

Antioxidant Activities, Total Flavonoid and Total Phenolic Contents of Whole Plant of *Kyllinga Erecta* Shumach

Oluwafemi Kola Augustus^{1*}, Jesumoroti Omobolanle Janet², Tinubu Busayo Ebenezer³, Uadia Jeremiah Ogboma³

¹Department of Chemistry, Rhodes University, Grahamstown, South Africa

²Department of Chemistry, Wesley University of Science and Technology, Ondo, Nigeria

³Department of Chemistry, Adekunle Ajasin University, Akungba-Akoko, Nigeria

*Corresponding author: augustusoluwafemi@yahoo.com

Abstract *K. erecta* S.; plant family Cyperaceae is locally used as food and medicinal flavor by the Hausas of the Northern Nigeria. This work aims to investigate the phytochemicals present in the whole plant extract, hence, determine the antioxidant activity, total flavonoid and total phenolic contents. The phytochemical screening was conducted on the crude extract using standard methods. The non-polar, moderately polar and highly polar (group 'N, M and P') fractions as well as a crystalline isolate (C) were subjected to antioxidant activity using DPPH and FRAP methods. TFC and TPC were determined in N, M and P only. Phytochemical screening result showed the presence of vital secondary phyto-constituents. The test fractions were able to scavenge DPPH radicals in a concentration dependant faction, the results were recorded as percentage inhibition and they all showed significant radical scavenging capacity (IC₅₀: 0.311, 0.035, 0.297 and 1.089 mg/ml, respectively for N, M, P and C). N, M, P and C were able to reduce Ferric Chloride in a concentration dependent manner as well and the results were recorded as Ascorbic Acid Equivalent. The TFC and TPC were presented as Quercetin Equivalent and Gallic Acid Equivalent, respectively. The TFC were 37.50 and 43.75 mg, respectively of Quercetin in one gram of sample for N and M at 0.91 mg/ml while P had 59.03 mg/g, QE at 0.55 mg/ml. 50.000 and 46.430 mg/g, GAE, respectively were the total phenolic content of N and M at 0.09 mg/ml, respectively while 80.355 mg/g, GAE was recorded for P.

Keywords: secondary phyto-constituents, percentage inhibition, 1,1'-diphenyl-2-picryl-hydrazyl, ferric ion reducing antioxidant power, quercetin

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1. Introduction

Kyllinga erecta Shumach, a specie of Cyperaceae is a robust perennial sedge with contiguous culms arising from a rhizome, the culms sometimes swollen at the base; distributed throughout the world region in damp-grassy area. [1] The Hausas of Northern Nigeria call it 'Aayaa-ayaa' and the Yorubas of South-Western Nigeria call it 'Dogbodogbo'. The rhizome is aromatic with a rather bitter taste. [2] It has aromatic roots; sold in Northern Nigeria markets under the Hausa name "Turare" meaning scent, for use as fumigants and added to food and medicine as flavour. [3] Most spices and natural flavourings agents possess antioxidant effect and properties [4-9].

Antioxidant substances can be sub-categorized into synthetic and natural depending on their sources. It is now widely accepted that antioxidant substances from natural sources are safe and can help improve nutritional values of diets. [10] Dietary antioxidant supplement are good means of strengthening the antioxidant defense and repair

systems. [11] Consciously or unconsciously, one can attempt to solve the problem of poor adherence or compliance to correct diet for improved health against cardiovascular diseases, cancer and aging, by balanced amount of natural antioxidant spices as food condiment.

Extensive literature review on *Kyllinga erecta* S. showed that two diterpenoids; 1 β -hydroxymanoyloxide and 11 α -hydroxymanoloxide have been isolated from the rhizome and the structures were elucidate. [12] 16-hydroxy-manyloxide-a derivative of manyloxide ambrenolide and norambrenolide were also isolated from the ethylacetate extract of *K. erecta* S. [13] as well as the isolation of 16-hydroxy-13-epi-manyloxide which was a new derivative of 13-epi-manyloxide from the ethylacetate extract of *K. erecta* S. rhizomes. [14] 11 α -hydroxyl-13-epi-manyloxide was isolated from the methylene chloride extract of the weed's rhizomes and the deoxygenation of this isolated compound gave 13-epi-manoloxide. [15] The oils of *K. erecta* S. species from Chad were reported to have large quantity of cyperene-type compound and manool oxides. [12,16] Investigation of essential oils of aerial part and rhizomes of *K. erecta* S. using capillary gas

chromatography and gas chromatography spectrometry-thirty one (31) and forty nine (49) compounds were identified from the aerial parts and rhizomes, respectively [17].

The present study, for the first time, reports the total flavonoid and phenolic contents as well as the antioxidant activities of fractions of whole plant of *K. erecta* S. (fractionated based on polarity) extract.

This work was triggered by the fact that synthetic antioxidant additives have been proven to have some effects of abnormal enlargement of liver size and increase microsomal enzyme activities in human, [18,19,20,21] therefore the need for sources of natural antioxidant supplements.

2. Material and Methods

2.1. Reagents

The 1,1'-diphenyl-2-picryl-hydrazyl (DPPH) used was purchased from Sigma-Aldrich, Chemie GmbH, Steinheim, Germany. All other reagents/Chemicals used were of analytical grade. Water used was glass distilled.

2.2. Extraction

Fresh whole plant material was collected at Arigidi-Akoko, Ondo State, Nigeria and was identified and authenticated by Dr O.A. Obembe, a scientist in the Department of plant Science, Adekunle Ajasin University, Akungba-Akoko, Nigeria.

The plant materials were sanitized and air-dried. The air-dried plant materials were crushed to break the pulp in order to create greater surface area for solvent extraction.

The crushed plant materials were subjected to cold extraction using methanol, the extract were filtered through Whatman No.1 using suction pump and the filtrate was concentrated (solvent recovered).

2.3. Phytochemical Screening

The secondary metabolites present in the concentrated extract were qualitatively investigated. The phytochemical investigation was conducted based on standard methods. [22] The presence of Alkaloids, Saponins, Flavonoids, Cardiac glycosides, Phenolic compounds, Phytosterols, Coumarins, Terpenoids, Steroid, Quinine, Phlobatanins, Anthroquinones and Phytosteroids were investigated.

2.4. Fractionation of Crude Extract

The methanolic extract (crude) was subjected to fractionation using Normal-Phase Column Chromatography. Silica gel of mesh size 60-200 was used in packing the column. Hexane, ethylacetate and methanol were used in eluting the fractions by gradually increasing the polarity of the mobile phase while monitoring the separation with thin layer chromatography. The fractions obtained were sub-grouped in non-polar (N), moderately polar (M) and highly polar (P) fractions. A crystalline isolate obtained was labeled 'C'.

2.5. Antioxidant Assays

The total Flavonoid and total Phenolic contents (TFC and TPC) of the non-polar, moderately polar and highly

polar fractions of *K. erecta* S. were determined. 1,1'-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay was conducted on sample N, M, P and C to verify their radical scavenging capacities. The reducing powers for the four test fractions were determined using Ferric ion Reducing Antioxidant Power (FRAP).

2.5.1. Sample Preparation

0.10 g of samples N and M (non-polar and moderately polar fractions) and 0.06 g of samples P (highly polar) as well as of C (crystalline isolate) were weighed in a clean beaker and dissolved in DMSO and the solutions were made up to 50 ml mark using glass distilled water. The mixtures were centrifuged for about 30 minutes until clear solutions were obtained. The samples were preserved by refrigeration prior to analysis.

2.5.2. DPPH (1,1'-diphenyl-2-picrylhydrazyl Radical Scavenging Ability)

Slight modification was made to the method described by Gyamfi *et al.* [23] in order to determine the DPPH radical scavenging ability of the test samples (N, M, P and C). Precisely, appropriate dilutions of samples were prepared (100, 200, 300 and 400 μ L respectively) and each separately mixed with 600 μ L of 0.4M DPPH radicals. The mixture was left in the dark for 30 minutes and the absorbance was recorded at 516 nm in UV-Visible spectrophotometer. The DPPH scavenging ability was calculated using equation 1.

$$\text{DPPH radical scavenging ability} = \frac{(A_r - A_s)}{A_r} \times 100 \quad (1)$$

Where A_r is the absorbance of the reference and A_s is the absorbance of the sample.

2.5.3. FRAP (Ferric ion Reducing Power)

Little modification was done on the method described by Pulido *et al* [24]. Different sample volumes (50, 100, 200 and 250 μ L, respectively) were prepared by dilution. The samples were separately mixed with 2.5 mL of 200 nM of 1 % potassium ferricyanides. The mixtures were incubated at 50°C for 20 minutes. 250 μ L of 10 % trichloroacetic acid (TCA) was added. This was centrifuge for 10 minutes. 5 mL portion of the supernatant was mixed with an equal volume of water and 200 μ L of 0.1 % ferric chloride. The same procedure was followed for the standard (ascorbic acid solution) and the absorbance was recorded at 700 nm against the reagent blank in UV-Visible spectrophotometer. The reducing power was calculated and expressed as ascorbic acid equivalent (AAE) in accordance with the method of Oboh *et al* [25].

2.5.4. TFC (Total Flavonoid Content)

Slight modification was made to the method of Meda *et al.* [26] in order to determine the TFC of *K. erecta* S. fractions (N, M and P). 250 μ L, each of appropriately diluted test samples was mixed with 500 μ L of methanol, 50 μ L of 10 % Aluminum Chloride, 50 μ L of 1 M Potassium acetate and 250 μ L of distilled water. The mixtures were allowed to incubate at room temperature for 30 minutes. Quercetin was used as the reference standard. The absorbance of the reaction mixtures was subsequently measured at 415 nm and the TFC was recorded as

Quercetin equivalent (milligram of quercetin per gram of test sample).

2.5.5. TPC (Total Phenolic Content)

The TPC was evaluated by slightly modifying the method used by Singleton *et al.* [27] To be concise, 200 μ L of appropriately diluted test fractions (N, M and P) were oxidized with 2.5 μ L of 10 % (v/v) Folin-Ciocalteu reagent and neutralized by 2.0 μ L of 7.5 % Sodium carbonate. The mixtures were incubated for 40 minutes at 45°C and the absorbance recorded at 765 nm. The TPC was calculated as Gallic acid equivalent (milligram of Galic acid per gram of test samples).

3. Results

3.1. Yield

After extraction, the plant extract yield was calculated to be 8.71 %.

3.2. Preliminary Phytochemical Investigation

Table 1. Phytochemical screening of *K. erecta* S. +ve indicates the presence of test phytochemical and -ve indicates its absence in the plant extract

Phytochemical	Result
Alkaloids	+ve
Anthroquinones	-ve
Cardiac glycosides	+ve
Coumarin	+ve
Flavonoids	+ve
Phenolic compounds	+ve
Phlobatanins	-ve
Phytosteroids	-ve
Phytosterols	+ve
Reducing sugar	-ve
Tannins	-ve
Terpenoids	+ve
Saponins	+ve
Quinones	+ve

3.3. Antioxidant Properties

3.3.1. DPPH

The DPPH antioxidant capacity for the non-polar, moderately polar, highly polar, fractions, respectively and for the crystalline isolate were evaluated and the results are as presented in Table 2a and b (mean of duplicate measurements \pm standard deviations).

Table 2a. Percentage inhibition of DPPH by samples N and M

Concentration (mg/ml)	Percentage Inhibition (% \pm SD)	
	N	M
0.17	42.66 \pm 1.30	52.98 \pm 0.32
0.33	54.82 \pm 4.22	66.28 \pm 0.97
0.50	56.65 \pm 0.32	69.72 \pm 0.65
0.67	58.03 \pm 0.32	70.18 \pm 2.59

Table 2b. Percentage inhibition of DPPH by samples P and C

Concentration (mg/ml)	Percentage Inhibition (% \pm SD)	
	C	P
0.10	21.33 \pm 1.62	41.05 \pm 0.32
0.24	25.46 \pm 0.97	44.27 \pm 0.32
0.30	27.06 \pm 0.65	52.06 \pm 0.32
0.40	30.05 \pm 0.32	55.28 \pm 4.22

N, M, P and C were able to scavenge the DPPH radicals in a concentration dependent fashion. At 0.17, 0.33, 0.50 and 0.67 mg/ml, respectively, the percentage inhibitions were 42.66, 54.82, 56.65 and 58.03 %, respectively for N and 52.98, 66.28, 69.72 and 70.18 %, respectively for the M. At 0.10, 0.24, 0.30 and 0.40 mg/ml, respectively, the percentage inhibitions were 41.05, 44.27, 52.06 and 55.28 %, respectively for the highly polar fraction. 21.33, 25.46, 27.06 and 30.05 %, respectively were the recorded percentage inhibitions for C at 0.10, 0.24, 0.30 and 0.40 mg/ml, respectively.

3.3.2. FRAP

The ferric reducing antioxidant strengths of the non-polar, moderately polar and highly polar fractions as well as that for the crystalline isolate are as presented in Table 3a and Table 3b.

Table 3a. Ferric Ion Reducing antioxidant power of sample N and M

Concentration (mg/ml)	AAE (mg/g)	
	N	M
0.05	5.580	10.745
0.09	5.785	12.395
0.18	10.125	14.670
0.23	12.395	14.050

Table 3b. Ferric Ion Reducing antioxidant power of sample C and P

Concentration (mg/ml)	AAE (mg/g)	
	C	P
0.03	13.085	9.985
0.05	8.265	15.150
0.11	7.920	17.560
0.14	16.875	19.970

At 0.05, 0.09, 0.18 and 0.23 mg/ml, respectively, the reducing powers were 5.58, 5.785, 10.125 and 12.395 milligram, AAE per gram, respectively for N while 10.745, 12.395, 14.670 and 14.050 milligram, AAE per gram, respectively were recorded for M. The reducing powers for P were 9.85, 15.150, 17.560 and 19.970 milligram, AAE per gram, respectively at 0.03, 0.05, 0.11 and 0.14 mg/ml, respectively. For the crystalline isolate, the reducing strength at 0.03, 0.05, 0.11 and 0.14 mg/ml, respectively were 13.085, 8.265, 7.920 and 16.875 milligram, AAE per gram, respectively.

3.3.3. TFC

At 0.91 mg/ml, the total flavonoid contents were 37.50 and 43.75 mg/g, QE, respectively for the N and M while P at 0.55 mg/ml had 59.03, QE as its total flavonoid content (Table 4).

Table 4. Total flavonoid Contents

Sample	Concentration (mg/ml)	QE (mg/g)
N	0.91	37.500
M	0.91	43.750
P	0.55	59.030

3.3.4. TPC

The total phenolic contents of N and M were recorded at 0.09 mg/ml, respectively. For the highly polar fraction (P), the total phenolic content was recorded at 0.05 mg/ml and the results are as presented in tables 5. The total phenolic content for the non-polar fraction was 50.00 mg/g, GAE while the moderately polar fraction's total phenolic content was 46.43 mg/g, GAE. 72 80.36 mg/g, GAE was recorded as the total phenolic contents of the highly polar fraction (Table 5).

Table 5. Total Phenolic Contents

Sample	Concentration (mg/ml)	GAE (mg/g)
N	0.09	50.000
M	0.09	46.430
P	0.05	80.355

4. Discussion

The low yield can be attributed to the fibrous nature of the plant materials. The presence of vital phytochemicals was observed. Preliminary phytochemical screening was conducted to ascertain the secondary phyto-constituents present in the crude extract. The result showed that *K. erecta* species from Arigidi-Akoko, Nigeria is rich in: Alkaloids, Cardiac glycosides, Coumarins Flavonoids, Phenolic compounds, Phlobatanins, Phytosterols, Terpenoids, Saponins and Quinones. Anthroquinone, Reducing Sugar, Tanin and Phlobatanins were absent. Some of these secondary metabolites are good antioxidant agents [28].

The nature and ratio of solvent system used during fractionation determines the nature of substances eluted. Less polar solvent system is expected to elute less polar solutes and vice-versa.

Polarity is the relative ability of molecules to engage in interactions. [29] Type and polarity of solvent system engaged in fractionation would determine the kind eluted substances and it is expected to have specific (high or low) antioxidant properties, total phenolic and total flavonoid contents.

Antioxidants are molecules that have the capacity of reducing the causes of oxidative stress. Oxidative stress can be caused by environmental factors such as diseases, infections, aging [reactive oxygen species (ROS) production]. ROS are free radicals and other oxygenated molecules that arise from these factors [30].

Flavonoids and phenolics are influential secondary phyto-constituents that are potential health promoting compounds because of their antioxidant properties. Flavonoids and other polyphenolics are of dietary importance because they possess free radical scavenging ability which as a result works against oxidative cell damage. [30,31,32] Therefore, the need to evaluate the total flavonoid contents in medicinal plants, food flavouring weeds and botanicals.

Flavonoids are effective as antioxidants because of their structural features such as conjugation which stabilizes radicals, ortho-dihydroxylsubstituted B-ring that creates allowance for chelation of pro-oxidants and α,β -unsaturated ketone as well as 3-OH on C-ring. In this work, the polar fraction has the highest flavonoid content

while the moderately polar fraction has the lowest flavonoid content.

The total phenolic content experiment is governed by Folin-Ciocaltau reaction. The Folin-Ciocaltau is a reagent that contains mixture of phosphomolybdate and phosphotungstate. The reagent measures the reducing capacity of the test sample by reacting with the reducing substance in the test samples. Phenolate ions present in the samples reduce $\text{Mo}^{(VI)}$ to $\text{Mo}^{(V)}$ by transfer of electron to produce a blue colour whose absorbances were measured at 765 nm.

There was a significant difference amongst various solvent fractions. The polar fraction has the highest phenolic content (in agreement with Ghasemzadeh *et al.*, 2011, Hegazy *et al.*, 2012, Kamaraj *et al.*, 2012, Kaneria *et al.*, 2012) [33,34,35,36] while the moderately polar fraction possesses the lowest phenolic content. The higher the flavonoid and phenolic contents of a test sample, the better antioxidant agent such sample is.

In DPPH antioxidant method, the radical scavenging capacity of fractionated extract of *K. erecta* S. species from Arigidi-Akoko, Nigeria and of a crystalline isolate was evaluated. The experiment predicts the ability of the test samples to scavenge free-radicals (ability to quench OH^\cdot , ROO^\cdot and other ROS). This antioxidant investigative method is very common and acceptable; this is as a result of the fact that DPPH molecule itself is a stable free radical in methanol and aqueous solution and it has the ability to pick up an electron or hydrogen radical to form a stable diamagnetic molecule as well as giving a strong absorption band in the visible region of the electromagnetic spectrum [37,38].

The Inhibitory concentration at 50 % (IC_{50}) was extrapolated from the equation of linear plot of concentration against percentage inhibition and it showed that the non-polar fraction had IC_{50} of 0.311 mg/ml and the moderately polar fraction had IC_{50} of 0.035 mg/ml. The polar fraction had IC_{50} of 0.297 mg/ml while the crystalline isolate had IC_{50} of 1.089 mg/ml.

The lower the inhibitory concentration at 50 % (IC_{50}) value, the higher the radical scavenging strength; the moderately polar fraction had the highest antioxidant strength followed by the polar fraction then the non-polar fraction but they all showed significant radical scavenging ability. Figure 1a and Figure 1b show the trend of percentage inhibition increase as concentration increases.

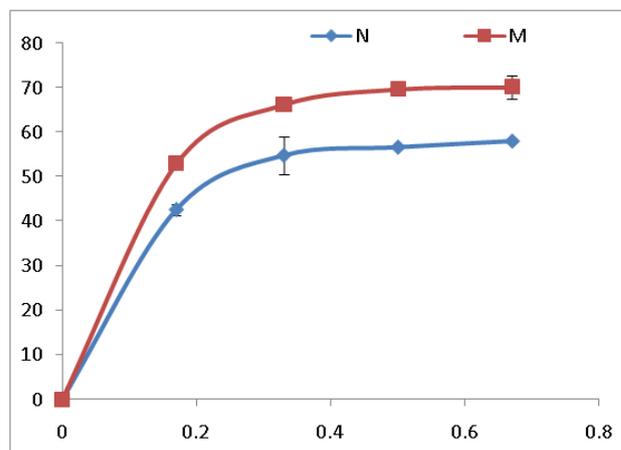


Figure 1a. Graphical relationship between Concentration (x-axis) and percentage inhibition (y-axis) for N and M

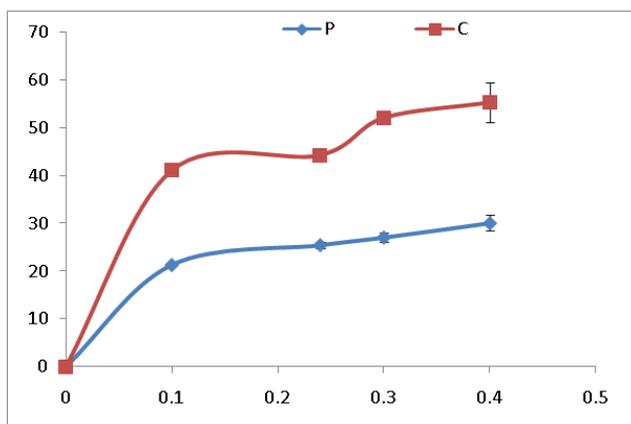


Figure 1b. Graphical illustration of the relationship between Concentration (x-axis) and percentage inhibition (y-axis) for P and C

DPPH radical scavenging capacity alone cannot conclusively affirm the antioxidant strength when exploring for new and natural antioxidant source. Reducing power also play a role. The reducing power of the test samples (N,M, P and C) were assessed by investigating their ability to reduce Fe^{3+} of $FeCl$ to Fe^{2+} .

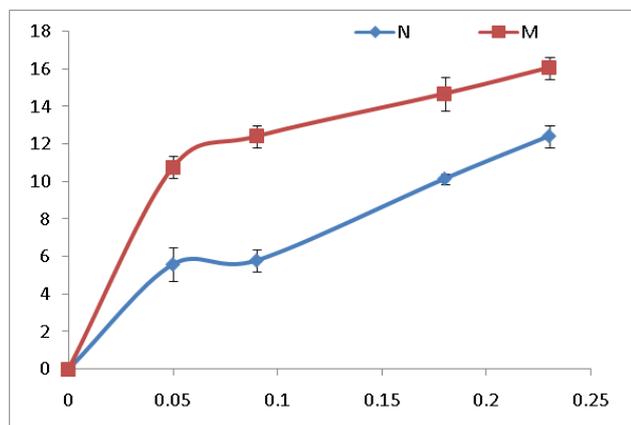


Figure 2a. Graphical illustration of Concentration (x-axis) against Reducing Power (y-axis) for N and M

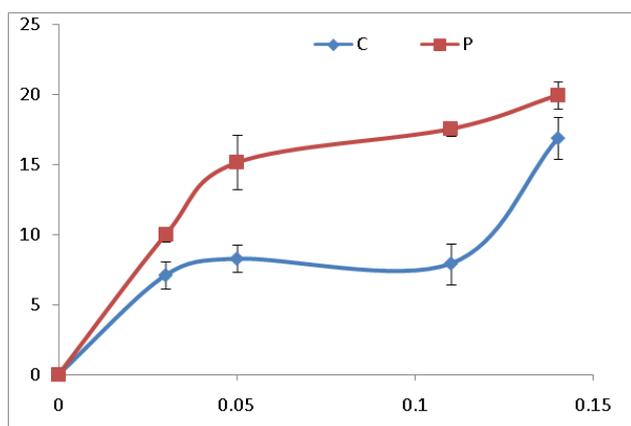


Figure 2b. Graphical Illustration of Concentration (x-axis) against Reducing Power (y-axis) for P and C

Reducing ability is a new defense mechanism by antioxidation which works by electron transfer and hydrogen ion transfer. This was studied by way of observing the ferric-to-ferrous ion reducing potentials; the concentration of the electron-donating antioxidant agent available in test samples correspond to the values in the

ferric reducing antioxidant property experiment. [39,40] The graphical relationship between concentration and reducing powers of the test samples are as presented in Figure 2a and Figure 2b.

5. Conclusion

Kyllinga erecta S.; is established here to be a rich source of secondary phyto-constituents. This positions the weed as a good natural antioxidant source. It has high flavonoid content and high phenolic contents. It has high reducing power hence; its antioxidant activity is high. Support is recommended for its use as a food and medicinal flavouring agent because it is a potential health improving natural flavouring agent as it could show a beneficial effect on cardiac health.

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