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INHIBITION OF LIPID PEROXIDATION AND IN-VITRO ANTIOXIDANT CAPACITY OF AQUEOUS, ACETONE AND METHANOL LEAF EXTRACTS OF GREEN AND RED Acalypha wilkesiana Muell Arg.

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ABSTRACT

Objective: The therapeutic potency of a medicinal plant is widely implicative of its anti-oxidative power. This study investigated the phytoconstitution, phenolic contents and the antioxidant potency (in-vitro) of aqueous, acetone and methanol leaf extracts of green and red Acalypha wilkesiana (A.w.) Method: Phytochemical screening was carried out as well as investigation of the antioxidant activity of the extracts by estimating the level of phenolics, ferric reducing antioxidant power (FRAP), diphenyl-2-picryl-hydrazyl (DPPH), 2,2-azino-biz(3-ethylthianzoline-6-sulfonic acid) (ABTS), nitric oxide (NO) as well as lipid peroxidation inhibitory potencies. Results: Preliminary phytochemical screening revealed the presence of flavonoid, saponin, alkaloid and carotenoid in the extracts. The highest total phenol content was observed in the aqueous extracts of red (29.72 ± 3.39mg/g) and green A.w. (22.56 ± 0.66mg/g). Total flavonoids content and FRAP results also followed the same trend; (344.60 ± 7.01mg/g and 706.46 ± 1.04mmol/g respectively) for the red A.w. and (339.37 ± 9.50mg/g and 679.14 ± 0.45mmol/g respectively) for the green A.w. meanwhile the highest total flavonol content was observed in the methanol extract of red A.w. with a value of 213.19 ± 2.44mg/g. The highest inhibitory effect on DPPH radicals, ABTS radicals and lipid peroxidation were expressed by the aqueous extract of red Acalypha wilkesiana with IC50 values of 0.59mg/ml, 0.64mg/ml and 0.62mg/ml respectively, followed by the aqueous extract of green Acalypha wilkesiana with IC50 values of 0.60mg/ml, 0.68mg/ml and 0.78mg/ml respectively. The aqueous extracts of green and red A. w. exhibited the highest inhibition towards NO with IC50 of 0.42mg/ml and 0.43mg/ml respectively. Conclusion: Results from this study suggest that aqueous extracts of these plants (with a higher potency observed in the red) possess high antioxidant levels.

INTRODUCTION:

Plant based approach in the management of free radical-induced diseases is continually attracting scientific attentions. Various experimental reports have implicated involvement (in part or full) of free radicals such as hydrogen peroxide (H2O2), superoxide anion (O2._), hydroxyl radical (.OH), nitric oxide (NO) etc. in the pathogenesis of various diseases such as cancer, malaria, neurodegeneration etc (Gutteridge, 1994). Free radicals, being dual in role could either be beneficial as are well understood to be involved in the normal physiological functioning of the body cells at low concentration or detrimental as excessive production could mediate damage to cellular components such as DNA and RNA, lipids and proteins, triggering structural and functional deterioration of vital organs, presented as diseases (Sen et al., 2010). The antioxidant defense system of the body naturally helps to scavenge and prevent the deleterious effect of free radicals. Such antioxidants produced by the body include glutathione and enzymes (e.g. superoxide dismutase, catalase etc). Proper physiological functioning of the cellular environment is normally necessitated by a balance between free radicals and antioxidants. Should free radicals production overwhelm the body's antioxidant defense, oxidative stress is triggered, at which level the quest for external sources of antioxidants becomes imperative. Butylated hydroxytoluene, butylated hydroxyanisole and a number of synthetic antioxidants are reported to be harmful to human health (Lobo et al., 2010). It is thus scientifically advantageous to embrace the use of less or non-toxic, effective natural components with antioxidant values (Lobo et al., 2010).

Folkloric medicine practices involve the use of natural products and plants with various pharmacological actions. Medicinal advantages of these plants have been explained by researchers as being owned to their antioxidative phytoconstitutions such as phenolics and flavonoids (Sulaiman and Balachandran, 2012). Acalypha wilkesiana is one among numerous plants with antioxidant properties. It belongs to the family of Euphorbiaceae and the genus Acalypha. It is alternatively called fire dragon, copper leaf or Jacob's coat (Makoshi et al., 2016). It is an outdoor plant, native to Fiji and Islands in the south pacific but has gained availability in various regions of the world

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including America, Asia and tropics of Africa (Forcados et al., 2016). Acalypha wilkesiana reportedly possess antidiabetic and hypocholesterolemic properties (Ikewuchi and Ikewuchi, 2010), antibacterial properties (Din et al., 2013) and antioxidant properties (Anokwuru et al., 2015). Acalypha wilkesiana (Mull Arg) species is presented to nature in two colors; the green and the red (or coppery green + red splashes) given the same specific naming and both are reportedly potent medicinally. This research tends to question the colorational difference by investigating to know and compare the antioxidant power of both plants using different solvents for extraction.

MATERIALS AND METHODS

Plant Materials

Leaves of green and red Acalypha wilkesiana were collected in March, 2019 from the Engineering and Engineering technology department/campus, Olusegun Agagu University of Science and Technology (OAUSTECH), Ondo State, Nigeria and authenticated at the department of plant biology of the university.

Chemicals

2, 2' – azino - bis - (3 – ethylbenzothiazoline – 6 - sulphonic acid) (ABTS), 2, 2 – Diphenyl – 1 -picrylhydrazyl (DPPH), ferric chloride (FeCl3), thiobarbituric acid (TBA) aluminum chloride, Folin-Ciocalteu reagent, sodium carbonate (Na2CO3), potassium persulphate (K2S2O8), trichloroacetic acid (TCA), gallic acid, quercetin and ascorbic acid were products of Sigma Co. (St. Louis, MO, USA). Sulphuric acid used, methanol, acetone and butanol were of analytical grade and purchased from Merck Co. (Darmstadt, Germany).

Preparation of plant extracts

Collected leaves of Acalypha wilkesiana were washed properly and kept under shade for two weeks until dried. Leaves were then ground to powder using an electric blender and 350g immersed in 1000ml of distilled water/methanol/acetone for 48 hours at room temperature. The extracts obtained were then subjected to rotary evaporator-aided dryness, weighed and stored at 4oC for subsequent analysis. Percentage yield was calculated using the formula;

$$\text{Yield (\%)} = (W1 / W2) * 100$$

Where W1 = weight of extract residue obtained after solvent removal

W2 = weight of ground sample taken

Table 1: Percentage yield of extraction

Extracts	Aqueous Green.	Aqueous Red.	Methanolic Green.	Methanolic Red.	Acetone Green	Acetone Red
Percentage yield	13.07	13.41	10.70	11.78	12.84	11.39

Total Phenolics content Determination

Using gallic acid as standard, Folin-Ciocalteu colorimetric method was adopted in the determination of total phenol contents of the extracts as described by Yafang et al. (2011). This method relies on the transfer of electron from phenolic compound, in an alkaline medium forming a bluish coloration constituted by a phosphotungstic/phosphomolybdenum complex where the depth of the coloration is directly proportional to the concentration of the phenolic compounds. Briefly, 0.2ml of extract was mixed with 1ml of Folin-Ciocalteu reagent, 1ml of saturated sodium carbonate Na2CO3 (7.5%) was added after 4 minutes. Mixture was thereafter allowed to stand at room temperature for 120 minutes after which absorbance was read at 760 nm and total polyphenols in different extract expressed as mg of gallic acid equivalent per gram of extract (mg GAE/g).

Total Flavonoid Content Determination

Flavonoids quantification of aqueous, methanolic and acetone extracts was done by the aluminium chloride reagent (AlCl3) method as described by Ayoola et al. (2008). 1ml of extract dissolved in corresponding solvent was added to 1ml of 2% AlCl3 in methanol. Mixture was allowed to incubate at room temperature for 10 minutes and absorbance was measured at a wavelength of 430nm in a u.v. visible spectrophotometer. Total flavonoid was expressed as mg of quercetin equivalent per gram (mg QE/g).

Total Flavonol Determination

The method described by Yermakov et al. (1987) was used in the determination of the flavonol contents of the extracts. 2ml of sample in ethanol was mixed with 2ml of 2% Aluminium trichloride (AlCl3) and 6ml of 5% sodium acetate. Flavonol content was estimated from the calibration curve of quercetin prepared by mixture of 2ml of varying concentration of quercetin with 2ml of 2% AlCl3 and 6ml of sodium acetate (C2H3NaO2). Mixtures stood for 150 minutes incubation time at 20oC and absorbance was then read at 440nm.

Ferric Reducing Antioxidant Power (FRAP) estimation

FRAP assay was carried out using a modified method of Benzie and Strain (1996). This assay method leverages on the ability of the extract to reduce ferric tripyridyltriazine (Fe (III) - TPTZ) complex to ferrous tripyridyltriazine (Fe (II) - TPTZ) at a low pH. A blue coloration of produced Fe (II) - TPTZ was read at 593nm. FeSO4 was used in generating calibration curve and ascorbic acid was reference control. Briefly, 1.5ml of newly prepared FRAP solution [25ml of 300 mmol acetate buffer pH 3.6, 2.5ml of 10 mmol 2,4,6 - tripyridyltriazine (TPTZ) in 40 mmol HCl and 2.5ml of 20 mmol ferric chloride (FeCl3.6H2O) solution] and thoroughly mixed with 1ml of the extract. Mixtures were incubated for half an hour at 37oC and absorbance read at 593nm. FRAP values were expressed as mmol Fe2SO4 equivalent per gram of extract (mmol/g)

Diphenyl – 2 – picryl - hydrazyl (DPPH) Radical Scavenging Activity

2, 2 - diphenyl-1-picryl-hydrazyl (DPPH) is a characteristic stable free radical owned to delocalization of spare electron over the molecule giving rise to a deep violet color. Any substrate (AH) capable of donating a hydrogen atom to DPPH will yield a reduced form, with loss of the characteristic violet color. Assay of extracts' ability to scavenge DPPH was carried out according to the method of Gyamfi et al. (1999). Briefly 50ul of different concentrations (0.25, 0.50, 0.75 and 1.0mg/ml) was mixed with DPPH solution (2.4mg in 100ml methanol). Mixture was then allowed to stand for 30 minutes at room temperature. Absorbance (of Control and Samples) was measured at wavelength 517nm using gallic acid as standard. Inhibition of DPPH in percentage was calculated thus;

Percentage inhibition of DPPH = (Ac – As) / Ac * 100

Where Ac = Absorbance of Control [DPPH + methanol]

As = Absorbance of sample [DPPH + extract/ standard]

Inhibitory concentration of 50% DPPH radical (IC50) was calculated as extracts' effective concentration scavenging half population of DPPH free radical

2, 2'- azino–bis (3 – ethylbenzothiazoline – 6 – sulfonic acid) [ABTS] Radical Scavenging Power

The assay method of Re et al. (1999) was employed in determining the ABTS free radical scavenging ability of the extracts. Reacting ABTS stock solution (7mmol) with potassium persulfate - K2S2O8 (2.45mmol, final concentration) in the dark for 16 hours was used in generating ABTS radical cations (ABTS.+) and absorbance was adjusted to 0.700 with ethanol at 734nm. Ascorbic acid was used as standard. 0.2ml of varying concentrations (0.25, 0.50, 0.75 and 1mg/ml) of extracts in DMSO were added to 2ml of ABTS.+ solution, mixture was allowed to stand for 15 minutes after which absorbance was read at 734nm. Results were expressed as percentage inhibition of ABTS using the formula

% ABTS inhibition = (Ac – As) / Ac *100

Where, Ac = Absorbance of control

As = Absorbance of sample

Inhibitory concentration of 50% ABTS radical (IC50) was calculated as extracts' effective concentration inhibiting half population of ABTS free radical

Nitric Oxide Scavenging Ability Determination

Sodium nitroprusside is understood to decompose in aqueous solution at physiological pH to yield nitric oxide (NO). Interaction of nitric oxide with oxygen leads to production of nitrite ion which is normally quantified using Griess reagent. Scavengers of nitric oxide do compete with O2 consequently leading to reduced generation of nitrite ion. Briefly 10 mmol sodium nitroprusside in phosphate buffered saline was mixed with varying concentrations (0.25, 0.50, 0.75 and 1.0mg/ml) of the extracts and incubated for 150 minutes at room temperature. Assay control contained same amount of reaction mixture but with distilled water in place of extract. Following the incubation time, 0.5ml of griess reagent [1% sulfanilide, 2% H3PO4 and 0.1% N (1-naphthyl ethylene diamine dihydrochloride)] was introduced and absorbance reading was done at 546nm wavelength. Ascorbic acid was used as standard and result was calculated as

% NO Inhibition = (Ac – As) / Ac * 100

Where Ac = Absorbance of control

As = Absorbance of sample

Inhibitory concentration (IC50) was also calculated as extracts' effective concentration inhibiting half population of NO.

Inhibition of Lipid Peroxidation assay

Egg yolk homogenate was the lipid rich medium in the modified thiobarbituric acid reactive species (TBARS) assay adopted here to measure the lipid peroxide formed as described by Ruberto et al. (2000). 500ul of 10% v/v egg yolk homogenate was added to 100ul of sample, content volume was made up to 1ml with distilled water. The reaction mixture received an addition of 0.05ml of Fe2SO4 and incubated at 37oC for 30 minutes. 1.5ml of acetic acid was added after which 1.5ml TBA in SDS was also introduced. The resulting mixture was vortex mixed and heated for 60 minutes at 95oC. It was allowed to cool and 5ml of Butanol was added and entire mixture centrifuged for 10 minutes at 3000 revmin-1. Absorbance of the supernatant was measured at 532nm and inhibition (in percentage) was calculated with the formula;

% inhibition of lipid peroxidation = (Ac – As) / Ac * 100

Where; Ac = Absorbance of control

As = Absorbance of sample

Inhibitory concentration (IC50) was calculated as extracts' effective concentration inhibiting lipid peroxidation by half.

Phytochemical Screening

Qualitative phytochemical screening for the presence of alkaloids, saponins, tannins, anthraquinones, flavonoids, carotenoids, cardiac glycosides, steroids etc were carried out using standard methods of Sofowora (1993) and Trease and Evans (2002).

Statistical Analysis

Assays were carried out in triplicates and results expressed as mean ± standard deviation. Data were analyzed using one way ANOVA. Differences in mean values were ascertained by Duncan multiple range test on graph pad prism (Graph pad software Inc. San Diego, USA). Values were considered statistically significant at $P < 0.05$. IC50 values were calculated following extrapolation from linear regression.

Results:

Table 2: Phytoconstituents of aqueous, acetone and methanol extracts of Acalypha wilkesiana

Extracts / PHYTOCHEMICALS	Aq. green *A. w.*	Aq. red *A. w.*	Meth. green *A. w.*	Meth. red *A. w.*	Acet. green *A. w.*	Acet. red *A. w.*
saponin	+	+	+	+	+	+
Tannin	+	+	+	+	+	-
Anthraquinone	-	-	-	-	-	-
Cardiac glycoside	-	+	+	+	+	-
Steroid	+	+	+	+	-	-
Carotenoid	+	+	+	+	+	+
Terpenoid	+	+	+	+	-	+
Flavonoid	+	+	+	+	+	+
Alkaloid	+	+	+	+	+	+

+ = Present, - = Absent

Table 3: Polyphenolic contents and Ferric reducing antioxidant power of Aqueous, acetone and methanol leaf extracts of green and red Acalypha wilkesiana (A. w.)

SAMPLE -(extract)	Total Phenol (mgGAE /gof extract)	Total flavonoid (mgQE /gof extract)	Total flavonol (mgQE /gof extract)	FRAP (mmol/gof extract)
Aqueous Green *A. w.*	22.56 ± 0.60^{b}	339.37 ± 9.50^{b}	207.10 ± 9.53^{b}	679.14 ± 0.45^{b}
Aqueous Red *A. w.*	29.72 ± 3.39^{a}	344.60 ± 7.01^{a}	168.26 ± 7.54^{c}	706.46 ± 1.04^{a}
Methanol Green *A. w.*	14.10 ± 2.17^{e}	252.52 ± 3.73^{d}	196.08 ± 5.53^{b}	611.90 ± 7.09^{c}
Methanol Red *A. w.*	15.58 ± 1.53^{e}	271.99 ± 0.73^{c}	213.19 ± 2.44^{a}	314.95 ± 5.08^{e}
Acetone Green *A. w.*	8.52 ± 7.01^{d}	123.88 ± 5.58^{f}	112.04 ± 8.27^{f}	292.09 ±9.39^{f}
Acetone Red *A. w.*	15.17 ± 5.78^{e}	150.90 ± 1.31^{e}	172.63 ± 2.05^{d}	399.53 ± 2.46^{d}

Values are expressed as mean ± standard deviation ± SD. (n = 3)

Values (in a column) with different superscript are significantly different ($p < 0.05$)

Figure 1: Percentage scavenging ability of DPPH radical of aqueous, acetone and methanol leaf extracts of Acalypha wilkesiana. (Values are expressed as mean ± SD, n = 3)

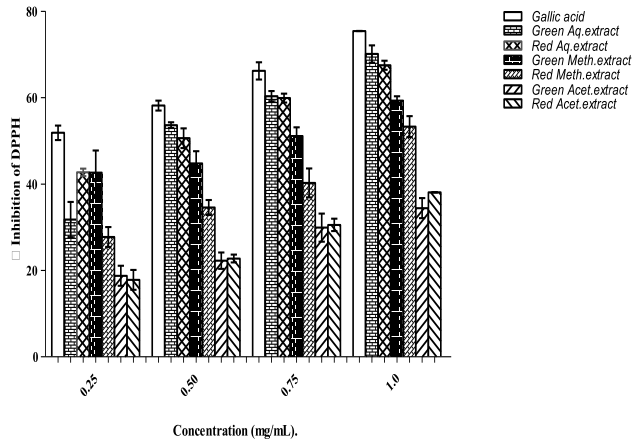

Figure 1: Percentage scavenging ability of DPPH radical of aqueous, acetone and methanol leaf extracts of *Acalypha wilkesiana* (Values are expressed as mean ± SD, n = 3)

Table 4: IC50 values of DPPH inhibition of aqueous, acetone and methanol leaf extracts of Acalypha wilkesiana.

SAMPLE – (extract)	IC $_{50}$ (mg/ml)
Aqueous green *A. w.*	0.60
Aqueous red *A. w.*	0.59
Methanol green *A. w.*	0.70
Methanol red *A. w.*	0.90
Acetone green *A. w.*	1.40
Acetone red *A. w.*	1.29

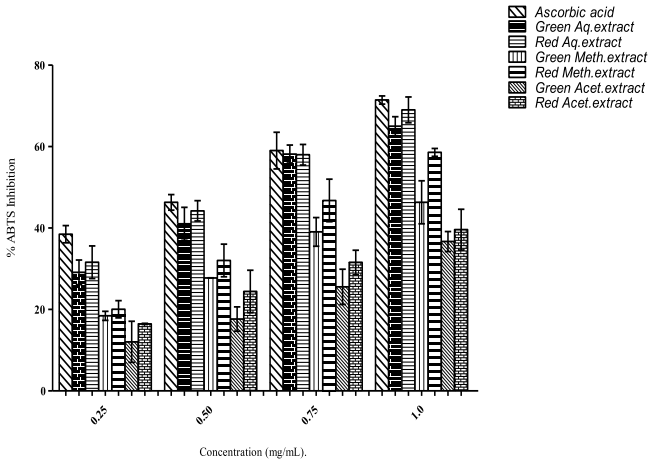

Figure 2: Percentage inhibition of ABTS radical of aqueous, acetone and methanol leaf extracts of *Acalypha wilkesiana* (Values are expressed as mean ± SD, n = 3)

Table 5: IC50 values of ABTS inhibition of aqueous, acetone and methanol leaf extracts of Acalypha wilkesiana.

SAMPLE – (extract)	IC $_{50}$ (mg/ml)
Aqueous green *A. w.*	0.68
Aqueous red *A. w.*	0.64
Methanol green *A. w.*	1.02
Methanol red *A. w.*	0.82
Acetone green *A. w.*	1.41
Acetone red *A. w.*	1.23

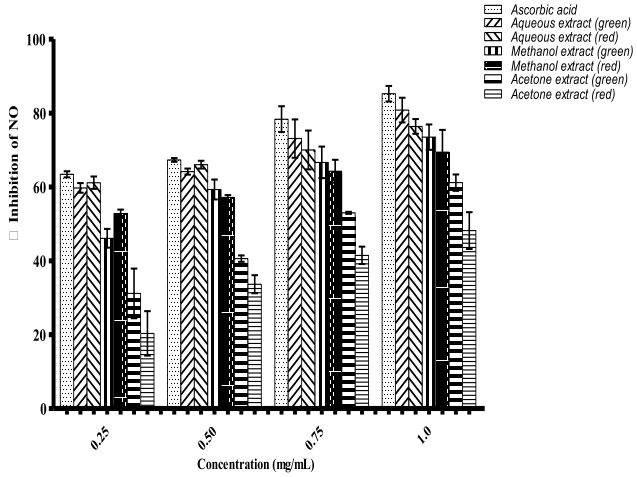

Figure 3: Percentage inhibition of NO of aqueous, acetone and methanol leaf extracts of *Acalypha wilkesiana* (Values are expressed as mean ± SD, n = 3)

Table 6: IC50 values of NO inhibition of aqueous, acetone and methanol leaf extracts of Acalypha wilkesiana.

SAMPLE – (extract)	IC $_{50}$ (mg/ml)
Aqueous green *A. w.*	0.42
Aqueous red *A. w.*	0.43
Methanol green *A. w.*	0.51
Methanol red *A. w.*	0.52
Acetone green *A. w.*	0.72
Acetone red *A. w.*	0.97

Table 7: Percentage inhibition of lipid peroxidation of aqueous, acetone and methanol leaf extracts of *Acalypha wilkesiana*

CONC. (mg/mL) / SAMPLE (extract)	0.25 (% inhibition)	0.50 (% inhibition)	0.75 (% inhibition)	0.10 (% inhibition)
Gallic acid	33.66 ± 4.37	49.95 ± 1.36	56.01 ± 3.22	69.20 ± 0.91
Aqueous green *A. w.*	22.66 ± 2.93	31.48 ± 1.61	47.51 ± 2.05	63.45 ± 2.61
Aqueous red *A. w.*	35.82 ± 0.49	53.33 ± 3.72	57.40 ± 5.02	68.12 ± 3.30
Methanol green *A. w.*	19.89 ± 0.80	39.42 ± 3.10	45.01 ± 3.31	55.43 ± 4.02
Methanol red *A. w.*	20.94 ± 1.19	29.83 ± 1.29	38.12 ± 2.00	47.55 ± 4.24
Acetone green *A. w.*	12.23 ± 0.44	25.88 ± 0.65	33.09 ± 2.71	41.62 ± 3.17
Acetone red *A. w.*	16.15 ± 2.29	24.63 ± 4.10	29.96 ± 0.01	39.05 ± 0.59

Values are expressed as mean ± standard deviation = SD. (n = 3)

Table 8: IC50 values of "inhibition of lipid peroxidation" of aqueous, acetone and methanol leaf extracts of Acalypha wilkesiana.

SAMPLE – (extract)	IC $_{50}$ (mg/ml)
Aqueous green *A. w.*	0.78
Aqueous red *A. w.*	0.62
Methanol green *A. w.*	0.82
Methanol red *A. w.*	1.01
Acetone green *A. w.*	1.31
Acetone red *A. w.*	1.24

DISCUSSION

Scientific investigations into the fundamentals of medicinal plants have revealed close proximity between their therapeutic potencies and antioxidative phytoconstituents. Implicated phytochemicals, generally reflecting antioxidant activity of plants are polyphenols and carotenoids (Jovanovic et al., 1994). Acalypha wilkesiana are reportedly antidiabetic

and hypocholesterolemic (Ikewuchi and Ikewuchi, 2010), antibacterial (Gotep et al., 2010), hepatoprotective at a safe dose of 100mg/kg (Ikewuchi et al., 2011; Ogbuehi et al., 2014), antimalarial (Ogbuehi et al., 2014) etc. These documented efficacies are at least in part due to the rich antioxidant phytochemicals reportedly present in Acalypha wilkesiana (Ogbuehi et al., 2014) with no exceptions to flavonoid and carotenoid. This was supportedly revealed by our findings of the presence of flavonoid and carotenoid as part of the major phytochemicals in the tested extracts (table 2).

We investigated and compared the antioxidant potency of the green and red Acalypha wilkesiana species using established methods. We understand that Acalypha wilkesiana (Muell Arg) species is presented as green colored and red colored (or coppery green with prominent red splashes) and both have been individually investigated for their therapeutic values, however, to the best of our knowledge there is yet to be a comparative antioxidant report on them.

The antioxidant power of a plant is directly related to its phenolic content due to the OH group, facilitating the donation of hydrogen to unstable free radicals (Ayoola et al., 2008; Hegazy and Ibrahim, 2012). We thus quantified the total phenolics present in the aqueous, methanol and acetone extracted leaves of both Acalypha wilkesiana plants. The aqueous extracts of both plants had the highest total phenolic content with a significantly higher ($p < 0.05$) value in the red Acalypha wilkesiana. Methanol extracts were next, with same result trend as observed in the aqueous extracts. Flavonoid content was also consequently highest in the aqueous extract followed by the methanol extract and acetone extracts - the least, with the red extracts having higher values than their counterparts. Flavonoids are active water soluble antioxidants (Flora, 2009; Oseni & Okoye, 2013), inhibiting peroxidation and mopping up reactive oxygen species, its hydrophilicity could possibly be the reason flavonoids was most concentrated in the aqueous extracts. Contrastingly the flavonol content of the methanol extract of red and green Acalypha wilkesiana was highest of the six extracts, suggesting that the major flavonoid content of the aqueous extracts might possibly not be of the flavonol origin as opposed to the case in the methanol extracts (table 3).

We investigated the Ferric Reducing Antioxidant Power (FRAP) of the extracts. A good antioxidant is believed to sufficiently reduce Fe3+ to Fe2+ due to the ability of phenolic compound to donate hydrogen. The FRAP value of the extracts were significantly different ($p<0.05$) from one another with the aqueous extracts expressing the highest value, followed by the methanol extracts. In each case (aqueous and acetone), the red showed a higher potency in reduction of Fe3+ except for methanol extracts where the green Acalypha wilkesiana had a higher value (table 3)

DPPH radical scavenging ability is one of the scientifically adopted standards in screening the antioxidant strength of samples (Lee et al., 2003) as unstable DPPH radicals accept electron from donor to become stable. DPPH radicals were presented against four different concentrations of each extracts of the two plants. Values were expressed as percentage inhibition (figure 1) and inhibitory strength of extracts were reflected by their IC50 values. The aqueous extracts of the red and green Acalypha wilkesiana showed the highest inhibition of DPPH with IC50 values of 0.59 mg/ml and 0.60 mg/ml respectively (table 4), the methanol extracts showed the second highest inhibitory potency and the acetone extracts showed the least efficacy towards DPPH having the highest IC50 values (1.29 mg/ml and 1.40 mg/ml for the green and red respectively).

A more flexible free radical scavenging model allowing for screening of both non polar and polar samples (Re et al, 1999; Oboh & Omorege, 2011) was also adopted in our antioxidant experimentation; the ABTS (2, 2-azinobis (3-ethylbenzothiazoline-6-sulfonate) assay. All samples were further investigated for their scavenging abilities towards ABTS radicals and result obtained for each extract was concentration dependent with the aqueous extract being comparable with the standard Ascorbic acid (figure 2). The efficacy of the extracts based on their IC50 values was as follows; (aqueous red > aqueous green) > (methanol red > methanol green) > (acetone red > acetone green) (table 5). In each case, red Acalypha wilkesiana proved to be more promising. Efficacies of the extracts correlated well with the amount of phenolics expressed.

Nitric oxide, an important signaling molecule transmitting signals to cells in the immune and nervous systems of the body, owned to its minute size - enhancing its permeability through membranes of cells facilitating its signaling function and the possession of a free radical confers higher reactivity on it than other signaling molecules. The body synthesizes nitric oxide through the help of the enzyme nitric oxide synthase from L-arginine, via metabolism to Citrulline and via a five electron oxidative reaction (Knowles et al., 1989). NO is also generated from macrophages, neurons, as well as endothelial cells, regulating a number of processes physiologically. Production in excess of NO is associated with modifications (structurally and functionally) of cell components. Anti radical agents are capable of quenching nitric oxide generated experimentally from nitroprusside in aqueous solutions via a competition with oxygen. Aqueous extracts of red and green Acalypha wilkesiana showed promising results towards nitric oxide with the lowest IC50 (0.43mg/ml and 0.42mg/ml respectively), followed by methanol extract with the acetone extracts having the highest IC50 values (table 6) and least inhibition of NO.

The inhibition of lipid peroxidation effect of the extracts was also investigated. A good antioxidant specie should capable donate electron to unstable free radicals inhibiting them from stealing free electrons from lipids in the cell membranes and consequently preventing oxidative degradation of lipids. Polyunsaturated fatty acids from egg yolk react with oxygen to form malondialdehyde which react with thiobarbituric acid producing a pink coloration. Our extracts were tested against lipid peroxidation, and concentrations capable of inhibiting lipid oxidation by 50% was calculated in mg/ml. Aqueous extracts showed the best potency against lipid peroxidation with IC50 of 0.62 mg/ml and 0.78 mg/ml for the red and green plant respectively. Methanol extracts displayed the second best efficacy and acetone extracts the least (table 8) with the red extracts showing better results for aqueous and acetone extracts.

CONCLUSION

The results of the lipid peroxidation assay showed that Acalypha wilkesiana contain potent inhibitory agents especially the aqueous and methanolic extracts, which is in conformity with the in-vivo reports of Ogbuehi et al. 2014. From the results of total phenolic and anti radical assays, the aqueous extracts of green and red Acalypha wilkesiana obviously proved to be most potent antioxidatively with a higher efficacy from the red plant. We thus suggest that aqueous extract of green and red Acalypha wilkesiana can be adopted in the management of free radical induced diseases.

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