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Husk Fibers of Wild Mature, Dwarf, Young and Hybrid Varieties of The Cocos Nucifera As A Potential Source Of Natural Antioxidant: An In Vitro Comparative Study

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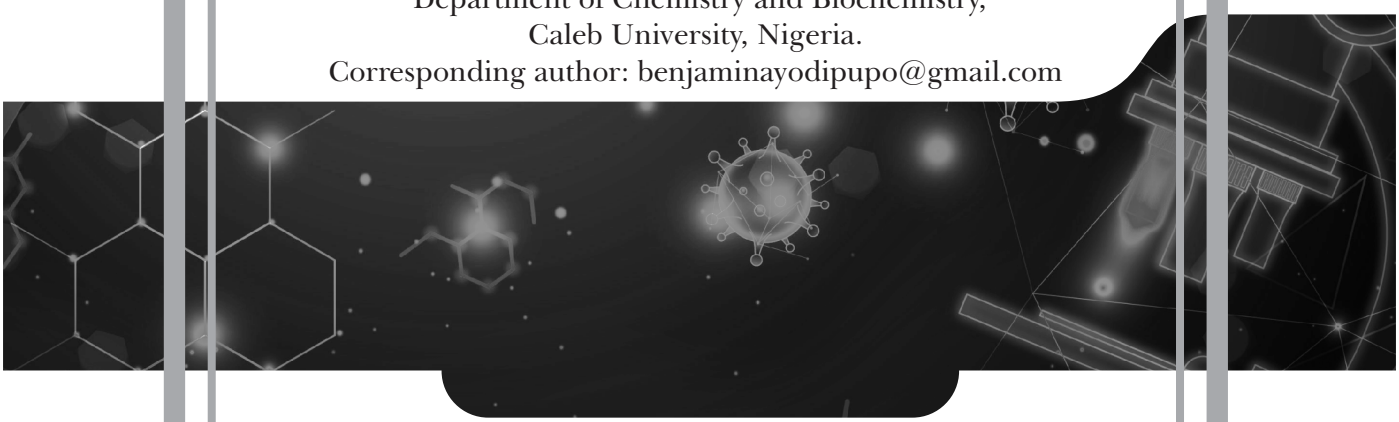

Abstract

Cocos nucifera (coconut) husk fiber is the dry and fibrous external material that is processed typically to obtain the solid albumen and endosperm (coconut-water). It has been reported previously that the husks of the coconut palm are discarded massively as waste and considered as a major agro-waste of tropical countries. In this study, we investigated and compared the antioxidant activities of the n-hexane and acetone-water extracts of varieties of Cocos nucifera husk fibre - wild mature (WM), dwarf (DWF), young (YNG), and hybrid (HYB). The antioxidant activities of extracts were evaluated using a range of in vitro free radical scavenging assay models, total antioxidant capacity, and reductive ability. The results obtained were statistically analysed at a significance level of $p < 0.05$. The highest values for the total flavonoid content, total phenolic content, and total antioxidant capacity for the n-hexane extract was obtained from the wild mature husk fibres while the lowest was from dwarf species. The acetone-water extracts of the young husk exhibited the highest values for the total flavonoid, phenolic content, and antioxidant capacity while the hybrid species showed the lowest. At varying concentrations, the coconut varieties and extracts displayed different 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals scavenging activities. All varieties displayed reducing potential, although with varying efficacy. Also, it was observed that the IC50 for the nitric oxide free radical scavenging activity of the n-hexane extracts and acetone-water extract were similar in the order of HYB > DWF > WM > YNG.

Keywords: Cocos nucifera, husk fiber, antioxidant, free radicals, Inhibition Concentration at 50% (Ic50)

Introduction

The trees of Cocos nucifera dwell in all Nigerian states but were dominant in the Southwest region. In Nigeria, the fruit is taken in conjunction with common foods like granulated cassava (garri), roasted maize, roasted plantain, bread, boiled rice, and groundnut. In obtaining the ingestible portion of Cocos nucifera, the husk is excised and then disposed of. So many in Nigeria are not aware of the medicinal significance of the husks, and their use has been relegated in local communities to enhance flames for cooking and the manufacturing of ropes (Rejees and Saju, 2017; Ede and Agbede, 2015). Cocos nucifera husk is medicinal, and studies have shown that the stems, seeds, flowers, barks, roots, and other parts of a plant are of medicinal value in traditional medicine (Femi-Adepoju et al., 2021; Hussin et al., 2021; El-Aziz et al., 2019; Abubakar and Majinda, 2016; Chandrasekaran et al., 2011). It was, however, reported that the roots of Cocos nucifera possess antipyretic and diuretic

agents, and the stem possesses antihelmintic and anti-inflammatory activities (Lima et al., 2015). In India, heating the shells of Cocos nucifera synthesize oils that are used against ringworm infections (Chakraborty and Mitra, 2008). Also, in Ghana, coconut water was reported to treat diarrhea (Lima et al., 2015). The water of coconut was recently postulated to act as a biostimulant and anti-cancer agent (Estévez, 2021, Babalola, 2020).

It is evident that from past studies the different parts of Cocos nucifera have shown to be of medicinal value. It hence infers their increased participation in the mopping of free radicals as they may be considered a natural and better source of antioxidant activity. Butylated-hydroxytoluene, butylated-hydroxyanisole, tertiary butylhydroquinone are common synthetic antioxidants and are used in food processing but have been reported to have toxic effects like liver damage and mutagenesis (Li et al., 2021; Rani et al., 2021). As such, the utilization of antioxidants from a natural source needs to be encouraged.

Although in Brazil, the husk fiber of C. nucifera was observed in traditional medicine in diarrhea and arthritis treatment (Esquenazi, 2002), Moumita and Adinpunya (2007) reported that the husks of the coconut palm are discarded massively as waste and it is considered as a major agro-waste of tropical countries. In this present study, we attempt to investigate and compare for effectiveness the therapeutic potential of this agro-waste and its varieties.

Materials and Methods

Sample Collection and Identification

Fresh husk fibres of different species of Cocos nucifera were obtained from Nigerian Institute for Oil Palm Research (NIFOR). The leaves were identified and authenticated in the herbarium of the Department of Botany, University of Lagos, Akoka, Lagos State.

Sample Preparation and Extraction

The husk fibres of different species of Cocos nucifera were air-dried for a period of fourteen days and ground into powder. The powdered samples were weighed on a weighing balance and further stored at room temperature. The solvent extraction of the plant sample was prepared by soaking 1000 g of the plant sample in 10 L of n-hexane at room temperature for three days using the maceration process with occasional shaking after which it was filtered using a muslin cloth. The filtrates were collected in a bottle and concentrated at the Department of Pharmacognosy, College of Medicine using the rotary evaporator. The dried residues were re-extracted using an acetone-water mixture and the filtrates were as well concentrated.

Antioxidant Activity Assay

DPPH Radical Scavenging Activity

The DPPH radical scavenging activity of plant extracts was measured as described by Mensor et al. (2001) with slight modification. A 1 mL aliquot of the extract and standard at different concentrations (25, 50, 75, 100 μg/mL) in test tubes was added to 2 mL of 1 mM DPPH in methanol. The mixtures were vortexed and incubated in a dark chamber for 30 min after which the absorbance was measured at 517 nm against a DPPH control containing only 1 ml of methanol in place of the extract. Ascorbic acid and Gallic acid were used as standards.

The inhibition of DPPH was calculated as a percentage using the expression:

$$\%\,I = [A\,control - A\,sample]/A\,control \times 100$$

% I is the percentage inhibition of the DPPH radical; A control is the absorbance of the control and A sample is the absorbance of the sample.

Reducing Power Activity

This was determined according to the method of Oyaizu et al. (1986) with slight modification. A 1 mL aliquot of the extract or standard at various concentrations (25, 50, 75, 100 μg/mL) was mixed with 2.5 mL phosphate buffer (pH 6.6) and 2.5 mL potassium ferricyanide. The mixture was incubated at 50 ^{o} C for 20 min and trichloroacetic acid (10%, 2.5 mL) was added to the mixture which was further incubated for 10 minutes. The resulting mixtures were centrifuged for 20 min at 3000 rpm. Filtrates (2.5 mL) gotten after centrifugation were measured into different test tubes and 2.5 mL of distilled water was added to each tube. 0.5 mL of 0.1% ferric chloride was as well added to each tube and the tubes were incubated for 10 min. The absorbance was read at 700 nm using a spectrophotometer and ascorbic acid with gallic acid serves as standards. Higher absorbance of the reaction mixture indicated the reductive potential of the extract.

Nitric Oxide Radical Scavenging Assay

The scavenging effect of the extract on nitric oxide radical was measured according to the method of Ebrahimzadeh et al. (2009) with slight modification. A 1 mL aliquot of sodium nitroprusside (5 mM) in 0.5 mL phosphate buffered saline (PBS) was mixed with different concentrations of extracts and distilled water. This was incubated at room temperature for 150 mins after which 0.5 mL of Griess reagent was added. After the addition of the Griess reagent, it was further incubated for 30 mins. The absorbance of the pink chromophore formed was read at 540 nm in a spectrophotometer.

Ascorbic acid and gallic acid were used as positive standards.

The percentage inhibition was calculated as:

% Inhibition = [Absorbance of control-Absorbance of Sample] / Absorbance of Control * 100

Determination of Total Flavonoid Content

The total flavonoid content was determined with a colorimetric method described by Jia et al. (1999) and Zucca et al. (2013) with slight modification. An aliquot of 1mL of the extract in 4 mL of distilled water was incubated and added to 0.3 mL of 5% NaNO2. After the addition of 0.3 mL of 5% NaNO2, 0.3 mL of 10% AlCl3 was added and was further incubated for another 10 min, followed by 2 mL of 1 M NaOH, and the resulting volume in each tube was made to 10 mL with distilled water. The solution was properly mixed and the colour intensity of the mixture read at 510 nm after 15 min while quercetin serves as the standard.

Determination of Total Phenolic Content

The total phenolic content of the extracts was determined using the method of Singleton and Rossi (1965) with slight modifications. Briefly, 4 mL of Folin C reagent was added to 1 mL of sample. After 5 min of incubation, 4 mL of 15% Na2CO3 was added and the solution was made up to 10 mL with distilled water. The reaction mixture was further incubated for 90mins and the absorbance was measured with a spectrophotometer at 750 nm.

Total Antioxidant Capacity

The total antioxidant capacity of the extracts was determined using the method of Prieto et al. (1999) with slight modification. An aliquot of 1 mL of the extract was mixed with 3 mL of TAC reagent (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath for 90 min. After this, the samples were cooled to room temperature and their absorbance was read at 695 nm using the spectrophotometer. The total antioxidant was expressed as equivalent of ascorbic acid.

Statistical Analysis

Experimental results were expressed as Mean $\pm$ Standard Deviation. All measurements were in duplicates. A two-way analysis of variance (ANOVA) was conducted to identify differences among the mean; the level of significance used was $p \leq 0.05$. Statistical analysis was performed using Graph pad prism 6.

Results

Comparative Analysis of The Antioxidant Activity of the N-Hexane Extracts and The Acetone-Water Extract

DPPH Radical Scavenging Activity

DPPH assay is one of the most widely used methods for screening antioxidant activity of plant extracts. The extracts demonstrated H-donor activity. It was observed in the n-hexane extract that the young species showed maximum inhibition at 25 μg/mL, the hybrid showed the highest scavenging activity at 50 μg/mL while the wild mature showed maximum inhibition at both 75 and 100 μg/mL compared to the standards (Figure 1a). In contrast, as shown in Figure 1b the acetone-water extract showed that the hybrid species had the highest concentration-dependent-inhibition at 25 and 50 μg/mL while the wild mature species had the highest at 75 and 100 μg/mL compared to the standards (ascorbic acid and gallic acid).

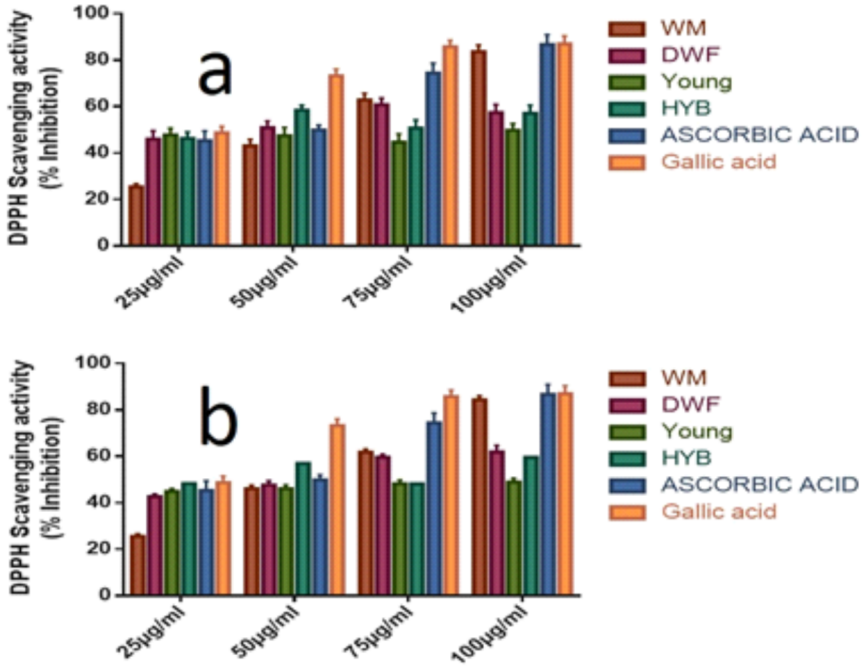

Figure 1.0 (a) DPPH radical scavenging activity of the n-hexane extracts of *C. nucifera in vitro* **(b)** DPPH radical scavenging activity of the acetone-water mixture extracts.

Reducing Power Activity

For the measurement of the reductive ability, the Fe^{3+}-Fe^{2+} transformation was investigated in presence of the extract. It was observed that n-hexane

and acetone-water extract of the different species of the *C. nucifera* possessed varying reducing ability at different concentrations. At 25 μg/mL, the young species has the highest reductive ability, while the hybrid species has the highest at 50, 75 and 100 μg/mL compared to the standards (Figure 2a). In contrast, the result in Figure 2b shows that the reductive ability of the various species of *Cocos nucifera* husk fibres is of close range with slight differences in their values; the hybrid species is of high reducing ability at the different concentrations.

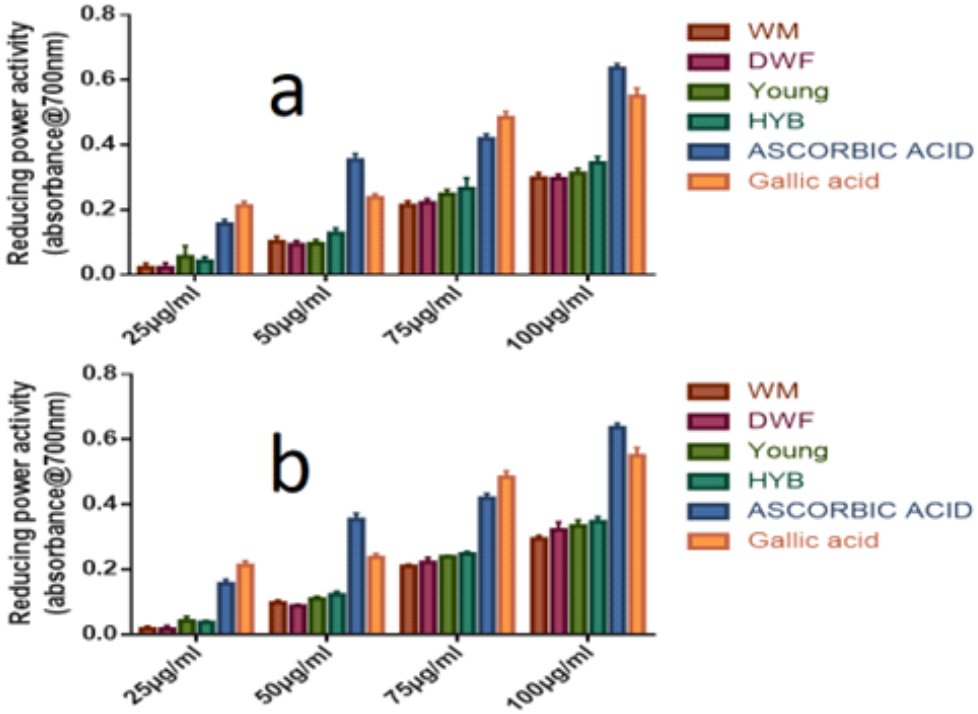

Figure 2.0 (a) Reductive ability of the n-hexane extracts **(b)** Reductive ability of the acetone-water mixture extracts.

Nitric Oxide Radical Scavenging Activity

Scavenging of nitric oxide radical is based on the generation of nitric oxide from sodium nitroprusside in buffered saline, which reacts with oxygen to produce nitrite ions that can be measured by using Griess reagent. From this study, it was observed that both extract methods display similar differences in the radical scavenging activity at different concentrations. It was observed in the n-hexane extract that the concentration dependent inhibition of young species is highest at 25, 50 and 75 μg/mL while at 100 μg/mL, both the wild and young species are at the maximum rate when compared with the

standards (Figure 3a). For the acetone-water extract, when compared to the standards, the percentage inhibition of young species was highest at the different concentrations (Figure 3b).

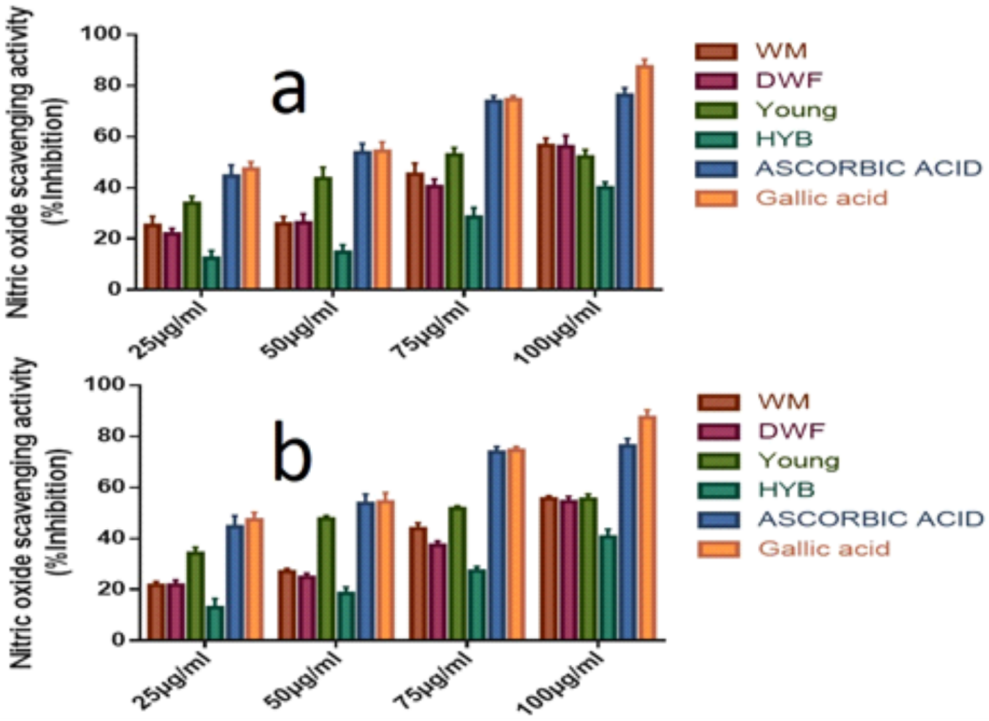

Figure 3.0 (a) Nitric-Oxide Scavenging Activity of the n-hexane extracts **(b)** Nitric-Oxide Scavenging Activity of the acetone-water mixture extracts.

Total Flavonoid Content

The result of the flavonoid content of the different species of the n-hexane extracts of *Cocos nucifera* husk fibres showed that the content of the wild mature species is of the highest concentration and the dwarf species is of the lowest concentration (Table 1.0). Also, from the acetone-water extract, it was observed that the flavonoid content of the young species of *Cocos nucifera* husk fibres is of the highest concentration when compared to other species (Table 2.0).

Total Phenolic Content

Comparing the results of the phenolic content of the two species, it was observed that the wild mature species of the n-hexane extracts of *Cocos nucifera* husk fibres were of a higher concentration in contrast to that of dwarf species of lowest concentration (Table 1.0). The concentration of phenolic compounds of the acetone-water extract determined showed that

the young species have the highest concentration with the dwarf species being the lowest (Table 2.0).

Total Antioxidant Capacity

The result from Table 1.0 suggests that the wild mature species of the n-hexane extracts of *Cocos nucifera* husk fibres possess the highest antioxidant activity compared to the dwarf species of the lowest concentration. Also, the result of the acetone-water extract suggests that the young species of *Cocos nucifera* husk fibres possess a higher antioxidant capacity when compared to the hybrid species (Table 2.0).

Table 1.0: Total flavonoid, total phenol, and total antioxidant capacity of the n-hexane extracts

n-hexane	Total Flavonoid	Total Phenol	Total Antioxidant Capacity
WM	27.84 ± 0.71	19.44 ± 0.33	34.93 ± 0.40
DWF	11.58 ± 0.45	11.55 ± 0.11	13.52 ± 0.40
YNG	20.89 ± 0.51	18.31 ± 0.28	22.34 ± 0.29
HYB	22.52 ± 0.39	15.07 ± 0.44	29.21 ± 0.52

Values represented as Mean ± Standard deviation (N=2) are significantly different (P≤0.05). WM-Wild mature, DWF-Dwarf, YNG-Young, and HYB-Hybrid.

Table 2.0: Total flavonoid, total phenol, and total antioxidant capacity of acetone-water extracts

Acetone	Total Flavonoid	Total Phenol	Total Antioxidant Capacity
WM	154.77 ± 1.93	41.33 ± 0.94	109.89 ± 1.04
DWF	162.17 ± 0.96	32.98 ± 1.05	112.75 ± 0.35
Young	174.21 ± 0.90	48.59 ± 0.94	137.54 ± 2.83
HYB	144.78 ± 1.41	45.39 ± 0.28	75.94 ± 1.79

Values represented as Mean ± Standard deviation (N=2) are significantly different (P?0.05), WM-Wild mature, DWF-Dwarf, YNG-Young, and HYB-Hybrid.

Discussion

Scientific reports have suggested and supported the use of antioxidant supplementation in the treatment of certain health conditions and as a wellness option by mopping out generated free-radical species to reduce the levels of oxidative stress (Gecer et al., 2021; Somade et al., 2020; Ramyaa et al., 2013). Many synthetic antioxidants have shown potential toxic and mutagenic effects (Sun et al., 2021; Motia et al., 2021; Li et al., 2021; Rani et al., 2021), which is causing a shift of attention towards naturally occurring antioxidants. Plant parts like the leaves, husk fibers, rhizomes, stem barks, and root tubers have been utilized for a long time and are patronized in most Nigerian cultures for traditional medicine. For so many years now other plants have been used as a traditional therapeutic option but the medicinal value of the husk of plants like Cocus nucifera Linn has not been known by traditional practitioners and patronisers, and this part is been relegated to an agro-waste even till this dispensation. It is therefore pertinent to investigate the antioxidant potential of the Cocus nucifera Linn and determine which of the varieties of the species would be more active and effective for traditional medical purposes.

Some phytochemicals such as phenols and flavonoids have antioxidant activity and are widely distributed in plants. The content of total phenolic compounds and the antioxidant activity have been reported to be higher in the exocarp regions such as husks and peels, and the seeds (Derakhshan et al., 2018). In this study, the mechanism of the total phenolic content (TPC), using Folin-Ciocalteau (FC) reagent, total flavonoid content, total antioxidant capacity, and the measurement of antioxidant activity by DPPH, reducing ability, and nitric oxide radical scavenging activity methods were employed to determine and investigate the anti-oxidant activity of this phytochemicals.

The DPPH result of multiple comparisons obtained from the n-hexane and acetone-water mixture extracts of husk fibres of four coconut varieties at different concentrations shows significant difference at $p < 0.01$. The DPPH-radical scavenging ability by the coconut varieties at the different concentrations, 25, 50, 75, 100 μg/mL, were in the order of young (YNG) ? hybrid (HYB) > dwarf (DWF) > wild mature (WM), HYB > DWF > YNG > WM, WM > DWF > HYB > YNG, WM > DWF > HYB > YNG for the n-hexane extracts respectively. While for the

acetone-water extract, the order was HYB > YNG > DWF > WM, HYB > DWF > YNG > WM, WM > DWF > YNG > HYB, WM > DWF > HYB > YNG. The IC50 value is the concentration (in μg/ml) of extracts that scavenges the DPPH radicals by 50%. It was reported by Alviano et al., 2004 that the aqueous extract of the husk fiber of Cocos nucifera L.(Palmae) possess free radical scavenging properties whose values were not reported, but whose EC50 value for was 10.0 ± 0.7μg/ml. Also, Oliveira et al. (2013) found the IC50 values of the ethanolic extracts of the husk fibres to varying from 8.6 to 55.9 μg/mL in the following order: yellow dwarf > hybrid > giant > green dwarf. Likewise, Chakraborty and Mitra (2008) found EC50 values ranging from 32.3 to 90.2μg/ml for the methanolic extracts of Cocos nucifera L. mesocarp In this study, IC50 values of the n-hexane extracts varied from 40-82 μg/mL in the order of DWF > YNG > WM > HYB and the acetone-water extracts varied from 45-110 μg/mL in the order of HYB> WM > DWF > YNG. It can be deduced that the n-hexane extracts possess free radical scavengers of higher activity since the high total phenolic content, total flavonoid content, and total antioxidant capacity produced low IC50 values.

The antioxidant potentials of the n-hexane and acetone-water extracts assessing the reducing power activities at different concentrations with a significant difference of $p < 0.01$ reveal that at 25 μg/mL, the young species of Cocus nucifera reduces Fe3+ to Fe2+ best while the dwarf species present the lowest activity, at 50 μg/mL, 75 μg/mL and 100 μg/mL, the hybrid species possess the highest activity. At 25 μg/mL, the young species possess a higher ability to reduce Fe3+ to Fe2+ while the dwarf species presents the lowest ability, and at 25 μg/mL, the hybrid species presents the highest reducing ability while the dwarf species presents the lowest. At 75μg/ml and 100 μg/mL, the hybrid species presents a higher reducing power compared to the wild mature species which present the lowest. Chakraborty and Mitra (2008) confirmed the reducing ability of the methanolic extracts of Cocos nucifera L. mesocarp.

The antioxidant capacity was also evaluated using nitric-oxide free radical scavenging assay for both the n-hexane and acetone-water mixture extracts with significant difference of $p < 0.01$. It was observed that the IC50 for the nitric oxide free radical scavenging activity of the n-hexane extracts ranges from 77-115

?mol/min in the order of HYB > DWF > WM > YNG and that of acetone-water extracts ranges from 75-120 ?mol/min in the same order. Adaramoye and Azeez (2014) demonstrated that the methanolic extract of Cocos nucifera husk fibre is a potent scavenger of nitric oxide in a dose-dependent from 10-250μg/ml when compared with Nauclea latifolia and Cymbopogon citratus. The nitric oxide generated from sodium nitroprusside during this reaction may react with oxygen to form nitrite. Consistent with the report by Adaramoye and Azeez (2014), C. nucifera extract inhibits nitrite formation by competing with nitric oxide for oxygen.

Overall, the result obtained in the present study indicates that the wild mature species, the dwarf species, the young species, and the hybrid species of the Cocos nucifera husk fibres are useful as they are a potential source of natural antioxidant. These indicates its medicinal potential as this will, thereby, limit it from being a waste product. It is suggested that more research should be conducted to further analyze and determine the specific antioxidant properties of different of the Cocos nucifera husk fibres based on their maturity.

Conclusion

These results showed that the husk fibres of Cocos nucifera of varying maturity are potential sources of natural antioxidants. Therefore, husk fibres of Cocos nucifera may be given more attention to compared to as a natural source of antioxidant compared to synthetic antioxidants like butylated-hydroxytoluene and butylated-hydroxyanisole which are food preservatives and may lead to cardiovascular diseases, asthma, hyperactivity, and possibly death, when ingested in high concentrations.

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