WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 11, Issue 1, 94-103. Research Article ISSN 2277– 7105

PHYTOCHEMICAL STUDY AND EVALUATION OF THE ANTIOXIDANT ACTIVITY OF TWO MEDICINAL PLANTS IN BENIN

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Article Received on
05 Nov. 2021,

Revised on 25 Nov. 2021,
Accepted on 15 Dec. 2021

DOI: 10.20959/wjpr20221-22520

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ABSTRACT

Walteria indica tropical perennial plant of the Fabaceae family and Pteleopsis suberosa Engl. and Diels (Combretaceae) tree of the genus *Pteleopsis* of the *Combretaceae* family are plants widely used in traditional medicine in Benin. In the present work we prepared hydro ethanolic extracts from the dried leaves of these two plants and the yields of crude extract (hydro ethanolic) are about 22.19% and 27.2% respectively. Quantitative estimation of flavonoids and total phenols by colorimetric method and Folin Ciocalteux method showed that both extracts are rich in these compounds. The evaluation of antioxidant power which was carried out by means of DPPH free radical scavenging method and FRAP Iron reduction method revealed that *Walteria indica* hydro ethanolic extract has higher reducing power than that of Pteleopsis suberosa hydro ethanolic extract and ascorbic acid.

KEYWORD: *Walteria indica Pteleopsis suberosa* flavonoids, antioxidant activity, Polyphenols.

INTRODUCTION

Free radicals, which are the source of various pathologies such as cancer, atherosclerosis, cataracts, diabetes, cardiovascular diseases, Parkinson's disease, and Alzheimer's disease (Ref), are permanently produced in the body by mitochondria or during phagocytosis. They can also be formed during the detoxification mechanism after exposure to certain chemical

species or under the effect of certain radiations. According to Pousset (2006), the use of natural products (fruits, vegetables) rich in antioxidants could play an important role in the prevention of these diseases, including antioxidants from plants. Unfortunately, in a difficult economic environment characterized by the high cost of drugs and health services, pharmacopoeia and traditional medicine are becoming a significant alternative in terms of health coverage for African populations. Nowadays, 80% of the population use herbal medicine at least once (Agban et al., 2013). Scientific research on medicinal plants then constitutes a promising approach, on the one hand for the development of phytomedicines financially accessible to these populations, and on the other hand for the discovery of new biologically active molecules.

We were interested in the study of the antioxidant activity of hydro ethanolic extracts of dried leaves of *Walteria indica* and *Pteleopsis suberosa.*

Walteria indica is a tropical perennial plant of the Fabaceae family with an upright habit that can reach one meter (1m) in height. It is covered with grey hairs. This plant is of Oceania origin. The leafy stems are used to treat diarrhoea in infants, women who have just given birth, colds, pneumonia and dry coughs. The decoction of the roots is used in the treatment of bilharzia and constipation. Somet Cataplasms of leaves or roots are applied to wounds and burns. This plant is recommended for stomach aches, diarrhoea, oral ailments, toothache and rheumatism. We recommend a decoction of thirty grams (30g) of leaves per litre of water, to be drunk during the day or used as a mouthwash.

Pteleopsis suberosa Engl. et Diels (Combretaceae) is a tree in the genus *Pteleopsis* of the *Combretaceae* family. It is a plant with anti-ulcer and anti-Helicobacter pylori activity. It is found in West Africa: Senegal, Guinea, Mali, Ivory Coast, Burkina Faso, southwestern Niger, Ghana, Togo, Benin, and southwestern Nigeria, and Guinea-Bissau. The trunk bark of Pteleopsis suberosa, Combretaceae shrub of the dry savannahs of West Africa, is traditionally used in Benin and Togo to treat several ailments including dermatoses.

MATERIALS AND METHODS

Plant material

The plant material consists of dried leaves of *Walteria indica* and Pteleopsis suberosa harvested in December 2020 in Abomey-Calavi. These two plants have been identified at the National Herbarium of the University of Abomey Calavi. The leaves of the two plants were

washed and dried at room temperature in a ventilated room of the Pharmacognosy laboratory for three weeks before being reduced to powder.

Extraction

The extraction was done for the hydro ethanolic extracts by mixing 50g of powder in 500 mL of a hydro ethanolic mixture (40V/60V respectively) for 48 hours with the aim of extracting as much as possible the polar compounds such as the polyphenols.

After filtration on Whatman N°1 paper, the filtrates obtained were evaporated using a rotary evaporator at 40°C. The residues of this filtrate were dried in the oven for 48 hours at 40°C to obtain the dry extracts.

Phytochemical screening

The presence of the main chemical groups in the extracts was investigated using the tests described by Bassene (2012): flavonoids (Shibata test) tannins (Stiasny reaction followed by ferric chloride reaction), carotenoids (Carr-Price reaction), anthracenes (Dragendorff reagent), sterols (Libermann-Buchard reaction), cardiotonic heterosides 'Baljet, Kedde and Raymond-Marthoud reaction) and saponosides (Foam Index).

Polyphenol content

The polyphenol content of the extracts is determined by the Folin - Ciocalteu method. 1 mL of Folin's reagent is added to 1 mL of the solution of each extract, then 3 minutes later 1 mL of 25% sodium carbonate. After 2 hours of incubation, the samples were centrifuged at 4000 rpm for 4 minutes. The absorbances were then read with a spectrophotometer at 670 nm. Three tests were performed for each concentration of product tested.

A calibration curve based on a dilution series of tannic acid (0.005-0.01-0.015-0.02-0.025-0.03-0.025-0.03-0.035-0.04 mg/ml) was treated the same way as the extracts. The results are expressed as milligram equivalent of tannic acid per gram of dry extract 'mg ETA/g).

Determination of flavonoids

The flavonoid content of the extracts was determined using the aluminium trichloride colorimetric method. A quantity of 100µL of the extract was mixed with 0.4 ml of distilled water and subsequently with 0.03 mL of 10% Aluminium chloride ($AlCl_3$) solution was added. To the mixture 0.2 mL of a solution of sodium nitrite ($NaNO_2$) 1M and 0, 25 mL of distilled water were added after a 5min rest period. The whole mixture was vortexed and the

absorbance was measured at 510 nm. The results are expressed as milligrams of catechin equivalent per g of dry plant material.

Antioxidant activity

DPPH test

Determination of free radical scavenging activity by DPPH assay was carried out using the method described by Molyneux (2003) slightly modified. An ethanolic solution of DPPH was prepared by dissolving 4mg of this product in 100ml of ethanol. Then to 50 uL of extract at a given concentration are added 950µL of the DPPH solution. The extracts as well as the reference (ascorbic acid) are tested at different concentrations (250 -125-62.5 - 31.25-15.62-7.81 ug/L); then the absorbances were measured at 517 nm after 30 min of incubation in the dark. Three tests were carried out for each concentration of product tested.

The antioxidant activity related to the DPPH radical scavenging effect is expressed in percentage inhibition (PI) using the following formula:

$PI = 100(A_0 - A_1) / A_0$ $A_0 =$ DPPH absorbance A_1 sample absorbance

The IC50 (concentration of the sample required to neutralize 50% of the free radicals) was obtained using the software Statgraphics Plus 5/0.

Frap test

The reducing power of the extracts is determined by FRAP method (Bassène 2012). Thus, 0.4mL of sample at different concentrations is mixed with 1mL of phosphate buffer (0.2M; pH=6.6) and 1mL of 1% potassium hexacyanoferrate [$H_3Fe(CN)_6$]. After incubating the mixture at 50°C for 30 minutes, 1mL of 10% trichloroacetic acid was added to it, then the tubes were centrifuged at 3000 rpm for 10 minutes. Then, 1mL of the supernatant from each tube was mixed with 0.2mL of 0.1% $FeCL_3$ solution and kept in the dark for 30 minutes before measuring the absorbances at 700 nm. The antioxidant activity related to the reducing power of the extracts is expressed as Reducing Power (RP) using the following formula: $RP = 100(A_a - A_b) / A_a$ A_a: absorbance of the extract A_b: absorbance of the blank

Statistical analysis

Significance tests are performed by Fisher's test through the software Stat View. A p-value < 0.05 was considered statistically significant.

RESULTS AND DISCUCTION

Phytochemical screening

Phytochemical screening revealed the presence of flavonoids, tannins and saponosides in the extracts of both plant species. Alkaloids, anthracenes, triterpenes, coumarins are also present in both leaf extracts on the other hand cardiotonic heterosides were not found in the two plant extracts which are the subject of the present study.

Table 1: Phytochemical screening of extracts.

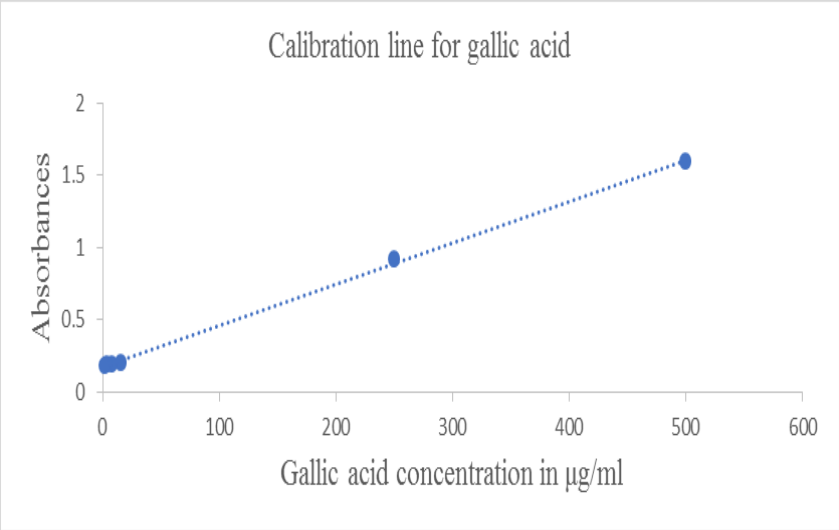
Classes	*Walteria indica* (hydroethanolic extract)	*Pteleopsis suberosa* (hydroethanolic extract)
Flavonoids	+	+
Tannins	+	+
Glycosylated coumarins	+	+
Triterpenes	+	+
Alkaloids	+	+
Anthracene derivatives	+	+
Cardiotonic glycosides	-	-
Saponosides	+	+
Lignans	+	+

Total polyphenol content

The determination of the total polyphenol content in both extracts was done by the Folin-Ciocalteux method for each extract. The content was reported in mg gallic acid equivalent/g dry plant material. These are extracts rich in total polyphenols. The hydro ethanolic extract of *Pteleopsis suberosa* is richer than that of *Walteria indica*.

Table 2: Total polyphénols content.

N°	Plants	Quantity of polyphenols in µg Eq AG/mg extract
1	*Walteria indica* (hydroethanolic extract)	205.925284
2	*Pteleopsis suberosa* (hydroethanolic extract)	232.362066



Picture 1: Gallic acid calibration line.

Determination of flavonoids

The flavonoid content determined by the aluminium trichloride method for each extract was reported in mg catechin equivalent/g dry plant material. The results reveal that both extracts show moderate contents (Table 3).

Table 3: Yield and Contents of total polyphenols and flavonoids.

Extracts	Yield (%)	Total polyphenols content in µg Eq AG/mg d'extract	Flavonoid content in mg QE
Walteria indica	22,19%	205,92	25,4
Pteleopsis suberosa	27,2%	232,36	32,8

Antioxidant activity

DPPH test

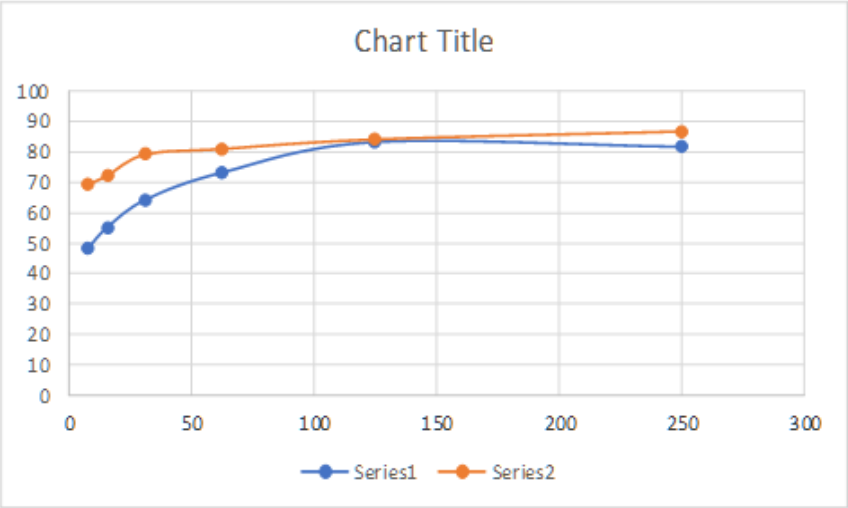
The antioxidant activity of the hydroethanolic extracts of the two plants and of the standard antioxidant (ascorbic acid) towards the DPPH radical was evaluated with a spectrophotometer by following the reduction of this radical which is accompanied by its passage from violet (DPPH°) to yellow colour (DPPH-H) measurable at 517nm. This reduction capacity is determined by a decrease in absorbance induced by antiradical substances.

The results of the antioxidant power of the tested extracts show that the percentage of inhibition of the extracts is more than 80% at concentrations in the range of 125µg/mL to 250µg/mL for both extracts. The IC 50 values determined in mg/ml expressing the effective

concentration of the antioxidant extract required for the entrapment and reduction of 50% moles of DPPH dissolved in methanol (Table).

According to the results recorded, both extracts are endowed with an antioxidant power, their respective EC50 is of 124,14 ±9,72 et 65 ±15,07 clearly stronger than that of ascorbic acid whose value is of the order of 0.235mg/mL.

The polyphenols contained in both extracts are responsible for the antioxidant activity of these extracts.



Picture 2: % DPPH inhibition as a function of different concentrations of extracts of *Walteria indica* and *Pteleopsis suberosa.*

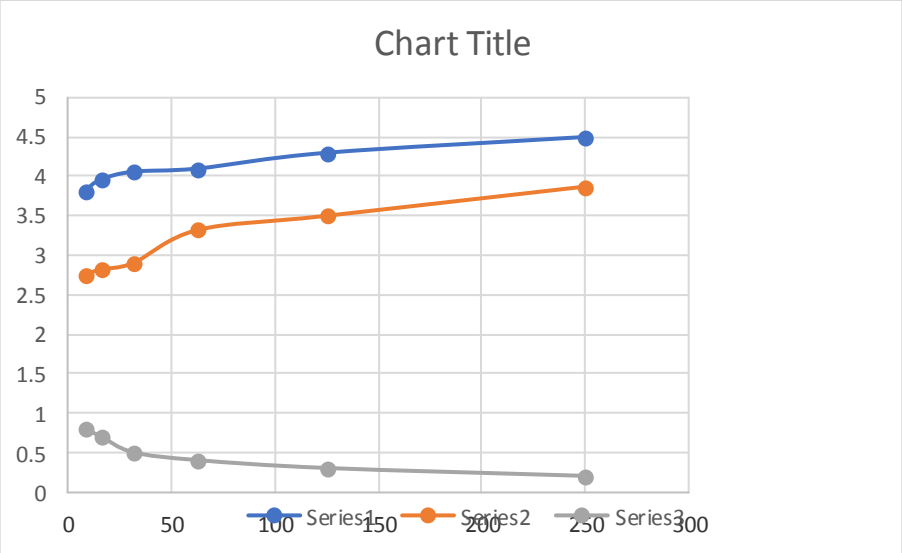
Table 4: IC 50 value of extract and ascorbic acid.

Extraits/Standard	IC50 ± Ecart type
Walteria indica	124,14 ± 9,73
Pteleopsis suberosa	65,02 ± 15,08
Acide ascorbique	0,235 ± 8,01

Frap test: The antioxidant activity of the extracts was evaluated by FRAP iron reduction method. The presence of reductants in the plant extracts causes the reduction of Fe $^{3+}$ ferricyanide complex to the ferrous form. ¨As a result, Fe $^{2+}$can be assessed by measuring the increase in blue colour density in the reaction medium at 700nm.

The reducing power of plant extracts is dose dependent (concentration dependent). The evaluation of reducing power of extracts showed a better activity of the extract hydroethanolic of *Walteria indica* compared to that of extract hydroethanolic of *Pteleopsis*

suberosa as shown in the picture 3. Indeed the extract hydroethanolic of *Walteria indica* at the concentration 7,8-15,62-31,25-62,5-125-250 µg/ml gave respective reducing powers 3 ,82%, 3,96%, 4,06%, 4,12%, 4,32%, 4,54%. At the same concentration the respective reducing powers 2,75%, 2,82% 2,9%, 3,32%, 3,5%, 3,87% have been observed for the extract de *Pteleopsis suberosa.*



CONCLUSION

The study of the antioxidant activity of the extracts of *Walteria indica* species and *Pteleopsis suberosa* according to the iron reduction method and the DPPH free radical scavenging method showed that the two hydro ethanolic extracts have a proven antioxidant activity. These extracts could therefore be an alternative to some synthetic antioxidant additives. Further research is needed to identify, isolate and purify these constituents.

CONFLICT OF INTEREST

We declare there are no conflicts of interest related to this work.

REFERENCES

1. Boxin OU, Dejian H., Maureen AF, Elizabeth KD. Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) anf ferric reducing antioxidant power (FRAP) assays: a comparative study. J Agric Food Chem, 2002; 5: 223-8.

2. Morteza Semnani K. Saeedi M, Shahnavaz B. Comparaison of antioxydant activity of extract from roots of liquorice (*Glycyrrhiza glabra L*.) to commercial antioxidants in 2% hydroquinone cream. J Cosmet Sci, 2003; 54: 551-8.
3. Chinaka ON, Julius 00, Motunrayo GA. In vitro antioxydant potentials of some herbal plants from Southern Nigeria J Med Sci, 2013; 13: 56-61.
4. Kone M, Toure A, Ouattara K, Coulibaly A, Phytochemical composition, antioxydant and antibacterial activities of root of *Uvaria chamae* P. Beauv. (Annonaceae) used in the treatement of dysentery in North of Cote d'ivoire. Int J Pharmacogn Phtochem Res, 2015; 7: 1047-53.
5. Sam SKG, Senthil KB., Ramachandran S., Saravanan M., Sridhar SK. Antioxydant and wound healing properties of *Glycyrrhiza glabra* root extract. Indian Drugs, 2001; 38: 355-7.
6. Yadav SB., Tripathi V., Singh RK, Pandey HP. Flavonoid glycosides from *Cuscuta reflexa* stems and their antioxydant activity. Indian Drugs, 2001; 38: 95-6.
7. Majhenic L., kerget M.S., et Knez Z. Antioxidant and antimicrobial activity of guarana seed extracts. Food Chemistry, 2007; 104: 1254-1268.
8. Banerjee A., Dasgupa N., De B. In vitro study of antioxidant activity of *Syzygium cumini* fruit. Food Chemistry, 2005; 90(4): 727-733.
9. Rezaeizadeh A., Zuki A.B.Z., Abdollahi M., Goh Y.M., Noordin M.M., et al Determination of antioxidant activity in methanolic and chloroformic extracts of *Momordica charantia*. African Journal of Biotechnology, 2011; 10(24): 4932-4940.
10. Bhupendra K.K., Mahesh G.T., Yogendra S. Free radical scavenging effect of various extracts of leaves of *Balanites aegyptiaca* (L) Delile by DPPH method. Asian Journal of Plant Science and Research, 2012; 2(3): 323-329.
11. Roberto G., Baratta M.T., Deans S.G., Doman H.J.D. Antioxidant and antimicrobial activity of *Foeniculum vulgare* and *Crithmum maritimum* essentiel oils. *Planta. Med*, 2000; 66: 687-693.
12. Bougandoura N., Bendimerad N. Evaluation de l'activité antioxydante des extraits aqueux et méthanolique de Satureja calamintha ssp. Nepeta (L) Briq. <<Nature & Technologie<< B-Sciences Agronomiques et Biologiques, 2013; 09(13): 14-19.
13. Huang D., Ou, B., Prior, R. L. The chemistry behind antioxydant capacity assays. Journal of Agricultural and Food Chemistry, 2005; 55: 1841-1856.

14. Marc Fr., Davin A., Deglène-Benbrahim L., et Fernand C. Methodes d'évaluation du potentiel antioxydant dans les aliments Erudit, M/S: médecine sciences, 2004; 20(4) : 458-463.
15. Idiko B., Maria -Loredana S., Dominica R., Simona et Codruta C. HPTLC quantification of some flavonoids in extractsn of Satureja hortensis L. obtained by use of differents techniques. Journal of PLanar Chromatography-Modern TLC, 2009; 22(1): 25-28.