contradiction with Siahpoosh and Fatemeh[49], 2016) on Iranian peel of range that presented $IC_{50}$ for DPPH•-RSC, $ABTS^{+}$ - RSC (at the 6 minutes) to be 491.52, 52.89 mg/ml respectively; values higher than $EC_{50}$ obtained with all the leaves' extracts of *C. sinensis.* This result demonstrates that leaves' extracts are more efficient in scavenging free radicals than the peels.

DPPH• assay is routinely practiced for assessment of free radical scavenging potential of an antioxidant molecule and considered as one of the standard and easy colorimetric methods for the evaluation of antioxidant properties of pure compounds despite its limited similarities with peroxyl radicals[37]. The DPPH• radical contains an odd electron, which is responsible for the violet color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized to yellow[50]. The diminishing coloration is also observed when the antioxidant scavenges $ABTS^{+}$ radicals also showed the scavenging ability of DPPH• in accordance with results obtained by ethanolic extracts of *Citrus sinensis* leaves from Barangay Bambanaba, Philippines[51].

Based on the fact that DPPH• scavenging assay, similar to the ABTS+ assay uses non-physiologically relevant free radicals, which has little resemblance to free radicals involved in oxidative processes in biological[37,38] and also on the fact that, no single method can fully help determining antioxidant mechanism of a substance[49]. Leaves' extracts were tested on free radicals from biological origin in nitric oxide radical (NO•-RSC) assay[38]. In fact, nitric oxide (NO•) is a free radical produced in mammalian cells and has a regulatory role in various physiological processes[52]. In fact, *C. sinensis* leaves' extracts scavenged nitric oxide radical

(Figure 3) with $EC_{50}$ ranging from 0.000401 to 7.08 mg/ml (Table 2). EA also presented lowest $EC_{50}$, better than $EC_{50}$ of *I. wombulu* peel kernels (15.41 mg /mL) and Vitamin C ($EC_{50}$= 1.78 mg/mL), obtained under similar experimental conditions[43]. NO•-scavenging action may be attributed to the ability of antioxidants present in extracts to compete with oxygen to react with nitric oxide, and thus inhibit the generation of nitrite. In fact, it has been proven that secondary metabolites which contribute to the pharmacological activities include flavonoids, steroids, hydroxyamides, alkanes and fatty acids, coumarins, peptides have been identified in fruits, peels, leaves, juice and roots of *C. sinensis*[40,41].

Vitamin C greatly contributes to the total antioxidant activity of orange juice[42,53]. This study revealed in Figure 4 that all the extracts mainly EE contains equivalent ascorbic acid or vitamin C antioxidant measured in TAC assay. TAC varies from 0.65 (0.5 mg/mL) to 1.32 µg EAA/mL (1 mg/mL) for EE that accounts for its antioxidant activity as confirmed with the positive correlation obtained between TPC and TAC (r= 0.988, P<0.001) compared to AE (r = 0 .449, p>0.05). The capacity EE to quench NO• free radicals can be justified by the presence of its high polyphenol content as proven with the positive correlation between TAC and TFC (r=0.997, p<0.001) as well as TPC and TAC (r= 0.988, p<0.001) justifying that polyphenols contributes to it as shown by the correlation between TPC and ABTS (r = 0.796, p<0.05) and between TAC and ABTS+-RSC (r=0.880, p < 0.05). TAC of HEE showed strong positive correlation with ABTS+ radicals (r = 0.948, p<0.001), absolute positive correlation with NO•-radicals (r = 1, p<0.001), but weak correlation with DPPH-RSC (0.381, p>0.05). The lowest $EC_{50}$ obtained with all the free radicals can be justified by the correlations observed between TAC and ABTS+-RSC (r = 0.958, p<0.001), TAC and DPPH-RSC (r= 0.934, p<0.001) as shown in Figure 5. In fact, the total antioxidant capacity of *C. sinensis* leaves' extracts was measured based on the reduction of Mo (VI) to Mo (V) and was found to be concentration dependent (Figure 4). This could be explained by the fact that related polyphenols and many flavonoids contribute significantly to the phosphomolybdenum reduction activity of medicinal plants through the transfer of electrons as shown by Khan *et al.*[54]. However, these results are in contradiction to those reported by Rosa *et al.*[55] , who obtained negative correlations.

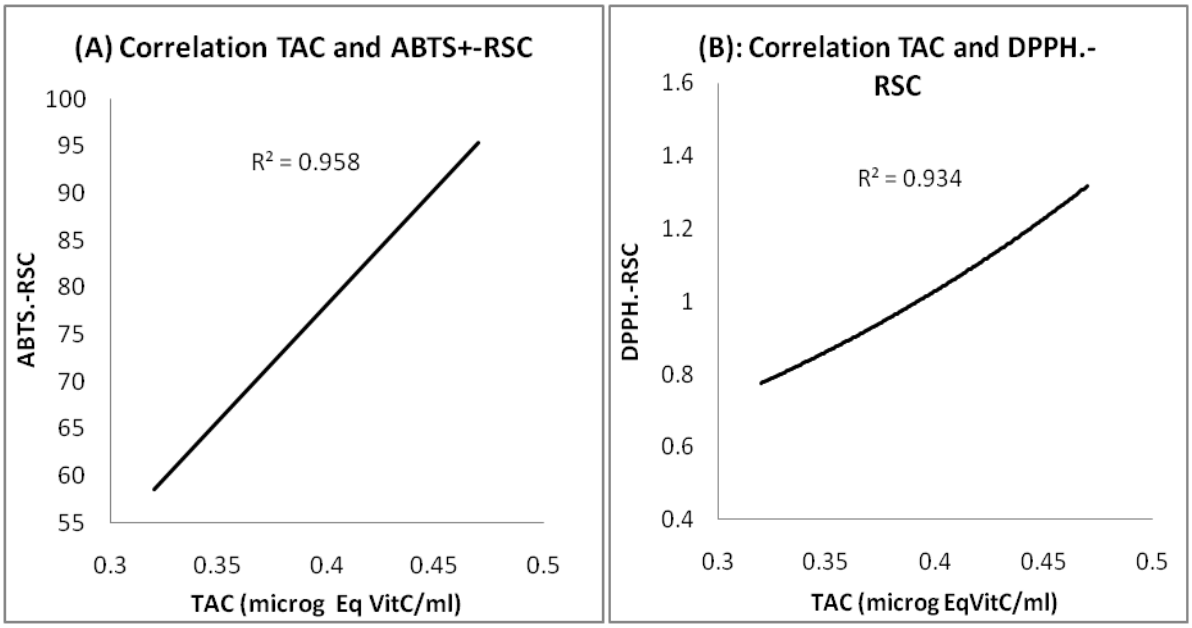


**Figure 5: Correlation between TAC-ABTS+ (A) and TAC-DPPH• (B) obtained with aqueous extract**

These results for clarity were also expressed in terms of antiradical, power (ARP) calculated as 1/EC50 , in which larger ARP values represented a larger, scavenging capacity[37] as observed in Table 2. A lower $EC_{50}$ value is associated with a stronger DPPH radical scavenging capacity under the same testing conditions. Although reporting results as EC50 value or ARP, however, has been criticized because results are highly dependent on initial DPPH concentration and definition of steady-state time point[37,38]. It however appears that Aqueous Extracts displayed the lowest $EC_{50}$ and highest ARP with DPPH•, ABTS+, and NO• radical. EE and HEE also possess high TPC, TAC and ability to scavenge radicals. Leaves' extracts, maybe, more than any other parts of the plant deserve to be tested *in vivo* against several pathologies.

## CONCLUSION

Aqueous, hydroethanolic and ethanolic leaves' extracts of *C. sinensis* possess high TPC and TAC. In addition, extracts also possess the ability to scavenge diverse radicals such as DPPH•, NO•, ABTS+ mainly with highest antiradical power and $EC_{50}$ from aqueous extracts. Leaves' extracts are potent source of natural antioxidants. However, further works should be carried out to characterize the specific active component responsible for these activities and to evaluate other potent biological properties of the leaves.

## AUTHOR CONTRIBUTIONS

Azantsa KGB conceived and designed the experiment, carried out experiments, statistical analyses and drafted the manuscript. Djuikoo NIL, Takuissu Guy, Kuikoua TW, Temdemnou E.

carried out experiments, drafted the manuscript. Judith LN and Julius O. supervised the work and proof-read the manuscript.

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