Original Research Article

ROLE OF CONDENSED TANNINS IN THE ANTIOXIDANT ACTIVITY OF SEEDS OF *COLA NITIDA* (VENT) SCHOTT & ENDL. (*MALVACEAE*)

ABSTRACT

Background

Native to West Africa, *Cola nitida* is a tropical tree of about 8-12 in height that grows in lowland rainforest. This plant is best known in Africa for its seeds, used in phytotherapy but for their socio-cultural importance.

Aims/Objective

This study investigated the antioxidant activity of condensed tannins of *Cola nitida* seeds by carrying out two antioxidant tests (DPPH and FRAP).

Methods

From a hydro-ethanolic extract of *Cola nitida* seeds, two samples were made. One treated with casein (EC) and another one without treatment (EWC). The researsh of condensed tannins were carried out by precipitation with Stiasny reagent. The total polyphenol and tannin contents were evaluated by the Folin-Denis method and the antioxidant power by DPPH and FRAP tests.

Results

Extract without treatment (EWC) showed more antioxidant activity than the extract treated with casein (EC). Thus, the IC_{50} of EWC which contains condensed tannins was 5.54 ± 0.005 µg/ml, while that of EC (without condensed tannins) reached 61.92 ± 0.165 µg/ml.

Conclusion

Cola nitida seeds are rich in condensed tannins that play an important role in the antioxidant activity.

Key words: *Cola nitida,* Seeds, Polyphenols, tannins, antioxidant activity.

1. INTRODUCTION

Natural products, especially those of plant origin, have always been an important source of bioactive molecules. Currently, about 25-30% of drugs available for the treatment of diseases are derived from natural products (plants, animals, bacteria and fungi) [1]. According to the World Health Organization [2], traditional medicine is very important but often underestimated in healthcare. It exists in almost every country in the world, and the demand for services in this area is growing.

Nowadays, numerous studies carried out in the field of ethnopharmacology had shown that most of the plants used in traditional medicine and tested in laboratories contained secondary metabolites that are active in pharmacological models.

Among these metabolites, polyphenols occupy a prominent place. They represent a large chemical family including tannins, flavonoids, anthocyanins, etc. Several biological activities have been attributed to polyphenols. These compounds had shown anti-carcinogenic, anti-inflammatory, antiatherogenic, anti-thrombotic, analgesic, antibacterial, antiviral, anticancer [3], anti-allergenic, vasodilator [4] and antioxidant activities [5].

The plant used for this study was *Cola nitida* (Vent) Schott. and Endl. The seeds were chosen by their several uses in Africa traditional medicine against many diseases such as dysentery,

cough, diarrhea, vomiting [6], and by the fact that they have potential for pharmaceutical and food development.

The aim of this study was to evaluate the role of condensed tannins in the antioxidant activity of *Cola nitida* seeds.

2. MATERIALS AND METHODS

2.1. Plant Material

The plant material consists of seeds of *Cola nitida* (Vent) Schott. and Endl. (*Malvaceae*) which were purchased at the Sandaga market in Dakar (Senegal). The drug was identified at the Laboratory of Pharmacognosy and Botany of the Faculty of Medicine, Pharmacy and Odonto-Stomatology (Cheikh Anta DIOP University of Dakar). *Cola nitida* is registered in the herbarium of the same institute under the number of 95. The seeds were crushed into small pieces and dried at room temperature before being ground into a powder using an electric grinder fitted with a medium-sized sieve. The powder was stored in a sachet protected from light.

2.2. Methods

2.2.1. Preparation of plant extracts

For extraction, 30 g of *Cola nitida* seeds powder were introduced into a cartridge and extracted with 700 ml of the ethanol-water mixture (80/20; v/v), for 24 hours in a Soxhlet apparatus [7]. The solution obtained was evaporated using a rotary evaporator at 65°C. The residue resulting from this operation was dried in an oven for 48 hours at 45°C to obtain the dry hydro-ethanolic extract (EC).

From the dry hydro-ethanolic extract, 60 ml of an aqueous solution at 50 µg/ml were prepared. The solution was then divided into two 100 ml Erlenmeyer flasks at the rate of 30 ml for each container. In one of the Erlenmeyer flasks, 3 grams of casein are added and the whole was submitted to magnetic stirring for 3 hours in order to fix the condensed tannins [8]. The Stiasny reaction was used to check whether all the condensed tannins have been fixed by the casein. This process led to the extract without condensed tannins (EC). The two solutions thus obtained (EWC and EC) were used for the tests of antioxidant activity and for the determination of total polyphenols.

2.2.2. Polyphenols and condensed tannins contents

The method used is that described by Joslyn [9], slightly modified. In a haemolysis tube, 2500 µl of sample at 50 µg/ml (EWC or EC) and 500 µl of Folin Denis reagent were mixed. After 3 min incubation, 500 µl of 25% sodium carbonate was added before centrifuging the tube at 4000 rpm for 4 min. The absorbance was measured at 760 nm against a blank (methanol) using a Bio System BTS/340 UV-visible spectrophotometer. The dosage was done in triplicate (n = 3). A calibration range carried out with gallic acid at different concentrations (0; 5 ;10 ;15 ;20 µg/ml) was treated in the same way as the samples.

Polyphenols contents were expressed in mg gallic acid equivalent per gram of dry extract (mg GAE/g) according to the formula: **T = C.V/M**

T: Total polyphenol content expressed in mg EAG/g,

C: concentration of polyphenols established from the equation of the calibration line in mg/l,

V: volume of extract in l,

M: mass of the extract studied per g of extract.

Tannins contents represented the difference between total polyphenols (in EWC) and residual polyphenols (in EC).

2.2.3. Antioxidant activity

2.2.3.1. DPPH assay

The determination of the DPPH free radical scavenging activity of samples was done using the described method [10]. An ethanol solution of DPPH was prepared by dissolving 4 mg of $DPPH^{\cdot}$ in 100 ml of ethanol, followed by incubation between 4-8° for at least 16 hours. An

aliquot of each sample (0.2 ml) at appropriate concentration was added to 0.8 ml of ethanol solution of DPPH. The two extracts (EWC and EC) were tested at different concentrations. The absorbance was measured at 517 nm after 30 min. Each experiment was done in triplicate.

The antioxidant activity related to the $DPPH^{\cdot}$ free radical scavenging effect was expressed as percentage of inhibition (IP) according to the following formula: IP (%) =100x(A0-A1)/A0.

A0: absorbance of the control

A1: absorbance of the sample

The IC_{50} values (concentration of sample required to scavenge 50% of free radicals) IC50 values were determined by plotting inhibition percentages versus concentrations IC50 values were determined by plotting a percent inhibition versus concentration curve using the software Statgraphics Plus 5.0.

2.2.3.2. FRAP assay

The ferric reducing antioxidant power was determined according to the described method [11]. An aliquot of 0.20 ml of each sample (EWC and EC) at appropriate concentration was mixed with 0.5 ml of phosphate buffered saline (0.2 M; pH: 6.6) and 0.5 ml of 1% potassium ferricyanide ($K_3Fe(CN)_6$). The mixture was incubated at 50 °C for 30 min and 0.5 ml of 10% trichloroacetic acid was added. After centrifugation for 10 minutes at 3000 rpm, the supernatant (0.5 ml) was mixed with distilled water (0.5 ml) and 0.1% ferric chloride (0.1 ml). The assays were performed three times with triplicate samples. Absorbance was measured at 700 nm. The reducing power (RP) expressed as percentage was obtained with the following formula: RP (%) =100x(A0-A1)/A0

A0: absorbance of the control A1: absorbance of the sample

2.2.4. Statistical analysis

The statistical analysis was done by Statview 4.5 software using the Fischer test. The difference was considered significant when $p<0.05$ compared to the negative control.

3. RESULTS

3.1. Extraction

After extraction of 30 g of *C. nitida* seeds powder, a dry extract weighing 3.78 g was obtained, giving a yield of 12.6 %.

3.2. Polyphenols and Condensed Tannins Contents

The total polyphenol content as well as the residual polyphenol content after precipitation of the tannins from the samples were calculated using the equation $y = 0.0341x + 0.0367$ ($R^2 = 0.9994$) from the calibration line obtained with gallic acid shown in Fig.1. As shown in fig. 2, EWC had a total polyphenol content of 64.72 ± 0.63 mg GAE/g. After treatment with casein (EC), tannins were eliminated and the residual polyphenol content was 37.53 ± 0.52 mg EAG/g. Thus, condensed tannins content was 27.19 ± 0.11 mg GAE/g.

3.3. Antioxidant activity

3.3.1. DPPH assay

The two samples (EWC and EC) of *Cola nitida* seeds significantly inhibit the DPPH radical ($p<0.05$ *versus* negative control) at all concentrations tested and, in a dose dependent manner as shown fig. 3.

The IC_{50} values had confirmed that the extract not treated with casein (($IC_{50:}$ 5.54 ± 0.005 µg/ml) had shown better ability to scavenge the DPPH free radical than the extract treated with casein ($IC_{50:}$ 61.92 ± 0.165 µg/ml) as illustrated in figure 4.

3.3.2. FRAP assay

The reducing activity of the hydro-ethanolic extract not treated with casein (EWC) and that of the hydro-ethanolic extract treated with casein (EC) are illustrated in fig.5. The results of the

FRAP test confirmed those of the DPPH test. Indeed, as EWC had a greater reducing power than EC at all tested concentrations.

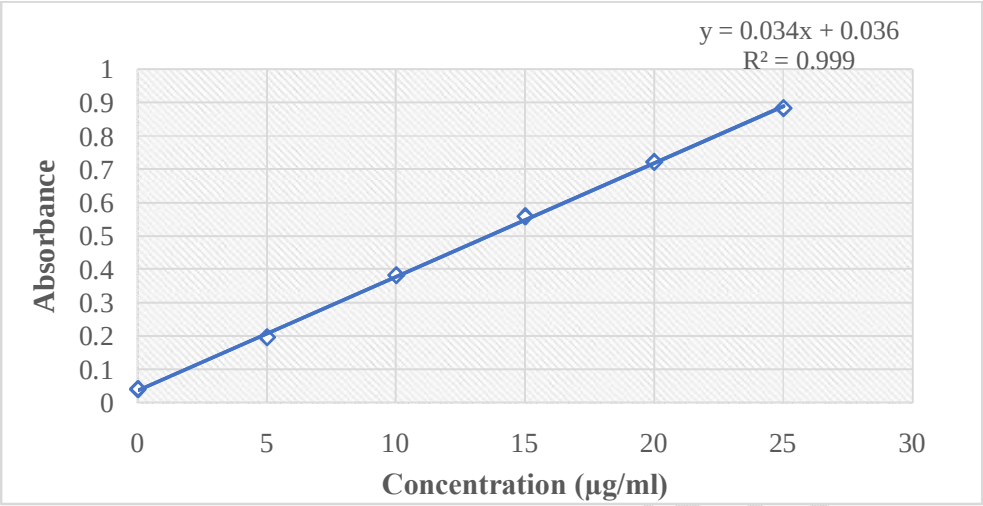

Figure 1: Calibration line obtained with gallic acid

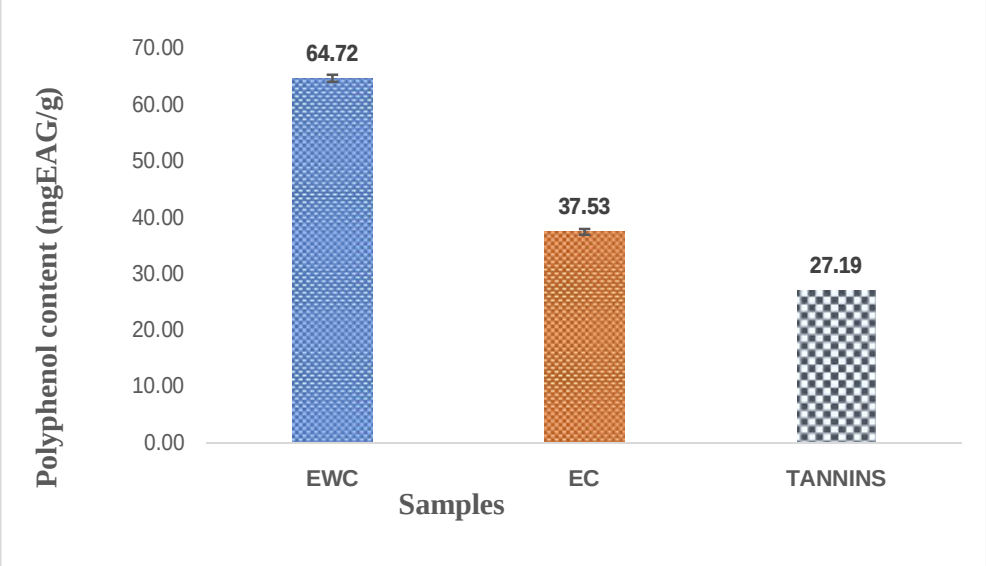

Figure 2: Total polyphenol (in EWC), Residual polyphenol (in EC) and condensed tannins contents

EWC: Extract not treated with casein **EC:** Extract treated with casein

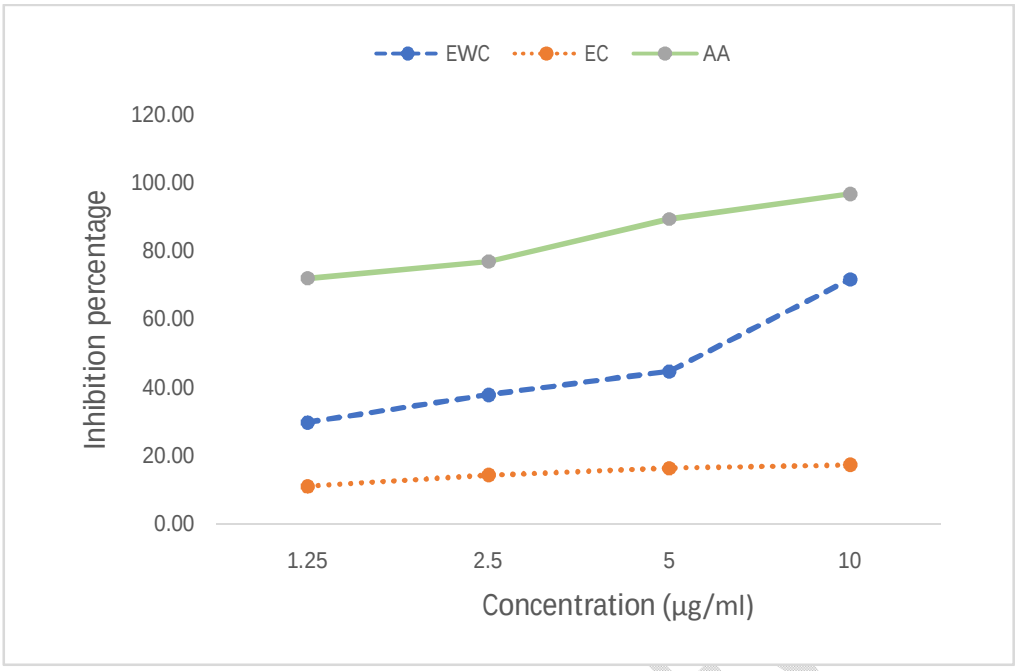

Figure 3: Inhibition percentage of samples on DPPH test
EWC: Extract not treated with casein **EC:** Extract treated with casein **AA:** ascorbic acid

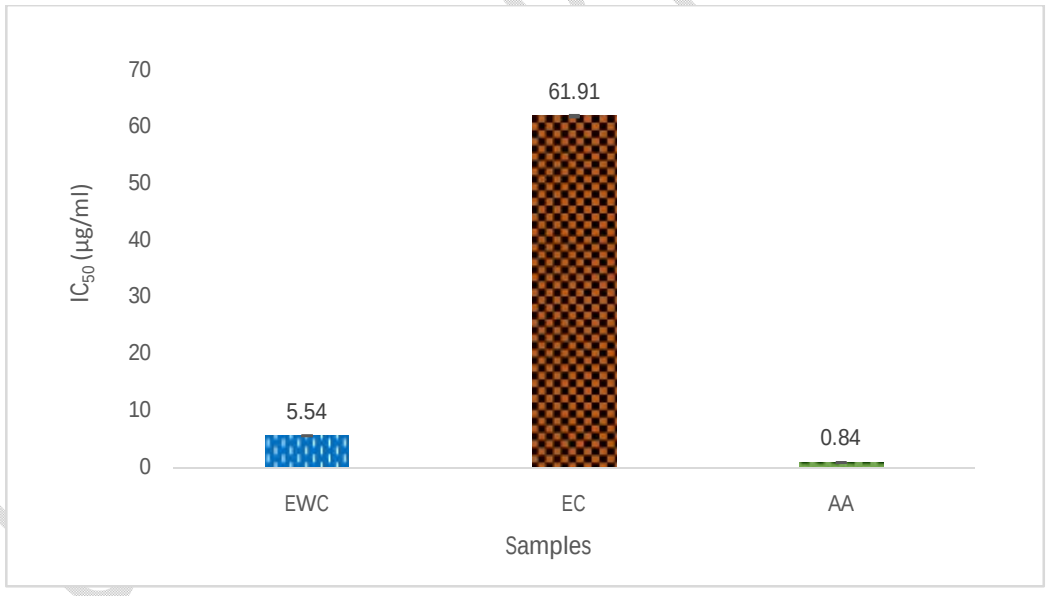

Figure 4: IC_{50} of samples on DPPH test.
EWC: Extract not treated with casein **EC:** Extract treated with casein **AA:** ascorbic acid

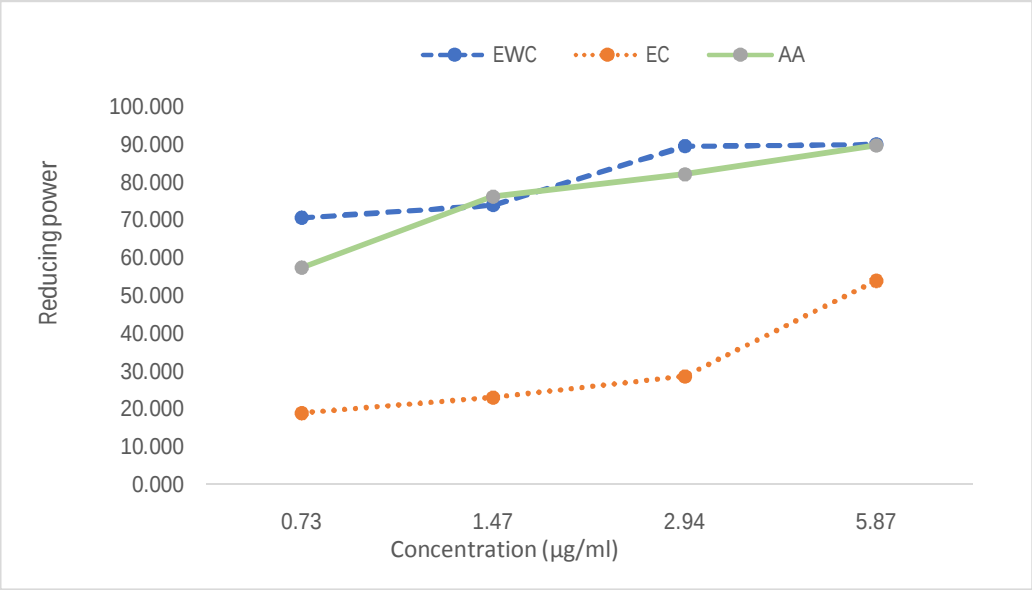

Figure 5: Reducing percentage of different samples in FRAP test
EWC: Extract not treated with casein **EC:** Extract treated with casein **AA:** ascorbic acid

4. DISCUSSION

According to Odebode [12], *Cola nitida* seeds are rich in phenolic compounds such as condensed tannins and flavonoids. The extraction of polyphenolic compounds is a crucial step for the valorization of these active ingredients recognized for their high antiradical power [13]. It depends on the method used and the appropriate choice of solvent preserving the biological properties of these phenolic compounds [14].

As part of our study, we first performed a Soxhlet extraction of *Cola nitida* seeds powder using an ethanol-water mixture (80/20; v/v) as solvent. These two polar solvents are likely to extract heterosides compounds such as the polyphenols contained in the seeds of *Cola nitida*. The yield of this extraction was 12.6%. Adesanwo and *al.* [15] had found a higher yield (17.6 %) using methanol as solvent. With dichloromethane, they obtained a yield of 0.77%. These results suggest that the most represented active principles in *Cola nitida* seeds are mainly polar compounds.

The main objective of our study was to highlight the antioxidant activity of the tannins of the hydro-ethanolic extract of *Cola nitida* seeds by the DPPH and FRAP methods.

The DPPH method is based on the ability of an antiradical to stabilize the purple DPPH free radical by transforming it into pale yellow DPPH, H+, by trapping a proton [16]. As for the FRAP method, it is based on the ability of a compound to reduce the ferric ion (Fe^{3+}) to the ferrous one (Fe^{2+}). The reducing capacity of ferric ion seems to be related to the degree of hydroxylation and extend to the conjugation in phenolic compounds [17].

The results obtained showed that EWC significantly inhibited the DPPH free radical and reduced ferric iron at all tested concentrations ($p<0.05$). After treatment with casein (fixation and elimination of tannins), the antioxidant activity drops significantly for both the DPPH method and the FRAP method. Thus, for the DPPH test, at the highest concentration tested (10 µg/ml), the inhibition percentage (IP) of EWC was 71.63 ± 0.06%. After treatment with casein (EC), the IP decreased to 17.21 ± 0.05% or 4.16 times less at the same concentration. The determination of the 50% inhibitory concentrations (IC_{50}) confirmed these results. Indeed, the IC_{50} of EWC was only 5.54 ± 0.005 µg/ml. After treatment with casein, it increased 11.15 times, with an IC_{50} value of 61.92 ± 0.165 µg/ml. The results of the FRAP test corroborate those of the DPPH method, with reducing powers of 90.06 ± 0.18% and

53.95 ± 0.06% for EWC and EC respectively. This means that the reducing power of EWC was 1.66 time greater than that of EC at the highest concentration tested (5,87 µg/ml).
The dosage of total polyphenols and residual polyphenols made it possible to deduce the tannin content of the EWC. Thus, the content of total polyphenols in this extract was 64.72 ± 0.6 mg GAE/g. After fixation of the tannins condensed by casein, it was estimated at 37.53 ± 0.52 mg GAE/g. The content of condensed tannins is then 27.19 ± 0.26 mg EAG/g, ie 42.2% of the total polyphenols.
These results showed that *Cola nitida* seeds are rich in condensed tannins that play an important role in the antioxidant activity noticed for this drug. These results agree with those of Dieng and *al.* [18], who had proved that the condensed tannins of *Piliostigma reticulatum* (DC) Hochst, had a clear antioxidant activity. So condensed tannins could be used against oxidative stress to prevent several diseases. In fatty foods, sorghum condensed tannins are already used as antioxidant additives for their conversation [19]. The use of natural antioxidants in food could be a good alternative to chemical antioxidants. Indeed, although authorized, these chemical additives would have long-term toxic effects. Certain nanoparticles of chemical antioxidants could cross the placental barrier and cause teratogenic effects [20]. However, condensed tannins have the disadvantage of binding to proteins, carbohydrates and mineral elements, thus reducing nutritional value of certain nutrients [21].

5. CONCLUSION

These studies showed that the hydro-ethanolic extract of *Cola nitida* seeds (EWC) had good antioxidant activity. Rid of condensed tannins (EC), this activity decreased sharply. Condensed tannins contribute significantly to the antioxidant activity of *Cola nitida* seeds.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. **Muanda F.N. (2010)**: Identification de polyphenols, évaluation de leur activité antioxydante et étude de leurs proprietés biologiques. *Thèse Doct. Pharm.*, Metz, 239p
2. **WHO (2013) :** WHO Strategy for Traditional Medicine 2014-2023. ISBN 978 92 4 250609 9 (classification NLM : WB 55) (www.who.int).
3. **Babar A., Hahne J., Paek K.V. (2007):** Methyl Jasmonate and Salicylic Acid Induced Oxidative Stress and Accumulation of Phenolics in Panax ginseng Bioreactor Root Suspension Cultures. *Molecules*; 12(3): 607–621.
4. **Falleh H., Ksouri R., Chaieb K., Karray-Bouraoui N., Trabelsi N., Boulaaba, M., Abdelly C. (2008) :** Phenolic composition of Cynara cardunculus L. organs, and their biological activities .*C. R. Biologies*. 331: 372-379.
5. **Gómez-Caravaca A.M., Gómez-Romero M., Arráez-Román D., Segura-Carretero A. and Fernández-Gutiérrez A. (2006):** Advances in the analysis of phenolic compound in products derived from bees. *J Pharm Biomed Anal;* 41 (4): 1220-34.
6. **Ayensu E.S.** Medicinal Plants of West Africa. 1st Edition. Algonac, Michigan: Reference Publications Inc; 1978. pp. 58–61. 130, 201, 222
7. **Redfern J., Kinninmonth M., Burdass D. and Verran J.** Using Soxhlet Ethanol Extraction to Produce and Test Plant Material (Essential Oils) for Their Antimicrobial Properties. *J Microbiol Biol Educ*. 2014; 15(1): 45–46.

8. **Sarr A., Dieng S.I.M., Badji K.D., Mbaye A.I., Diatta W., Ka A., Fall A.D. and Bassène E. :** Phytochemical Screening and Determination of Polyphenols in the Hydro-Ethanolic Extract of TrunkBark and Its Fractions of *Stereospermum kunthianium* Cham (*Bignoniaceae*), *Asian Plant Research Journal* 2021; 7(1): 1-9.
9. **Joslyn M.A.** Ash content and ashing procedures. in: Joslyn, m.a., ed., methods in food analysis. Physical, Chemical and Instrumental Methods of Analysis, 2nd Edition, *Academic Press*, New York. 1970 ;109-140.
10. **Nunes P.X., Silva S.F, Guedes J.R, Tolentino J., Augusto L., Quintans-Junior L.J. and Filho J.M.B. (2012) :** Biological oxidations and antioxidant activity of natural products. Phytochemicals as nutraceuticals - Global Approaches to Their Role in Nutrition and Health 2012, 278. Doi: 10.5772/2375
11. **Bassène E.** Initiation à la recherche sur les substances naturelles : extraction, analyses, essais biologiques. Presses Universitaires, Dakar 2012.
12. **Odebode A.C (1996) :** Phenolic compounds in the kola nut (*Cola nitida and Cola acuminata*) (*Sterculiaceae*) in Africa. *Rev. Biol. Trop.*, 44(2): 513-515
13. **Bonnaillie C., Salacs M., Vassiliova E., Saykova I. (2012):** Etude de l'extraction de composés phénoliques à partir de pellicules d'arachide (*Arachis hypogea* L.). *Revue de Génie Industriel*, 7 : 35-45.
14. **Mahmoudi S., Khali M., Mahmoudi N. (2013):** Etude de l'extraction des composés phénoliques de différentes parties de la fleur d'artichaut (*Cynara scolymus L.*). *Nature & Technologie*, 9: 35-40.
15. **Adesanwo J.K., Ogundele S.B., Akinpelu D.A. and McDonald A.G.** Chemical Analyses, Antimicrobial and Antioxidant Activities of Extracts from *Cola nitida* See. Journal of Exploratory Research in Pharmacology 2017 ;2(3):67-77doi: 10.14218/JERP.2017.00015
16. **Prior R.L., Wu X., Schaich K**. : Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agriculture and Food Chemistry*. 2005;53(10):4290-303.
17. **Benzie I.F. and Choi S.W.** Antioxidants in food: content, measurement, significance, action, cautions, caveats, and research needs. *Advances in Food and Nutrition Research.* 2014;71:1-53.
18. **Dieng S.I.M., Mathieu C., Sarr A., Diatta-Badji K., Fall A.D.** Condensed tannins content and their influence on the antioxidant activity of bark hydroethanol extract of *Piliostigma reticulatum* (Dc) hochst and its *fractions. Pharmacognosy Journal*. 2020; 12(2):361-368. DOI: 10.5530/pj.2020.12.57
19. **Sikwese F.E. & Duodu K.G. 2007.** Antioxidant effect of crude phenolic extract from sorghum bran in sunflower oil in the presence of ferric ions. *Food Chem.*, 104**,** 324-331.
20. **Guillard A., Gaultier E., Cartier C., Devoille L., Noireaux J., Chevalier L., Morin M., Grandin F., Lacroix M.Z., Coméra C., Cazanave A., de Place A., Gayrard V., Bach V., Chardon K., Bekhti N., Adel-Patient K., Vayssière C., Fisicaro P., Feltin N., de la Farge F., Picard-Hagen N., Lamas B. & Houdeau E.** Basal Ti level in the human placenta and meconium and evidence of a materno-foetal transfer of food-grade TiO2 nanoparticles in an ex vivo placental perfusion model. *Part. Fibre Toxicol* 17, 51 (2020). https://doi.org/10.1186/s12989-020-00381-z
21. **Ojo M.A.** Tannins in Foods: Nutritional Implications and Processing Effects of Hydrothermal Techniques on Underutilized Hard-to-Cook Legume Seeds–A Review. *Prev Nutr Food* Sci. 2022 ; 27(1): 14–19.