PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTIOXIDANT ACTIVITY OF THE AQUEOUS EXTRACT OF *DETARIUM SENEGALENSE* ROOT BARK

UNDER PEER REVIEW

ABSTRACT

Aims: To screen for the presence of bioactive antioxidant phytochemicals and determine the antioxidant activity of *Detarium Senegalense* root bark.
Place and Duration of Study: Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Nigeria, between April 2018 and December 2018.
Methodology: Two solvents, water mixed with chloroform (70:30) was used for extraction. The plant extract was screened for the presence of phytochemicals by standard qualitative analysis and evaluated for *in vitro* antioxidant activity by determining the reducing power, total antioxidant capacity, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and nitric oxide radical scavenging activity in comparison with ascorbic acid and gallic acid.
Results: The reducing power and nitric oxide scavenging activity of the extract increased in a concentration/dose dependent manner and was significantly (P<.05) lower when compared to ascorbic acid and gallic acid at all concentrations tested. The total antioxidant capacity (TAC) of the extract also increased as the concentration increased. Interestingly, at 1000µg/ml, the extract (201.45±0.95) was found to be significantly higher (p<.05) than that of ascorbic acid (198.36±0.83), although lower than gallic acid (266.50±0.84). The % DPPH inhibition of the extract was also significantly lower when compared to ascorbic acid and gallic acid at all concenterations tested. Overall, the results showed the extract was able to scavenge free radicals in a dose dependant manner and revealed the presence of tannins, steroids, alkaloids, terpenoids, saponins and phenols whose synergistic effect may be responsible for the antioxidant activity of the extract.
Conclusion: From the study, it is concluded that the aqueous extract of *Detarium Senegalense* root bark possess appreciable/considerable antioxidant properties and could be exploited as source of antioxidant additives or supplements. However, there is need for further work to clarify and isolate the different classes of phytochemical constituents and also to investigate it's *in vivo* potential.

1. INTRODUCTION

The pathology and development of various malignant diseases such as cancer, diabetes, neurodegeneration, cardiovascular diseases, rheumatoid arthritis, kidney disease, eye disease, and ageing are strongly linked to oxidative stress [1]. When the body's production of reactive oxygen species (ROS) is out of balance, it interferes with its ability to detoxify reactive intermediates or repair the harm ROS can do to organ and cellular systems. This condition is known as oxidative stress. [1]. In simpler terms, oxidative stress is the imbalance in pro-oxidants and antioxidant species. Also, excessive reactive oxygenated/nitrogenated species production, which counteracts the organism's defence systems, is known as oxidative stress [2]. When these reactive oxygen/nitrate species are produced in excess, they react/interact with transition metals to produce highly reactive oxygen species that can cause extensive damage to key biomolecules, such as; lipid peroxidation, protein carbonylation, carbonyl

(aldehyde/ketone) adduct formation, nitration, sulfoxidation, DNA impairment (such as strand breaks or nucleobase oxidation) [1, 2].

Thankfully, nature has evolved elegant regulatory mechanisms for preserving the needed equilibrium in antioxidative redox states. Hence, we are not defenceless against the oxygen radicals and other activated-oxygen species to which we are constantly exposed. All aerobic organisms, including people, employ some main antioxidant defences to try and fend off oxidative damage [2, 3]. Antioxidants are reducing substances that can interact with reaction intermediates to halt or stop oxidation reactions directly or interact with the oxidizing agent to stop the reaction altogether [1]. Some of these antioxidant compounds are directly produced in the body's machinery as endogenous antioxidant enzyme systems, for example, thioredoxin, glutathione, superoxide dismutase and catalase systems. However, these endogenous systems are not infallible as the number of cancer, and other oxidative stress-related disease cases continue to surge.

According to Jones, [1] using herbal/dietary antioxidants can improve the protection of cellular redox equilibrium. According to epidemiological data, eating a wide variety of fruits and vegetables is linked to a lower risk of developing chronic diseases, the majority of which are caused by oxidative stress. A large array of antioxidant molecules, including flavonoids, carotenoids, nitrogen-containing compounds, and organosulfur compounds, can be found in whole grains, veggies, and fruits [4]. Dietary antioxidants work primarily by scavenging free radicals [5]. Studies have shown that dietary antioxidant intake and the onset of cardiovascular illnesses are inversely related. [6,7, 8]. Antioxidants from food are crucial in lowering the chance of developing cancer. Consuming fruit and vegetables was linked to a lower risk of malignancies of the lung, colon, breast, cervix, esophagus, oral cavity, stomach, bladder, pancreas, and ovary, according to an epidemiologic evaluation of 200 cancer studies [9]. Almost every other chronic disease linked to oxidative stress has been demonstrated to be prevented or attenuated by dietary antioxidant consumption [1]. Herbs have continuously been evaluated phytochemically to reveal their antioxidant capacity.

In light of the demonstrated antioxidant potency of food/herbal sources, it is therefore imperative that more plants are investigated for their bioactive phytochemical constituents and antioxidant capacity. This would ultimately increase the pool of antioxidant sources needed to fend off oxidative stress. Detarium senegalense (D.Senegalense) is one example of a functional plant that can play a significant role in protection from oxidative stress. Various studies have been conducted on different parts of D.Senegalense to determine its phytochemical constituents and, ultimately, its medicinal use. In south-eastern Nigeria, the seeds serve as soup thickeners and flavouring agents [10]. In a study by Wang et al, D.Senegalense was reported to have considerable potential in food, and pharmaceutical industries [11]. Also, according to Burkhill, a decoction of the stem bark is effective in the treatment of veneral diseases, urogenital infections, wounds, haemorrhoids, diarrhoea, pneumonia, malaria and rheumatism [12]. Despite the numerous studies on the different parts of Detarium Senegalense, there is little or no data on the root bark's antioxidant capacity and phytochemical constituents. Therefore this study was designed to investigate the phytochemical constituents and antioxidant capacity of the aqueous extract of the root bark of D.Senegalense.

2. MATERIALS AND METHODS

2.1. Collection of plant materials: Fresh roots of *Detarium Senegalese* were collected from the botany garden of the Forestry department of the Michael Okpara University of Agriculture, Umudike. The plant material was identified and authenticated by Mr Ibe Kalu Ndukwe of the Forestry Department, Michael Okpara University of Agriculture, Umudike.

2.2. Sample preparation: The root of *Detarium Senegalese* was debarked, air-dried for two weeks, oven-dried ($60^{o}C$ - $80^{o}C$ overnight, to remove all moisture), and pulverized using an electronic milling machine. 350g of the powdered root bark was poured into a plastic bucket and macerated with 2.4L of distilled water and chloroform in the ratio of 70:30 (1.68L of distilled water + 0.72L of chloroform) and

UNDER PEER REVIEW

allowed to stand for 72 hours with intermittent stirring at room temperature. The mixture was filtered with Whatman 41 filter paper and concentrated to semi-solid residue in a water bath at 60oC to get the semi-solid extract.

2.3. Quantitative and qualitative phytochemical screening: The root extract was subjected to both quantitative and qualitative phytochemical screening using standard phytochemical methods as outlined by Harborne [14], Evans and Fauci [15] and Siddiqui and Ali [16]. Phytochemicals tested for include: Alkaloids [14], tannins, saponins [15], terpenoids, and flavonoids [16].

The total phenolics content of the extract and the fractions were determined using the method of McDonald et al., (2001) with slight modifications, as described by Aliyu et al. [17].

2.4. Invitroantioxidant Assays

Phosphomolybdate Assay (*Total* Antioxidant *Capacity)*

The total antioxidant capacities of the extracts were determined by the phosphomolybdenum method according to the procedure described by Prieto, *et al* [18], using gallic acid and ascorbic acid as standards.

DPPH (2.2-Diphenyl-1- picrylhydrazy) Radical Activity

The free radical scavenging capacity of the extract was analyzed by using the DPPH test according to the method of Sun et al [19].

Reducing Power

Fe^{3+} reducing power of the extracts were determined according to the method of Oyaizu [20].

Nitric Oxide Scavenging Activity

Nitric oxide was measured by the colorimetric assay based on the Griess reaction as described by Ozdestan and Uren [21].

2.5. STATISTICAL ANALYSIS

The tests were performed in triplicates, and the results obtained were expressed as mean ± standard deviation (S.D). The statistical comparison among the aqueous extract of Detarium Senegalense root bark, ascorbic acid and gallic acid was performed using paired sample T-test (IBM SPSS statistics 22) at $P<.05$ level.

UNDER PEER REVIEW

3.0 RESULTS AND DISCUSSION

3.1. Phytochemical Screening

Table 1: Qualitative Phytochemical Constituents Of aqueous extract of *Detarium senegalensis* Root Bark (DsRB). .

S/N	CONSTITUENT	EXPERIMENTAL METHOD	PRESENCE
1	Saponins	Frothing Test Emulsion test	++
2	Tannin (Catecholic)	Ferric chloride test	++
3	Flavonoids	Ammonium test 1% Aluminium chloride test	- -
4	Alkaloids	Picric acid test Wagner's test Dragindroff test	++ + +
5	Steroids		++
6	Terpenoids		+++
7	Phenol		++

key:*+ presence in trace concentration; ++presence in moderately high concentration; +++ presence in very high concentration; -Absent*

Table 2: Total Phenolic Content Of aqueous extract Of *Detarium senegalensis* Root Bark.

PARAMETER	% Percentage phenol content (%w/w)	Gallic acid equivalent(µg/ml)
Total Phenolics	0.36	72.52

Aqueous extract of *Detarium Senegalense* Root Bark (DsRB) which was qualitatively assessed for phytochemicals, revealed the presence of tannins, steroids, alkaloids, terpenoids, saponins and phenols. These metabolites were also found in the stem bark of D.senegalense in a study conducted by Uchegbu and Okwu, 2012 [10]. The tannins present have high antioxidant potential due to their high molecular weight and ability to donate hydrogen electrons or atoms [22]. Also, there is increasing evidence which indicates that plant saponins have high antioxidant activities due to their free radical scavenging abilities. A recent study by Brindhadevi et al, 2022 [23], confirms the antioxidant and anticancer potential of saponins. Although alkaloids and steroids may not correlate sufficiently with antioxidant activity, they are pharmacologically important metabolites. Alkaloids found in plants are most known for their strong pharmacological effects. The analgesic, antispasmodic, and antibacterial properties of isolated pure plant alkaloids and their synthetic derivatives are well documented [24]. Steroids are significant substances, particularly in light of their interactions with other substances like sex hormones [25].

Table '2' shows the total phenol content of the aqueous root extract to be 72.52µg gallic acid equivalent/g of extract. There is a correlation between the phenolic content of plant extracts and their capacity to scavenge free radicals [26,27]. This is due to the high redox potential of polyphenolics, which enables them to function as reducing agents, hydrogen donors, and singlet oxygen quenchers [28]. Similarly, carotenoids (a terpenoid class) also scavenge free radicals with the same mechanism [29]. These metabolites provide sufficient pharmacological logic to consider the aqueous root bark extract of D.Senegalense as a good source of natural antioxidants and use in folk and herbal medicine.

3.2. *Invitro* Antioxidant Assays

UNDER PEER REVIEW

No one assay can adequately represent all of the antioxidants in a mixed or complex system since antioxidants are typically engaged in many mechanisms of action, including prevention of free radical formation, augmentation of scavenging capacity against free radicals, and reducing power. In the assessment of the antioxidant potency of plants, it is important to employ at least two different approaches [30]. In this study, three antioxidant assays, reducing power, total antioxidant capacity (phosphomolybdenum method), percentage DPPH inhibition and nitric oxide scavenging activity, were applied to evaluate the antioxidant properties of the aqueous extracts of Detarium Senegalense root bark (DsRB).

3.2.1 Reducing Power

Table 3: Reduction power of aqueous extract of *Detarium senegalense* Root Bark (DsRB) with two standards ascorbic acid and gallic acid, values are expressed as mean±standard deviation.

In the same row, the values affected with different letter (a-c) are significantly different at P<.05

REDUCTION POWER			
Concenteration(µg/ml)	***D.senegalense***	**Ascorbic acid**	**Gallic**
100	0.31 ± 0.004^{a}	0.55 ± 0.001^{b}	0.896 ± 0.003^{c}
250	0.47 ± 0.006^{a}	0.82 ± 0.004^{b}	1.48 ± 0.005^{c}
500	0.99 ± 0.02^{a}	1.53 ± 0.007^{b}	1.51 ± 0.02^{c}
1000	1.14 ± 0.028^{a}	1.54 ± 0.01^{b}	1.51 ± 0.05^{c}

In the same row, the values affected with different letter (a-c) are significantly different at P<.05

The primary factor in determining the antioxidant capacity of medicinal herbs is iron chelation. The dose-dependent reducing power of various fractions of the aqueous DsRb extract to reduce iron ion Fe (III) into Fe (II), in comparison with ascorbic acid and gallic acid, is shown in Table '3' above. The reduction power of the aqueous extract of DsRB was found to increase with increasing concentration. Despite being significantly ($p<.05$) lower when compared to ascorbic acid and gallic acid at all concentrations tested, the reducing power of the extract, a significant indicator of antioxidant activity, was also found to be appreciable at the highest concentration (1000µg/ml) for which the plant extract was tested (1.142±0.028µg/ml) when compared to the reducing power of Ascorbic acid (1.542±0.01µg/ml) and gallic acid (1.509±0.05µg/ml). The dose-dependent reducing power of the extract is in line with the trends from previous studies on medicinal plants [17, 31, 32].

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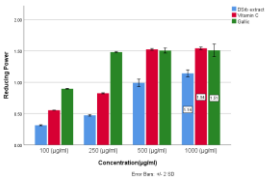

Figure 1: Reduction power of aqueous extract of *Detarium senegalense* Root Bark (DsRB) in comparison with two standards, vitamin C and gallic acid.

3.2.2 Total Antioxidant Capacity

Table 4: Total antioxidant capacity (phosphomolybdenum) of aqueous extract of *Detarium senegalense* root bark (DsRB) with two standards ascorbic acid and gallic acid, values are expressed as mean±standard deviation.

	TOTAL ANTIOXIDANT CAPACITY		
Concenteration(µg/ml)	***D.senegalense***	**Ascorbic acid**	**Gallic**
100	15.83±0.62[a]	36.03±0.81[b]	98.82±0.48[c]
250	30.98±0.67[a]	94.17±0.951[b]	148.87±1.10[c]
500	75.93±0.8[a]	103.04±1.53[b]	162.25±1.84[c]
1000	201.3±0.95[a]	199.02±0.83[b]	267.45±2.41[c]

In the same row, the values affected with different letter (a-c) are significantly different at P<.05

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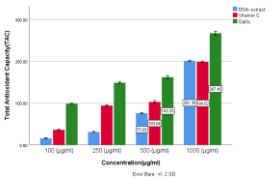

Figure 2: Total antioxidant capacity (phosphomolybdenum) of aqueous extract of *Detarium senegalense* root bark (DsRB) with two standards ascorbic acid and gallic acid.

Table 4 above shows the dose-dependent total antioxidant capacity of *Detarium Senegalense* in comparison with ascorbic and gallic acid. The total antioxidant capacity (TAC) test quantifies the concentration of electrons or radicals that a certain antioxidant donates or squelches. The fundamental idea behind the test is the reduction of Molybdenum (VI) to Molybdenum (V) by the plant extract that contains antioxidant chemicals [33]. The results presented in table '4' shows that there was an increase in the total antioxidant capacity of the plant extract as the extract concentration increased. At the highest concentration (1000µg/ml), the total antioxidant activity of plant extract (201.3±0.95) was found to be significantly ($p<0.05$) higher than that of ascorbic acid (199.02±0.83) but significantly ($p<0.05$) lower than gallic acid (267.45±2.41). This suggests the presence of effective antioxidants in various fractions of the extract.

3.2.3 Percentage DPPH Inhibition

Table 5: Percentage DPPH inhibition and IC_{50} of aqueous extract of *Detarium senegalense* root bark (DsRB), ascorbic acid and gallic acid, values are expressed as mean±standard deviation.

	Percentage DPPH inhibition		
Concenteration(µg/ml)	***D.senegalense***	**Ascorbic acid**	**Gallic**
1000	55.79±0.5[a]	87.61±1.58[b]	90.04±1.7[c]
2000	47.13±0.68[a]	89.02±1.3[b]	83.99±2.43[c]
5000	53.78±0.9[a]	91.54±2.12[b]	85.6±1.85[c]

UNDER PEER REVIEW

10000	68.08±1.47[a]	90.03±1.26[b]	91.34±1.6[c]
IC_{50} (µg/ml)	2.57[a]	1.67[b]	1.70[c]

In the same row, the values affected with different letter (a-c) are significantly different at P<.05

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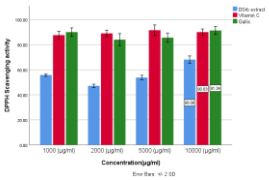

Figure 3: Percentage DPPH inhibition of aqueous extract of *Detarium senegalense* root bark (DsRB), in comparison with vitamin C and gallic acid.

The percentage DPPH inhibition assay is widely used in assessing free radical scavenging activity because of the ease of the reaction [34]. Antioxidants' ability to donate protons was thought to be the reason for their impact on DPPH radical scavenging. Antioxidants were able to convert the stable radical DPPH in the DPPH test into the yellow-coloured diphenylpicrylhydrazine [35]. In this study, the DPPH free radical scavenging effect of the aqueous extract of DsRb, as shown in Table '5', was evaluated. The results indicated that the % DPPH inhibition of the extract was significantly ($p<.05$) lower when compared to ascorbic acid and gallic acid at all concentrations tested. Nonetheless, The *D.senegalensis* extract showed appreciable % DPPH inhibition at the highest concentration of 10000 µg/ml, where the percentage inhibition was (68.08±1.47)% compared to ascorbic acid (90.03±1.26)% and gallic (91.34±1.6)%. The result of the DPPH scavenging activity assay in this study indicates that the plant was potently active and suggests that the plant extract contains compounds capable of donating hydrogen to a free radical to remove odd electrons, which is responsible for free radical reactivity [36].

3.2.4 Percentage Nitric Oxide Scavenging Activity

Table 6: Percentage nitric oxide scavenging activity and IC_{50} of aqueous extract of *Detarium senegalense* Root Bark (DsRB) with ascorbic acid, values are expressed as mean±standard deviation.

	%Nitric Oxide Scavenging activity	
Concenteration(µg/ml)	***D.senegalense***	**Ascorbic acid**
1000	44.79±1.49[a]	97.12±1.88[b]
2000	28.31±0.4[a]	96.63±1.9[b]
5000	46.53±2.15[a]	99.09±0.05[b]
10000	49.78±0.39[a]	99.42±0.08[b]
IC_{50} (µg/ml)	3.53[a]	1.52[b]

In the same row, the values affected with different letter (a-b) are significantly different at P<.05

At physiological pH, sodium nitroprusside in an aqueous solution spontaneously generates nitric oxide (NO), which reacts with oxygen to create nitrite ions that may be measured using Grries' reagent. Scavengers of nitric oxide compete with oxygen, reducing nitrite ions' production [31]. In order to evaluate the antioxidant potency through NO scavenging by the DsRb extract, the change of optical density of NO was monitored. The result in table '6' shows that the extract's percentage of nitric oxide scavenging activity was significantly ($p<.05$) lower when compared to ascorbic acid at all concentrations tested. The *D.senegalensis* extract showed appreciable %Nitric Oxide Scavenging activity at the highest concentration of 10000 µg/ml, where the percentage inhibition was (49.78±0.39)% compared to ascorbic acid (99.42±0.08)%. The table also shows the IC50 of the extract as (3.53µg/ml) compared to ascorbic acid (1.52 µg/ml).

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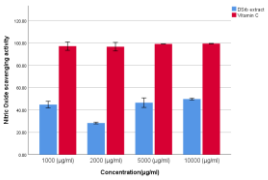

Figure 4: Percentage nitric oxide scavenging activity of aqueous extract of *Detarium senegalense* Root Bark (DsRB) in comparison with vitamin C

4. CONCLUSION

This study justifies the *in vitro* antioxidant potential of aqueous extract of the root bark of *Detarium Senegalense*, with its Total Antioxidant Capacity results comparable to those of the standard compound ascorbic acid. The root bark of *Detarium Senegalensis* is rich in phytochemicals with proven antioxidant activities. Further studies (Gas Chromatography-Mass Spectrometry) are needed to clarify and isolate the different classes of phytochemical constituents and investigate their *in vivo* potential in managing human diseases resulting from oxidative stress.

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UNDER PEER REVIEW

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PEER REVIEW

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