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Total polyphenol content and antioxidants capacity of three Africans antipyretic plants

Ayoman Thierry-Lenoir Djadji[1], Donald Brou Koua[1], Sandrine Any-Grah-Aka Sandrine[2], Etienne Kouakou Effo[1], Landry Sylvain Kouakou[1], Genevieve Irie-N'Guessan[1] and Gisele N'Doua Kouakou-Siransy[1]

[1]*Laboratory Pharmacology, Clinical Pharmacy and Therapeutic: Laboratory of Pharmacology, UFR Pharmaceutical and Biological Sciences, Felix Houphouet-Boigny University*
[2]*Laboratory of Pharmacotechnic, Galenic Formulation and Pharmaceutical Legislation*

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***Correspondence Info:**
Ayoman Thierry-Lenoir Djadji
Laboratory Pharmacology,
Clinical Pharmacy and Therapeutic,
Laboratory of Pharmacology, UFR Pharmaceutical and Biological Sciences, Felix Houphouet-Boigny University

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Abstract

Introduction: *Ceiba P. Crossopteryx F. Pseudocedrela K.* are used as a nutritional and medicinal (Antipyretic). The aim of this study was to perform the dosage of polyphenol content and their antioxidants capacity.
Methods: Aqueous extracts of three plants leaves were used to determine polyphenol content using Folin-Ciocalteu and antioxidants activities by DPPH radical scavenging and FRAP Methods.
Results: We found much more total phenol content in the leaves of *Pseudocedrela K.* respectively (352.57 mg/g). Antioxidants activities of *pseudocedrela* (279.9 mMol eq Trolox) by the Frap method was better than these others plants. (Trolox; R_2= 0.9968). The IC_{50} = 12.1 µg/mL of leaves of *pseudocedrela K.* was inferior to two others plants (*Ceiba* and *crossopteryx*). That means that aqueous extract of *Pseudocedrela* have more antioxidants activity than *Ceiba* and *crossopteryx*, but fewer than ascorbic acid.
Conclusion: The total polyphenol and others compounds in the leaves of *Pseudocedrela* may be used as natural antioxidants to prevent lead to oxidative stress lead.
Keywords: Antioxidants; Total phenol, and oxidative stress.

1. Introduction

The plants are a therapeutic alternative for the treatment of diseases low expensive and fewer sides effects. Several studies have shown the pharmacology activities of secondaries metabolites such as antioxidants known to have an action on free radicals.[1]

All body system may be influenced by the free radicals through contribution to many kinds of degenerative diseases including diabetes and cardiovascular damage [2], CNS complications[3,4], cancer [5] and many others illness [6, [6,7]. Most important way of producing free radicals in living systems is the Oxidation mechanisms [8]. Additional natural antioxidants (Total Phenol, Flavonoids and Tanins) as frees radical's scavengers may be needed to boost the defensive system and slow down aging. This hypothesis has evoked great efforts for finding the powerful free radical scavengers to overcome harmful effects of oxidative stress. Recently attentions have been focused on the therapeutic potential of medicinal plants which is believed to reduce free radical induced tissue injury by trapping them [9]. Higher Plants give most of the time a variety of antioxidant compounds of which, polyphenols are known to be the most potent one [10, 11].

These antioxidant compounds can be obtained from natural and chemical origins. Natural sources are much more expansive, harmless to use due to less toxicity and side effects, so the production of the antioxidant

compound from the natural sources such as plants and algae is in great request [12].

Ceiba pentandra (Malvaceae); *Crossopteryx febrifuga* (*Rubiaceae*), *Pseudocedrela Kostchyi* (*Meliaceae*) are three plants most common used in African traditional medicine to treat fever. They are also given for many others diseases due to oxidative stress (antiasthma, antidiabetic, anti-inflammatory[13-16]. in add; in our knowledge, it is the first study that performs polyphenol in this three plants in our state.

The main objective of this work is to determine polyphenols content and antioxidant activities of these three plants.

2. Materials and Methods

2.1 Plant materials

Samples of three plants: *Ceiba pentandra Gaertn (Malvacees), Crossopteryx Febrifuga* Afzel, ex G. Don benth and *Pseudocedrela kotschyi* Schweint. Harms (Meliaceae) were collected from Pakouabo (Bouafle, Côte d'Ivoire). It was identified and authenticated by National Floristic Center (NFC) at Felix Houphouet Boigny University (Abidjan.).

2.2 Chemicals

2,2'- diphenylpicryl-1-hydrazyl (DPPH), Ascorbic acid, Folin- Ciocalteu, wers purchased from Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA)..

2.3 Preparation of the sample

Leaves of three plants were dried in a dark ventilated room for 10 days. These parts were ground to fine powder using Restsch GM 300 TM grinder mill. Extraction was carried out by cold maceration of 100 g of fine powder with 1000 ml of distilled water for 24 hours. The macerate was successively filtered through fabric, hydrophilic cotton and finally Whatman paper. Subsequently, the filtrate was evaporated dried in a Memmert™ brand oven at 45°C for 3 days and the dark brown dried solids were stored in a refrigerator at 4°C for the antioxidants and total phenol content assays.

2.4 Dosage of total phenol

Total phenol was achieved by Folin- Ciocalteu test according to Amarowicz et al[17] and Chun et al [18] with some changes. One mL of the extract and sample (solved in 60% acetone, 5 mg/100 mL) was mixed with 200 µl Folin-Ciocalteu reagent and 1 mL of aqueous Na_2CO_3. The mixtures were left at room temperature for 30 min and the phenol contents were determined by colorimetric method at 715 nm. The calibration curve was prepared using Gallic acid solutions at concentrations of 1- 0.01562 mg/mL in 60% acetone. Total phenol contents were expressed in terms of gallic acid equivalent (mg g-1). Samples were analysed in three replications.

2.5 Antioxidant activity

2.5.1 Radical scavenging: 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging

DPPH assay antioxidant activity of the different extracts was measured according to Aderogba et al [19] with some modifications. In order to obtain dilutions, different sample concentrations were prepared in methanol and 5 mL of each concentration were added to 5mL of 0.004% methanolic solution of DPPH. After completion of reaction at room temperature for 30 min, bleaching of DPPH was monitored at 517 nm against a blank. Ascorbic acid equivalent antioxidant capacity was calculated by using ascorbic acid as a reference compound to prepare the standard curve.

2.5.2 Ferric reducing antioxidant power

The ferric reducing antioxidant power (FRAP) assay was done according to Benzie et al [20] with some modifications. The stock solutions included 300 mM acetate buffer (3.1 g $C_2H_3NaO_2$ $3H_2O$ and 16 mL $C_2H_4O_2$), pH 3.6, 10 mM TPTZ (2, 4, 6- tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3$ $6H_2O$ solution. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution, and 2.5 mL $FeCl_3$ $6H_2O$ solution and then warmed at 37°C before using. Aqueous extracts from our plants (150mL) were allowed to react with 2850 mL of the FRAP solution for 30 min in the dark condition. Readings of the colored product [ferrous tripyridyltriazine complex] were then taken at 593 nm. The standard curve was linear between 25 and 800 mM Trolox. Results are expressed in mM TE/g fresh mass. Additional dilution was needed if the FRAP value measured was over the linear range of the standard curve.

2.6 Statistical analysis

All the assays were carried out in triplicate and the experimental results obtained were expressed as mean±SD. IC_{50} value was calculated by plotting nonlinear regression curve using GraphPad Prism 7 software.

3. Results

3.1 Total polyphenols content

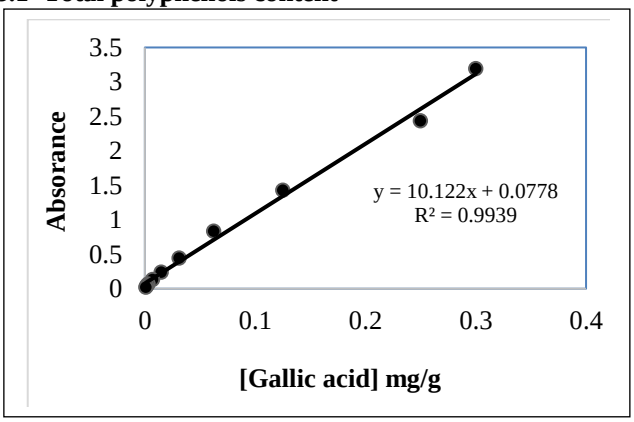

Figure I: Stantard line in gallic Acid

Table: I Total phenol and flavonoids content of aqueous of three plants

Plants (Leaves)	Total phenol content (mg/g)
Ceiba pentandra	22.325±4.64
Crossopteryx Febrifuga	126.65±1.86
Pseudocedrela Kotschyi	352.57±4.86

We found much more total phenol content in the leaves of *pseudocedrela kotschyi* respectively (352.57 mg/g) and than others plants.

3.2 Antioxidants activities

3.2.1 Ferric reducing antioxidant power (FRAP)

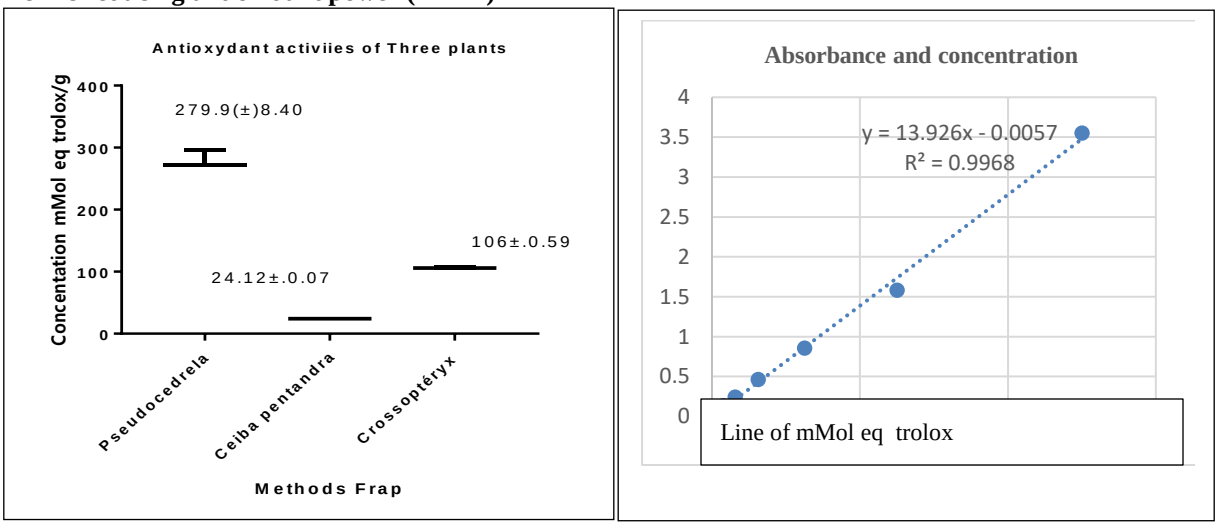

Figure 2: Ferric reducing antioxidant power (FRAP)

Antioxidants activities of *pseudocedrela* (279.9 mMol eq trolox) by the Frap method was better than these others plants. (Trolox; $R^{2=}$ 0.9968)

3.2.2 Antioxidant DPPH

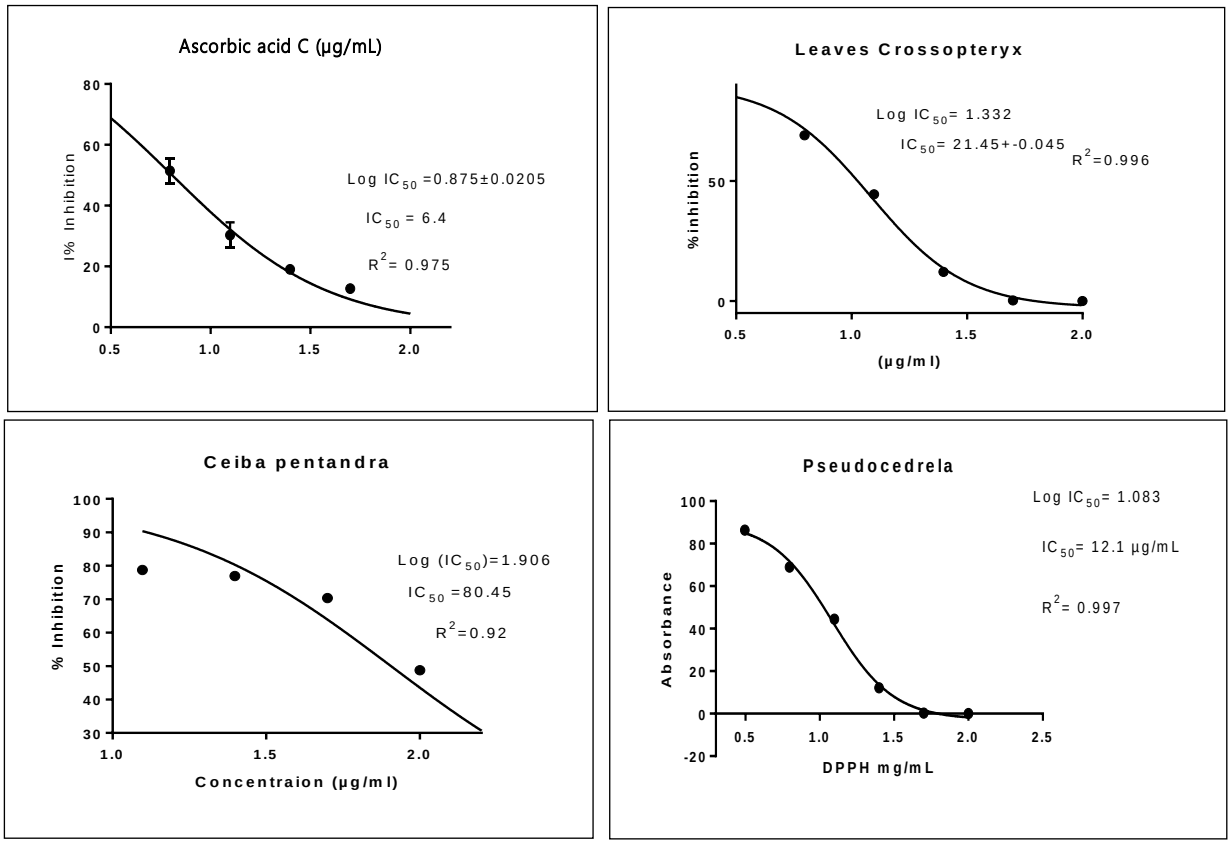

Figure 3: IC_{50} of threes plants comparatively to acid acsorbic

The IC_{50} = 12.1 µg/mL of leaves of *pseudocedrela K.* was inferior to two others plants (*Ceiba* and *crossopteryx*). That means that aqueous extract of *Pseudocedrela* have more antioxidants activity than *Ceiba* and *crossopteryx*, but fewer than ascorbic acid.

4. Discussions

Total phenols were assessed by Folin Ciocalteu method and results were reported as gallic acid equivalents by reference to the standard curve. Total phenols of three plants *Ceiba P. Crossopteryx F.* and *Pseudocedrela K.* (Table: I). According to our results, there is much more total phenol and flavonoids content in the leaves of *Pseudocedrela K.* respectively (352.57±4.86 mg/g) and than others plants. The Total phenolic compound of plant acts as primary antioxidants or free radical scavenger [21]. Various large works have been done in recent years and a significant correlation was found between TPC and antioxidant activity.[22].

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. For the estimation of the reductive ability we investigated the Fe3+ to Fe2+ transformation using the method of Oyaizu, where the change in the optical density of the final mixture is measured at 700nm. Increase in optical density indicates higher reductive ability. The reducing capabilities of the leaf extract of *Pseudocedral K.*(279 ± 8.40 mMol equivalent trolox was found to be in dose dependent manner when compared with Quercetin. [20,23]; This may be due to the presence of alkaloids, steroids, phenolic compounds, proteins and flavonoids [24]. We also found that compounds content varies from species to another species and also by the nature of part the plants used. Indeed, *Ceiba* leaves present fewer antioxidants and polyphenol content than the seed of this same plants according Kiran *et al* [13] and Loganayaki et al [14]. From the results it is made clear all of our three plants possess free radical scavenging activity through total antioxidant content.

Free radical scavenging activity of the extracts is concentration dependent and lower IC_{50} value reflects better protective action. The aqueous extract of *pseudocedrela* was able to reduce the stable free radical DPPH to the yellow-colored diphenylpicrylhydrazine with an IC_{50} of 12.1 ± 1.21 µg/ml, exhibiting better activity than antioxidant of *Ceiba P.* and *Crossopteryx febrifuga* respectively(80.4 ± 0.02 µg/ml), 21.45.4 ± 0.02 µg/ml), but lower activity than ascorbic acid (6.4 ± 0.93 µg/ml).

The collected data have shown the existence of a good harmony between antioxidant potency of samples and their total phenolic and flavonoid content. Generally, the higher antioxidant activity of the mentioned fractions might be attributed to their contents of total polyphenols and especially flavonoids. However according to [25], many other compounds may contribute to the reaction and cause a false positive error.

5. Conclusion

The results indicate that the water extract of *Pseudocedrela K.* possesses strong antioxidative properties in vitro. They are confirmed by polyphenols contents and corroborated by using spectrophotometer identifications.

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