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Full Length Research Paper

Comparative studies on the *in vitro* antioxidant properties of methanolic and hydro-ethanolic leafy extracts from eight edible leafy vegetables of Ghana

Joseph Francis Morrison* and Sylvester Kwadwo Twumasi

Department of Chemistry, Kwame Nkrumah University of Science and Technology, Kumasi-Ghana.

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Epidemiological studies indicate that consumption of fruits and vegetables has the ability to inhibit the damaging activities of free radicals in the human body. Eight edible leafy vegetables of Ghana namely: *Xanthosoma sagittifolium*, *Hibiscus Sabdariffa*, *Solanum macrocarpon*, *Talinum triangulare*, *Corchorus olitorius, Laportea aestuans, Ipomoea batatas,* and *Amaranthus cruentus* were assessed for their antioxidant properties. The experimental results indicate that hydro-ethanol is an effective solvent for extracting the phytoconstituents of the leafy vegetables. The total antioxidant capacity (TAC) and total phenol content (TPC) in the methanol extracts (METE) and hydro-ethanol extracts (HETE) from the selected leafy vegetables within the measured concentration range (0.1 - 3.0 mg/ml) decreased in the order *X. sagittifolium* > *I. batatas* > *L. aestuans* > *T. Triangulare* > *H. Sabdariffa* > *C. olitorius* > *S. macrocarpon* > *A. cruentus*. A high and positive correlation was observed between TPC and TAC in both the METE and HETE from all the selected leafy vegetables. The selected leafy vegetables showed strong antioxidant properties with respect to their free radical scavenging activity and Fe^{3+} reduction ability with hydro-ethanol extracts indicating higher antioxidant potential compared with their respective methanol extracts.

Key words: Hydro-ethanol extract, 1,1-diphenyl-2-picrylhydrazyl free radical scavenging activity, total antioxidant capacity, reducing power, edible leafy vegetables.

INTRODUCTION

Free radicals find their way into the human body via metabolic pathways within body tissues and also from external sources such as food, drugs and pollution from the environment (Miller and Britigan, 1997). There is strong evidence that suggests that free radicals, such as superoxide radical ($^{\cdot}O_2^-$) and hydroxyl radical ($^{\cdot}OH^-$) and non-free radical species, such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) within the human body facilitate cellular injury, aging, development of neurodegenerative and cardiovascular diseases (Ames et al., 1993; Stadtman, 1992, Cadenas and Davies, 2000; De la Fuente and Victor, 2000; Pinzino et al., 1999; Stanner et al., 2002).

Consumption of fruits and vegetables has proven to substantially reduce the risk of cardiovascular diseases, cancers (Gerber et al., 2002; Kris-Etherton et al., 2002; Serafini et al., 2002) and neurodegenerative diseases, including Parkinson's and Alzheimer's diseases (Di Matteo and Esposito, 2003; Ames et al., 1993).

In this study, comparative studies on the *in vitro* antioxidant activity on methanolic and hydro-ethanolic leaf extracts from eight indigenous edible leafy vegetables of Ghana were investigated to assess their antioxidant properties in different antioxidant property determination assays. The selected leafy vegetables are *Ipomoea batatas*, *Xanthosoma sagittifolium*, *Hibiscus Sabdariffa*, *Solanum macrocarpon*, *Laportea aestuans*, *Talinum triangulare*,

*Corresponding author. E-mail: akaimorrison@yahoo.com. Tel: +1 662 617 5158.

Abbreviations: TAC, Total antioxidant capacity; **TPC,** total phenol content; **METE,** methanol extracts; **HETE,** hydro-ethanol extracts; **AAE,** ascorbic acid equivalent; **F-C,** Folin-Ciocalteu; **TAE,** tannic acid equivalent; **RSA,** radical scavenging activity.

Corchorus olitorius and *Amaranthus cruentus* or *Amaranthus hybridus*.

The selected leafy vegetables are reported to contain high levels of micronutrients and vitamins; have medicinal uses, and are mostly used in soup and stew preparations in most African countries (Dokosi, 1998). Recent studies on some of the selected leafy vegetables suggest that these leafy vegetables possess antioxidants (Aiyeloja and Bello, 2006; Akindahunsi and Salawu, 2005a,b; Odukoya et al., 2007; Salawu et al., 2006). The present study is necessitated by lack of extensive research information on the selected leafy vegetables, especially in Ghana.

MATERIALS AND METHODS

Chemical reagents

The entire chemical reagents used were of analytical-reagent grade.

Sampling of the selected leafy vegetables

The shoots of the selected leafy vegetables except two were harvested from vegetable farms at Emena, a suburb of Kumasi from September 2008 to October 2008. However, within the same sampling period of the study, the shoots of *L. aestuans* were collected from a weed-infested area near Ayeduase Chief's palace, and that of *I. batatas* from Kumawu-Bodomase – all suburbs of Kumasi. The identities of the selected leafy vegetables were then verified at the Department of Horticulture, KNUST, Kumasi, Ghana.

Preparation of vegetable samples

The freshly cut leaves of the vegetable samples were washed with distilled water and air-dried to constant weight at ambient temperature for 2 months after which the leaves were pounded into powdered form (using mortar), labeled and kept in the refrigerator for future analyses.

Extraction

50 g of the air-dried powdered leaves of the vegetable samples were extracted with 500 ml methanol (99%), 500 ml distilled water-ethanol (98%) mixture (1:1) for 10 h using Soxhlet apparatus. The extracts were concentrated in a rotary evaporator apparatus (BUCHI Rotavapor, R-144) at approximately 60°C. The concentrated extracts were kept in a desiccator until analyses. The percentage yields of the samples were calculated.

Determination of total antioxidant capacity (TAC)

The total antioxidant capacity was evaluated using the method described by Prieto et al. (1999). Ascorbic acid was used as the standard antioxidant drug. 3 ml of the extract/standard drug (0.1, 0.3, 1 and 3 mg/ml) was placed in a test tube. 0.3 ml of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was then added and the resulting mixture was incubated at 95°C for 90 min. After the mixture has cooled to room temperature, the absorbance of each solution was measured in triplicate using the UV-visible spectrophotometer (LKB Biochrom, Cambridge, Cambridge, England, Model 4050) at 695 nm against a blank. The total antioxidant capacity was expressed as ascorbic acid equivalent (AAE) using the GraphPad Prism for Windows version 4.02 (GraphPad Software, San Diego, CA, USA).

Determination of total phenol content (TPC)

The content of total phenolic compounds in the leaf extracts from the vegetable samples (0.1, 0.3, 1.0 and 3.0 mg/ml) was quantitatively determined by colorimetric assay using Folin-Ciocalteu's (F-C) reagent (Singleton, 1977) with slight modifications. Tannic acid (0.01, 0.03, 0.1 and 0.3 mg/ml) was used as the reference drug. The leaf extract (1 ml) was added to 1 ml of F-C reagent (diluted five folds in distilled water) in a test tube. The content of the test tube was then mixed and allowed to stand for five minutes at 25°C in an incubator (Gallenkamp model IH, UK). 1 ml of 2% sodium bicarbonate solution was added to the mixture. The reaction mixture was then incubated at 25°C for 2 h and then centrifuged at 3000 rpm for 10 min to get a clear supernatant. The absorbance of the supernatant was then measured at 760 nm using the UV-visible spectrophotometer (LKB Biochrom, Cambridge, Cambridge, England, Model 4050). Distilled water (1 ml) was added to 1 ml F-C reagent (diluted five folds in distilled water) processed in the same way as done for the test leaf extracts and reference drug and used as blank. The measurements were done in triplicate. The content of total phenolic compounds was expressed as tannic acid equivalent (TAE) using the GraphPad Prism for Windows version 4.02 (GraphPad Software, San Diego, CA, USA).

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

Radical scavenging activity (RSA) of the extracts from the vegetable samples against stable DPPH• radical was measured using the method of Govindarajan et al. (2003) with slight modifications. The RSA of the leaf extract (0.1, 0.3, 1.0, 3.0 mg/ml in methanol) was compared with the RSA of n-propyl gallate (0.01, 0.03, 0.1, 0.3 mg/ml in methanol), a reference free radical scavenger. Briefly, 10 ml of the leaf extract was centrifuged at 3000 rpm using a centrifuge (Sanyo MSE, MISTRAL 3000E, UK) for 10 min and the supernatant collected. The supernatant of the extract (1 ml) was added to 3 ml methanolic solution of DPPH (20 mg/l) in a test tube. The reaction mixture was kept at 25°C for 1 h in an incubator (Gallenkamp model IH, UK). The absorbance of the residual DPPH solution was determined at 517 nm in a UV-visible spectrophotometer (LKB Biochrom, Cambridge, Cambridge, England, Model 4050). Methanol (1 ml) was added to 3 ml DPPH solution, incubated at 25°C for 1 h and used as control. Methanol was used as the blank. The measurements were done in triplicate. The results were expressed as %RSA against concentration and the EC_{50} determined.

Determination of reducing power

The reducing potential of the extracts (0.1, 0.3, 1.0 and 3.0 mg/ml in methanol) was determined using the method described by Oyaizu (1986), with slight modifications using n-propyl gallate as a reference drug. The extract/drug (1 ml) was mixed with 2.5 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide solution in a test tube. The mixture was incubated at 50°C for 20 min. Following this, 1.5 ml of 10% trichloroacetic acid solution was added to the incubated mixture and centrifuged at 3000 rpm for 10 min using the centrifuge (Sanyo MSE, MISTRAL 3000E, UK). 2.5 ml of the supernatant was mixed with 2.5 ml

Table 1. Methanolic and hydro-ethanolic extraction yields (% $^{w}/_{w}$) of the selected leafy vegetables.

Selected vegetables	Yield (%, w/w)	
	METE extract	HETE extract
X. Sagittifolium (XS)	8.1	8.7
I. batatas (IB)	4.4	7.5
L. aestuans (LA)	4.2	5.9
T. triangulare (TT)	7.3	8.3
H.sabdariffa (HS)	6.2	7.5
C. olitorius (CO)	6.7	7.1
S. macrocarpon (SM)	7.4	10.3
A. cruentus (AC)	6.3	9.4

METE, Methanolic extract; HETE, hydro-ethanolic extract

distilled water and 0.5 ml of 0.1% ferric chloride solution [$FeCl_3$ (aq.)] in a test tube. The absorbance was then measured at 700 nm using the UV-visible spectrophotometer (LKB Biochrom, Cambridge, Cambridge, England, Model 4050). Distilled water was used in place of the test drug/extract and used as the blank. The absorbance measurements were done in triplicate. Data was presented as concentration-absorbance curves and the EC_{50} was computed using the GraphPad Prism for Windows version 4.02 (GraphPad Software, San Diego, CA, USA).

Statistical analysis

The EC_{50} (the concentration of agonist that gives a response half way between bottom and top) of the extracts and reference antioxidant compounds were analyzed using an iterative computer least squares method, with the following nonlinear regression (three-parameter logistic) equation:

$$Y = \frac{a + (b - a)}{(1 + 10^{(Log\,EC50 - X)})}$$

Where, *X* is the logarithm of dose and *Y* is the response. *Y* starts at *a* (the bottom) and goes to *b* (the top) with a sigmoid shape. All the experimental data were analyzed statistically by one-way analysis of variance (ANOVA) and "Bonferroni's Multiple Comparison Test" at 95% confidence interval using the software, GraphPad Prism for Windows version 4.02 (GraphPad Software, San Diego, CA, USA). Correlation coefficient (r) was used to determine the relationship between two variables, TAE and AAE. All the points on graphical representation of experimental values were expressed as mean±sem. Differences in mean between paired results were accepted as significant at $P < 0.05$.

RESULTS AND DISCUSSION

Extraction yields

The percentage yields of extracts from the selected leafy vegetables using methanol and hydro-ethanol (mixture) as solvent systems for extraction are presented in Table 1. The extraction yields (Table 1) of the selected leafy vegetables shows that hydro-ethanol extraction was very effective since the percentage yields of hydro-ethanol extraction on all the leafy vegetable samples were higher than their respective methanol extraction yields. This marginal difference could be due to the polarity differences between the two solvent systems.

Determination of TAC and TPC

The results of the total antioxidant capacity (expressed as AAE) and total phenol content (expressed as TAE) of the extracts from the selected leafy vegetables are shown in Figures 1 and 2. The TAC and TPC in the methanol extracts (METE) and hydro-ethanol extracts (HETE) from the selected leafy vegetables within the measured concentration range (0.1 - 3.0 mg/ml) decreased in the order *X. sagittifolium* > *I. batatas* > *L. aestuans* > *T. Triangulare* > *H. Sabdariffa* > *C. olitorius*> *S. macrocarpon* > *A. cruentus* as shown in Figures 1 and 2.

In terms of TAC, Odukoya et al. (2007) reported the following order, *C. olitorius* (153.63 mg AA 100 g^{-1}) > *T. Triangular* (116.35 mg AA 100 g^{-1}) > *A. cruentus* (52.17 mg AA 100 g^{-1}) > *S. macrocarpon* (38.11 mg AA 100 g^{-1}). Salawu et al. (2006) also reported total antioxidant as gallic acid equivalent in the following order, *S. macrocarpon* (1.60 mg l^{-1}) > *C. olitorius* (1.10 mg l^{-1}) > *A. cruentus* (0.55 mg l^{-1}) >*T. Triangular* (0.20 mg l^{-1}).

From Table 2, the highest ascorbic acid equivalent was measured for *X. sagittifolium* 0.488 and 0.681 mg AAE ml^{-1} on the METE and HETE, respectively, while *A. cruentus* showed the least AAE, 0.228 and 0.312 mg/ml on its METE and HETE, respectively. Total antioxidant capacity is defined as a measure of the ability of substances extracted from food or herbal matrix to delay oxidation process in a controlled system (Miller and Rice-Evans, 1997; Pellegrini et al., 2003). Diets with high vitamin C (ascorbic acid) content are reported to have the capability of improving the pulmonary function and reduce the risk of cancer (Block and Menkes, 1989; Percival, 1998). Vitamin C is also reported to have neutralizing effect on hydrogen peroxide, hydroxyl and superoxide radicals (Percival, 1998). The appreciable levels of ascorbic acid equivalent of the extracts from the selected vegetables suggest their antiradical potential.

With respect to TAE, Odukoya et al. (2007) reported the following order, *C. olitorius* (503.72 mg TAE 100 g^{-1}) > *A. cruentus* (406.33 mg TAE 100 g^{-1}) > *S. macrocarpon* (190.07 mg TAE 100 g^{-1}) > *T. triangular* (21.83 mg TAE 100 g^{-1}). Salawu et al. (2006) also reported total flavonoid as quercetin equivalent in the following order, *S. macrocarpon* (3.08 mg l^{-1}) > *C. olitorius* (1.24 mg l^{-1}) > *A. cruentus* (0.33 mg l^{-1}) >*T. Triangular* (0.12 mg l^{-1}). Moreover, from Table 2, *X. sagittifolium* had the highest phenol content (0.706 and 0.485 mg TAE ml^{-1}) for the HETE and METE respectively while *A. cruentus* had the least phenol content (0.339 and 0.238 mg TAE ml^{-1}) for the HETE and METE, respectively. Studies carried out by

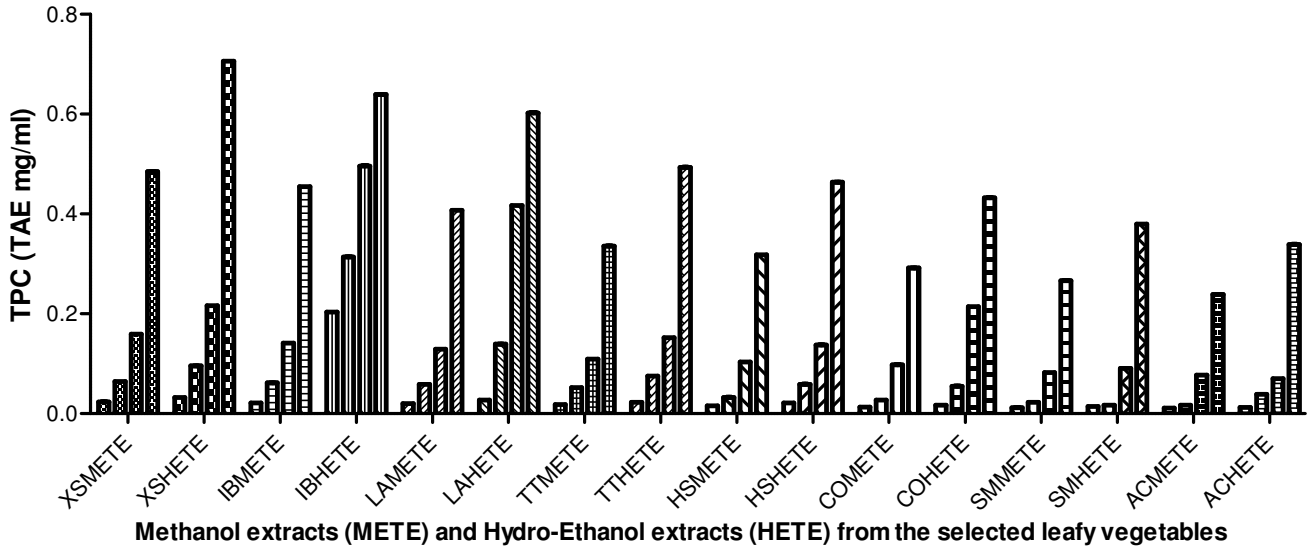


Figure 1. TAE (mg/ml) of extracts from the selected vegetables. XS, *Xanthosoma sagittifolium*; HS, *Hibiscus Sabdariffa*; SM, *Solanum macrocarpon*; TT, *Talinum triangulare*; CO, *Corchorus* olitorius; LA, *Laportea aestuans*; IB, *Ipomoea batatas*; and AC, *Amaranthus cruentus.*

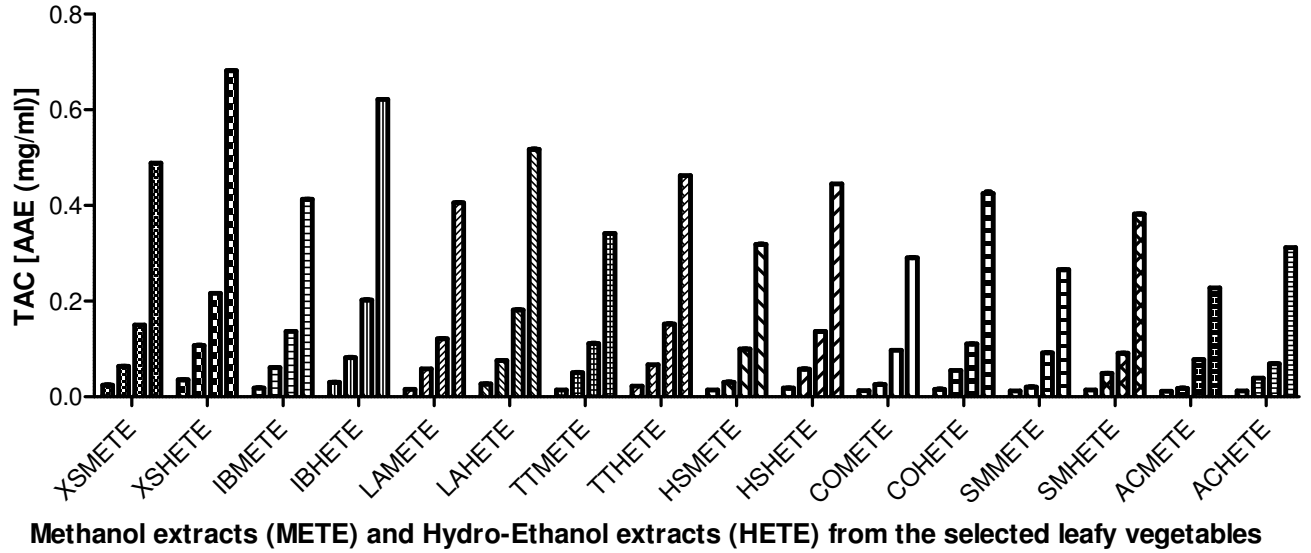


Figure 2. AAE (mg/ml) of extracts from the selected vegetables. XS, *Xanthosoma sagittifolium*; HS, *Hibiscus Sabdariffa*; SM, *Solanum macrocarpon*; TT, *Talinum triangulare*; CO, *Corchorus* olitorius; LA, *Laportea aestuans*; IB, *Ipomoea batatas*; and AC, *Amaranthus cruentus.*

Manach et al. (2004) and Rice-Evans et al. (1996) on dietary medicinal plants and edible leafy vegetables showed that phenolic compounds possess a special ability to inhibit oxidative stress since phenolic compounds readily undergo electron-donation reactions with reactive oxygen species. Dykes and Rooney (2007) also reported the ability of phenolic compounds to boost the immune system. The experimental results therefore suggest that the selected leafy vegetables possess antioxidant ability and can be used as dietary supplements.

Correlation between TPC and TAC

A high and positive correlation was observed between TPC and TAC in both the METE and HETE from all the selected leafy vegetables as shown in Figures 3a- p. The positive correlation between the TPC and TAC suggests that the phyto-constituents responsible for the total phenol content may also be responsible for the total antioxidant capacity of the vegetable extracts. Moreover, the TAE in all the selected leafy vegetables were higher

Table 2. AAE and TAE of METE and HETE from the selected leafy vegetables (3.0 mg/ml)expressed as Mean±sem.

Methanol extract (METE)	TPC expressed as TAE (mg/ml)	TAC expressed as AAE (mg/ml)
X. Sagittifolium	0.485±0.105	0.488±0.105
I. batatas	0.455±0.098	0.412±0.089
L. aestuans	0.407±0.087	0.406±0.088
T. triangulare	0.336±0.072	0.340±0.073
H.sabdariffa	0.318±0.070	0.318±0.070
C. olitorius	0.292±0.064	0.290±0.064
S. macrocarpon	0.267±0.059	0.261±0.059
A. cruentus	0.238±0.053	0.228±0.053
Hydro-ethanol extract (HETE)		
X. Sagittifolium	0.706±0.153	0.681±0.145
I. batatas	0.639±0.097	0.621±0.134
L. aestuans	0.602±0.131	0.517±0.111
T. triangulare	0.494±0.106	0.462±0.099
H.sabdariffa	0.463±0.101	0.445±0.097
C. olitorius	0.432±0.095	0.421±0.093
S. macrocarpon	0.380±0.087	0.365±0.084
A. cruentus	0.339±0.076	0.312±0.069

Table 3. Radical scavenging activity (RSA) and F^{3+} reducing potencies of the methanol and hydro-ethanol extracts from the selected leafy vegetables.

Test samples	RSA (EC_{50})/mg ml^{-1}		Fe^{3+} reducing potential (EC_{50})/mg ml^{-1}	
	METE	HETE	METE	HETE
n-Propyl gallate	0.01513	0.01513	0.01513	0.01513
X. Sagittifolium	0.1294	0.1186	0.1186	0.1186
I. batatas	0.1746	0.1307	0.1307	0.1307
L. aestuans	0.2326	0.1586	0.1586	0.1586
T. triangulare	0.1656	0.1799	0.1799	0.1799
H.sabdariffa	0.1745	0.1212	0.1212	0.1212
C. olitorius	0.1635	0.1452	0.1452	0.1452
S. macrocarpon	0.1541	0.1380	0.1380	0.1380
A. cruentus	0.1364	0.1378	0.1378	0.1378

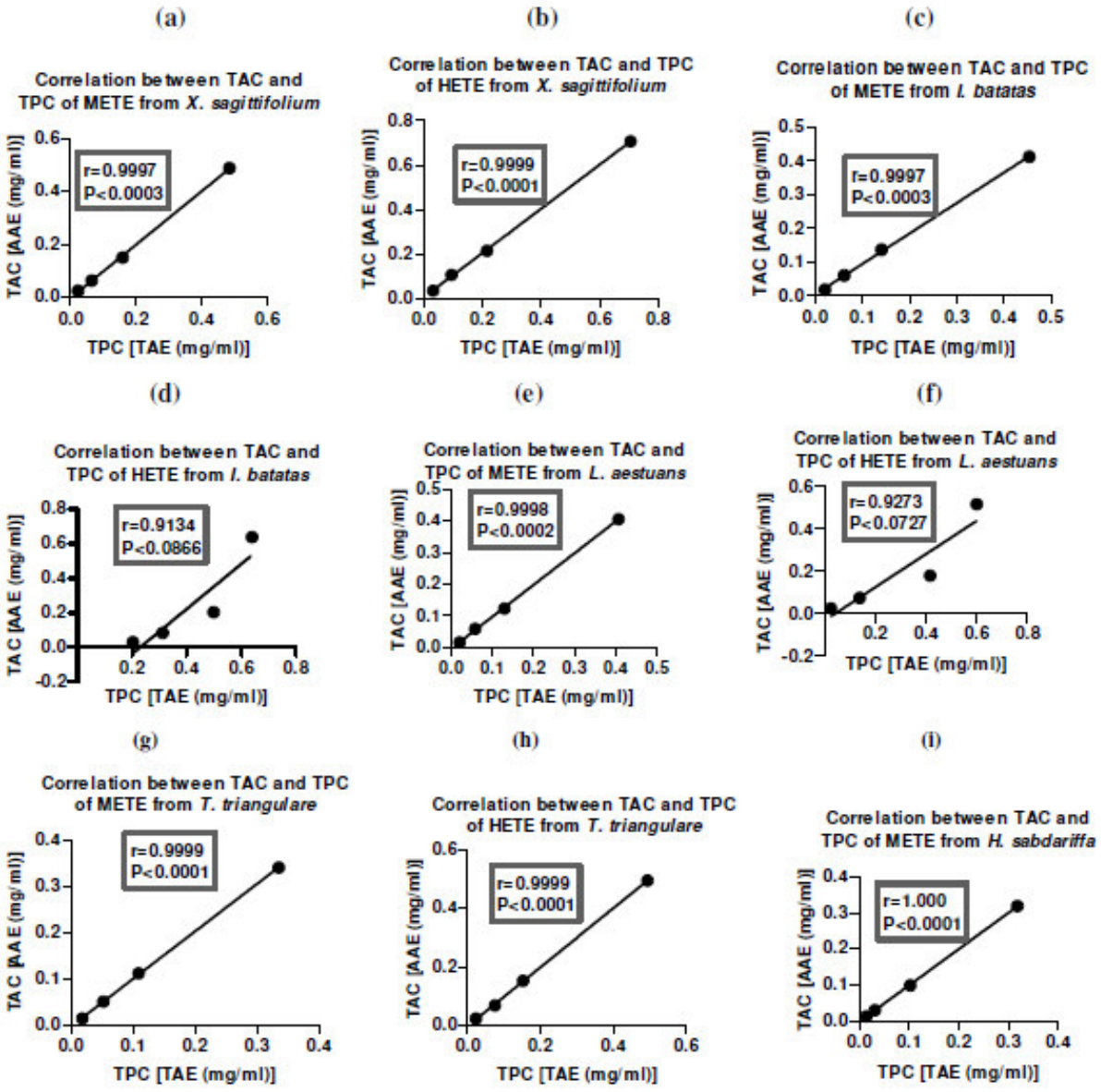
METE, Methanolic extract; HETE, hydro-ethanolic extract.

than their respective AAE. This observation was consistent with the findings of Odukoya et al. (2007) except that the TAE in *T. triangular* (21.83 mg TAE 100 g^{-1}) was far lower than its AAE (116.35 mg AA 100 g^{-1}).

Determination of DPPH radical scavenging activity and reducing power

The experimental results for RSA and F^{3+} to Fe^{2+} reducing ability of the METE and HETE extracts from the selected leafy vegetables are presented in Table 3. The experimental results revealed that the METE and HETE from the selected vegetables exhibited appreciable ability to scavenge the stable $DPPH^{\cdot}$ free radical in a dose-dependent manner from 0.1 - 3.0 mg/ml.

The smaller the EC_{50} value the greater the RSA and reducing ability of the leaf extract. It could be deduced from Table 3 that the RSA of the reference antioxidant compound, n-Propyl gallate showed approximately fifteen times potency (EC_{50}, 0.01513 mg/ml) and it's reducing power (EC_{50}, 0.2104 mg/ml) was relatively more pronounced compared with the values obtained for all the extracts from the selected leafy vegetables. The order of RSA of the METE from the selected leafy vegetables. was: *X. sagittifolium* > *A. cruentus* > *S. macrocarpon* > *C.*



olitorius > *T. triangulare* > *H. Sabdariffa* > *I. batatas* > *L aestuans*. METE from *X. sagittifolium* showed the strongest radical scavenging activity against DPPH free radical (EC_{50}, 0.1294 mg/ml) and the least radical scavenging activity was exhibited by *L. aestuans* (EC_{50}, 0.2326 mg/ml).

The HETE from the selected leafy vegetables showed a different trend. The RSA order was as follows: *X. sagittifolium* > *H. Sabdariffa* > *I. batatas* > *A. cruentus* > *S. macrocarpon* > *C. olitorius* > *L. aestuans* > *T. triangulare*. The strongest radical scavenging activity was again demonstrated by the HETE of *X. sagittifolium* (EC_{50}, 0.1186 mg/ml) and the HETE from *T. triangulare* showed the least RSA (EC_{50}, 0.1799 mg/ml). Moreover, the decreasing order of the reducing power of the METE from the selected vegetables at the 3.0 mg/ml was as follows: *L. aestuans* > *X. sagittifolium* > *I. batatas* > *T. triangulare* >*H. Sabdariffa* >*S. macrocarpon* > *A. cruentus* > *C. olitorius* with EC_{50} values of 1.150, 2.535, 2.778, 4.518, 5.148, 9.808, 14.80 and 36.15 mg/ml, respectively. A similar trend of reducing ability was observed for the HETE from the selected vegetables with slight variations: *L. aestuans* > *I. batatas* > *T. triangulare* >*H. Sabdariffa* > *X. sagittifolium* > *S. macrocarpon* > *A. cruentus* > *C. olitorius* with their respective EC_{50} values being 0.6894, 1.005, 4.091, 5.028, 5.374, 5.618, 10.68 and 14.12 mg/ml.

Generally, the HETE from the selected leafy vegetables showed a high RSA and greater reducing potencies than their respective METE from the leafy vegetables. The experimental results clearly show that the selected vegetables are very promising as natural antioxidants. This assertion is collaborated by earlier reports that tropical

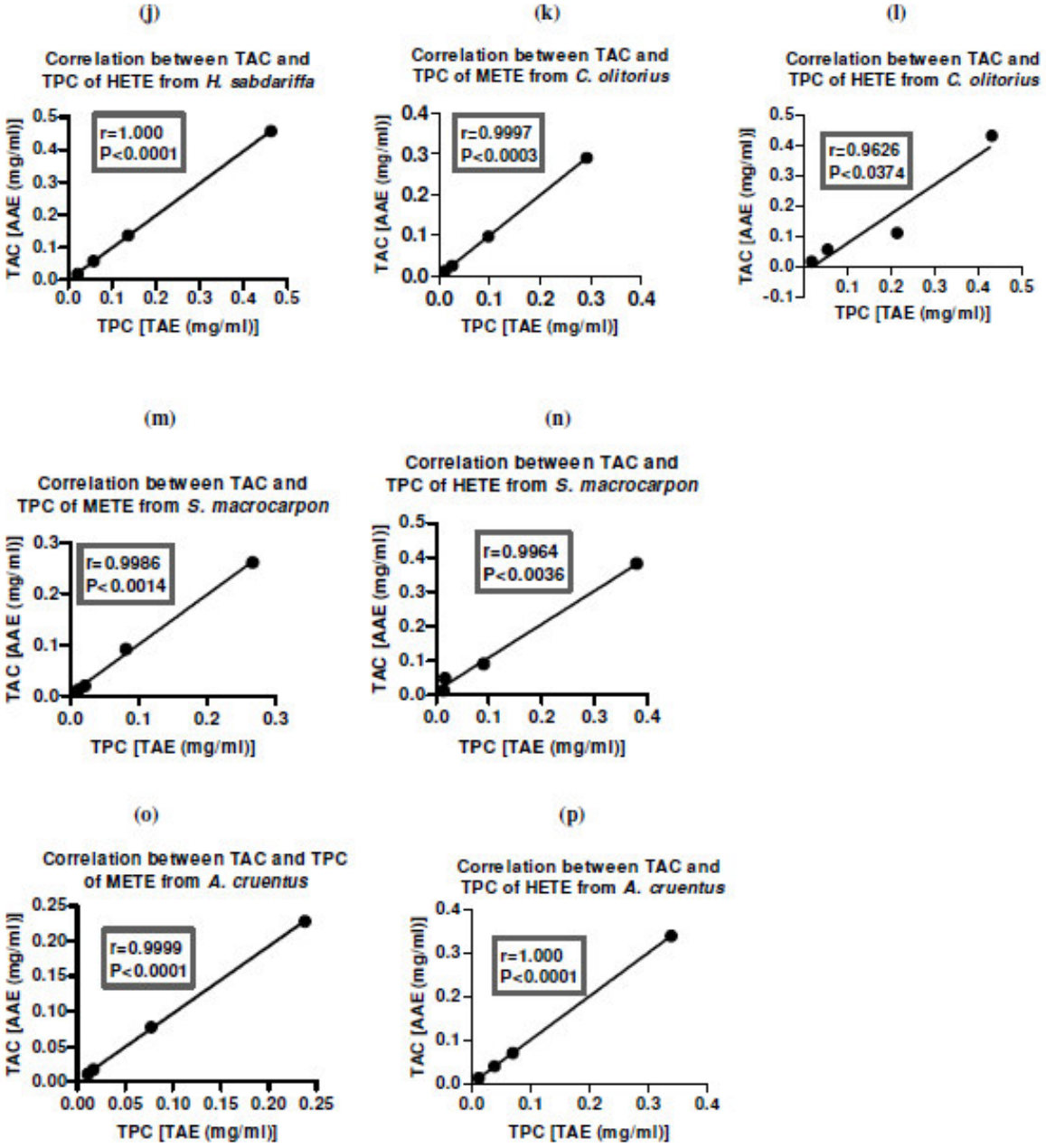


Fig. 3 (a) to **(p)**: Correlation between Total Phenol Content (TPC) and Total Antioxidant Capacity (TAC) in the METE and HETE from the selected vegetables

edible leafy vegetables have strong antioxidant activity (Akindahunsi and Salawu, 2005a, b; Odukoya et al., 2007 and Salawu et al., 2006).

Conclusion

Hydro-ethanolic extraction gave high percentage extract yield from all the selected leafy vegetables which suggest that hydro-ethanol is a preferred solvent system for extracting the phytoconstituents in the selected leafy vegetables. Extracts from the selected leafy vegetables all demonstrated appreciable total phenol content, total antioxidant capacity, radical scavenging activity and reducing ability with slight variations compared with the standard antioxidant compounds employed in the study. These findings agree to a large extent with similar works carried out in Nigeria by Akindahunsi and Salawu (2005a, b); Odukoya et al. (2007) and Salawu et al. (2006). This observation suggests that the selected leafy vegetables possess free radical scavenging potential and households in Ghana are encouraged to utilize them in their diets daily.

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