COMPARATIVE PHENOLIC, FLAVONOID CONTENTS AND ANTIOXIDANT ACTIVITY OF AQUEOUS METHANOL AND WATER EXTRACTS OF FOUR MEDICINAL PLANTS IN NIGERIA

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ABSTRACT: The comparative antioxidant activities of aqueous methanol and water extracts of *Acalypha wilkesiana, Cnidoscolus aconitifolius, Vernonia amygdalina* and *Solanun scabrum* leaves were studied. The total phenolic content (TPC) was determined using folin-ciocalteu method while total flavonoid content (TFC) was determined using aluminum chloride method. Antioxidant activity was determined using 2, 2-diphenyl-1-picryl hydrazine (DPPH) free radical scavenging and reducing power activity. The result of the study showed that the aqueous methanol extracted more phenols and flavonoids compared to the water extracts of the four plant leaves. More so, the DPPH free radical inhibition of the aqueous methanol extracts was higher than the water extracts of all the plant leaves studied. However, only the aqueous methanol extracts of *A.wilkesiana* and *S.scabrum* showed higher reducing power than their corresponding water extracts while the water extracts of *C.aconitifolius* and *V.amygdalina* leaves showed higher reducing power compared to their corresponding aqueous methanol extracts. In conclusion, this study showed that aqueous methanol has a higher capacity to extract more phenols, flavonoids and increase the free radical scavenging activities of the plant leaves.

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1.0. INTRODUCTION

Antioxidants are a group of substances, which inhibit or delay oxidative processes. Most of the potentially harmful effects of oxygen are due to the formation and activity of a number of chemical compounds, known as reactive oxygen species (ROS), which have a tendency to donate oxygen to other substances. Many such reactive species are free radicals and have a surplus of one or more free-floating electrons rather than having matched pairs and are, therefore, unstable and highly reactive. Free radicals produced from oxidation reaction start the chain reaction that damages the cell involved in immune suppression, cell membrane disintegration, membrane protein damage and DNA mutation, which can further initiate the development of many diseases such as cancer, liver injury, cardiovascular diseases, inflammation, diabetes, atherosclerosis etc.

Many antioxidant compounds, naturally occurring from plant sources, have been identified as a free radical or active oxygen scavengers. Phenolic compounds are abundantly present in human diet and acts as antioxidants and are widespread constituents of fruit, vegetables, cereals, olive oil, dry legumes, chocolate and beverages. Also they are found in both edible and non-edible plants. They may exert antioxidant effects as free radical scavengers, as hydrogen donating sources or as singlet oxygen quenchers and metal ion chelators. Phenolic compounds are known to counteract oxidative stress in the human body by helping maintaining a balance between oxidant and antioxidant substances (Attarde *et al*., 2010; Chaulya *et al*., 2010; Laloo and Sahu, 2011; Zheng and Wang, 2001). Although the phenolic, flavonoid contents and antioxidant activities of *Acalypha wilkesiana, Cnidoscolus aconitifolius, Vernonia amygdalina* and *Solanun scabrum* leaves have been reported (Anokwuru *et al*., 2011, 2012), comparative antioxidant activities of their extracts in water and aqueous organic solvent has not been reported and hence the aim of this study.

2.0. MATERIALS AND METHOD

2.1. Plant samples preparation and extraction

Fresh leaves of *Acalypha wilkesiana,* was obtained from Babcock University, Ilishan-Remo,

Ogun State. Fresh leaves of *Cnidoscolus aconitifolius, Vernonia amygdalina* and *Solanun scabrum* were purchased from Ilishan Remo market. The leaves of the four plants were thoroughly rinsed and air dried. They were ground to fine powder and 50 g of each plant sample was soaked in 80% methanol for 72 hours. Another 50g of each plant sample was soaked in water for 72 hours. After the extraction, the supernatants were filtered and all the filtrates concentrated using rotary evaporator at 40^{o}C. The crude extracts were weighed and stored till further use.

2.2.Determination of total phenolic content (TPC)

This was estimated as described by Singleton and Rossi, (1965). The assay is based on the reduction of Folin-Ciocalteu reagent (Phosphomolybdate and phosphotungstate) by the phenolic compounds. The reduced Folin-Ciocalteu reagent is blue and thus detectable with a spectrophotometer at 760nm.

PROCEDURE:

One ml aliquot of extracts (0.1mg/ml) was added in a volumetric flask containing 9 mls of water. One milliliter of Folin-Ciocalteu's reagent was added to the mixture and vortexed. After 5 min, 10 ml of 7% sodium carbonate was added to the mixture, and then incubated for 90 mins at room temperature. After incubation the absorbance against the reagent blank was determined at 750nm. A reagent blank was prepared using distilled water instead of the plant extract. The amount of phenolic compound in the extract was determined as Gallic Acid equivalent (mg/g of dry weight). All samples were analyzed in triplicates.

2.2.Determination of total flavonoid content (TFC)

The TFC was measured following a spectrophotometric method by Dewanto *et al.* (2002). Extract of each plant material (0.1mg/ml) was diluted with water (4 ml) in a 10 ml volumetric flask. Then 5% $NaN0_2$ solution (0.3 ml) was added to each volumetric flask at 5 min, 10% $AlCl_3$ (0.3 ml) was added and at 6 min, 1M NaOH (2 ml) was added. Water (2.4 ml) was then added to the reaction flask and mixed well. Absorbance of the reaction mixture was read at 510 nm. Total Flavonoid Content was determined as Quercetin equivalents (mg/g of dry weight). All samples were analyzed in triplicates.

2.3.ANTIOXIDANT ASSAY

2.3.1.Determination of DPPH radical scavenging activity

A solution of DPPH mixed with that of a substance that can donate a hydrogen atom, gives rise to the reduced form with change in colour, from deep violet to pale yellow.

PROCEDURE:

This was carried out according to the DPPH spectrophotometric method of Mensor *et al.,* 2001. One ml of a 0.3 mM DPPH methanol solution was added to a 2.5ml solution of the extract and allowed to react at room temperature for 30 min. The absorbance of the resulting mixture was measured at 518nm and converted to percentage antioxidant activity (AA%), using the formula:

$$AA\% = \frac{[(Abs_{control} - Abs_{sample}) \times 100]}{Abs_{control}}$$

Methanol (1.0 ml) plus extract solution (2.5 ml) was used as blank. 1 ml of 0.3mM DPPH plus methanol (2.5 ml) was used as the control. This assay was carried out in triplicates for each concentration. The IC_{50} value represented the concentration of the compounds that caused 50% inhibition of radical formation, which was obtained by interpolation from linear regression analysis (Stoilova *et al*., 2007).

2.3.2.Total reducing power

The total reducing power of the extracts were determined according to the procedure of Yen and Duh, (1993) as reported by Premanath and Lakshmidevi, (2010).

Various extracts (20 - 100 µg/ml) were mixed with phosphate buffer (500 µl, 20 mM, pH 6.6) and 1% potassium ferricyanide (500 µl), and incubated at 50^{0}C for 20 min; 500 µl of 10% Trichloroacetic acid were added, and the mixture was centrifuged at 2500 rpm for 10 min. The supernatant was mixed with distilled water (1.5 ml) and 0.1% ferric chloride (300 µl) and the absorbance was read at 700 nm. The experiment was repeated thrice. Increase in the absorbance of the reactions mixture indicated increase in the reducing power. The extract concentration providing 0.5 of absorbance (IC_{50}) was calculated from the graph of absorbance at 700 nm against extract concentration (Barros *et al*., 2007).

2.4.Statistical Analysis

Data were expressed as mean ± standard error. Analysis of variance was carried out on the values obtained in the experiment. Correlation analysis (Pearson) was carried out to determine the relationship between the assays carried out in this experiment. SPSS 15.0 was used to carry out these analyses.

3.0.RESULTS

3.1.Polyphenolic Content

The result of the phenolic and flavonoid contents of *A. wilkesiana*, *C. aconitifolius S. scabrum* and *V. amygdalina* are shown in table 1. The aqueous (80%) methanol extract gave higher phenolic content which was significantly different ($p<0.05$) from the water extract in all the four plants in this study. Similar trend was also observed in the flavonoid contents of the extracts. For the water extracts, *A.wilkesiana* gave the highest phenolic and flavonoid contents while *S.scabrum* gave the highest phenolic and flavonoid contents in the aqueous methanol extracts.

Table 1: Total Phenolic Contents (mgGAE/g) and Total Flavonoid Content (mgQE/g) *A. wilkesiana*, *C. aconitifolius S. scabrum* and *V. amygdalina*

Plant	Phenol		Flavonoid	
	Water	80% MeOH	Water	80% MeOH
A.wilkesiana	139 ± 1.8^{a}	204 ± 1.7^{b}	182 ± 0.3^{a}	201 ± 0.67^{b}
C.aconitifolius	75 ± 0.7^{c}	93 ± 1.5^{d}	66 ± 0.9^{c}	114 ± 1.5^{d}
S.scabrum	104 ± 3.5^{e}	210 ± 1.2^{f}	75 ± 0.7^{e}	253 ± 2.3^{f}
V.amygdalina	76 ± 1.5^{g}	117 ± 0.3^{h}	60 ± 0.7^{g}	172 ± 0.3^{h}

Data are expressed as the average of three determinations ± S.E. Data with different lower case letters on each row of each parameter analyzed are significantly different ($p<0.05$).

3.2.Antioxidant Activity

The result of percentage DPPH inhibition of the plants (see table 2) showed that the scavenging capacity of the aqueous methanol extracts for all the plants was higher than the water extracts. *A.wilkesiana* showed the highest scavenging activity in both water and aqueous extracts with IC_{50} values 5.0 and 1.76 µg/ml respectively while *C.aconitifolius* leaves showed the least scavenging activity with IC_{50} values 282.7 and 259.7µg/ml respectively.

The result of the reducing power of the plants (see table 2) showed that the aqueous extracts of *A.wilkesiana* and *S.scabrum* gave higher reducing power compared to their corresponding water extracts while the water extracts of *C.aconitifolius* and *V. amygdalina* gave higher reducing power compared to their corresponding aqueous methanol extracts. *C.aconitifolius* leaf gave the highest reducing power in the water extracts while A.wilkesiana gave the highest reducing power in the aqueous methanol extracts.

Table 2: Antioxidant activities (IC_{50}µg/ml) of *A. wilkesiana*, *C. aconitifolius S. scabrum* and *V. amygdalina* leaves

Plant	DPPH		RP	
	Water	80% MeOH	Water	80% MeOH
A.wilkesiana	$5.0\pm0.3^{\mathbf{a}}$	$1.76\pm0.2^{\mathbf{a}}$	$88.61\pm1.0^{\mathbf{a}}$	$3.06\pm1.2^{\mathbf{e}}$
C.aconitifolius	$282.7\pm4.7^{\mathbf{b}}$	$259.7\pm0.3^{\mathbf{e}}$	$51.14\pm4.7^{\mathbf{b}}$	$189.4\pm0.9^{\mathbf{f}}$
S.scabrum	$142\pm0.6^{\mathbf{c}}$	$6.4\pm0.03^{\mathbf{a}}$	$127.48\pm1.2^{\mathbf{c}}$	$86.1\pm0.6^{\mathbf{a}}$
V.amygdalina	$192.3\pm2^{\mathbf{d}}$	$87\pm0.3^{\mathbf{f}}$	$114.04\pm0.5^{\mathbf{d}}$	$144\pm0.8^{\mathbf{g}}$

Data are expressed as the average of three determinations ± S.E. Data with different lower case letters on each row and column of each parameter analyzed are significantly different ($p<0.05$).

Table 3: Correlation coefficients, R, for the relationships between assays

	TFC	DPPH	RP
TPC	0.911^{**}	0.854^{**}	0.515^{*}
TFC		0.844^{**}	0.288
DPPH			0.412^{*}

** Correlation is significant at the 0.01 level, *Correlation is significant at the 0.05 level.

4.0.DISCUSSION

Phenolic compounds are constituent of both edible and non-edible parts of plants (Amarowicz *et al*., 2010). They are widely distributed in plants and are known for their antioxidant and free radical scavenging abilities, which have beneficial implications for human health (Kubola and Siriamornpun, 2008). The higher total phenolic content and total flavonoid content in the aqueous methanol extracts of *A. wilkesiana*, *C. aconitifolius S. scabrum* and *V. amygdalina* leaves showed that the polyphenolics in these plant leaves are best extracted with an aqueous alcoholic solvent compared to the traditional maceration in cold water. It also suggests

that the therapeutic activities of the plants associated with the phenolic contents can best be potentiated with an aqueous organic solvent. This may be the reason why some local concoctions are prepared with alcoholic beverages (like dry gin). The strong correlation (see table 3) between TPC and TFC (r=0.911) showed that aqueous methanol extracts extracted more phenols and flavonoids than the water extracts.

The stable radical DPPH has been used widely for the determination of primary antioxidant activity, that is, the free radical scavenging activities of pure antioxidant compounds, plant, fruit extracts and food materials (Wong *et al*., 2006). The result of the antioxidant activity (see table 2) showed that the aqueous methanol extracts were able to scavenge more of the DPPH free radicals than the water extracts either by donating electrons or hydrogen. There are several reports of strong correlation between phenols and DPPH free radical scavenging activities of plants (Anokwuru *et al*., 2011; Cai *et al*., 2004; Kaur *et al*., 2008; Kubola & siriamornpun, 2008; Loo *et al*., 2007; Tawaha *et al*., 2007; Zheng and Wang, 2004). This study showed a very strong correlation (see table 3) which was statistically significant ($p<0.01$) between phenols (r=0.854) and inhibition of DPPH free radicals. The correlation between flavonoids and inhibition of DPPH free radicals (r=0.844) was also statistically significant ($p<0.01$). The scavenging ability of phenols is mainly due to the presence of hydroxyl groups (Subhasree *et al*., 2009). This result showed that the free radical scavenging activity of *A. wilkesiana*, *C. aconitifolius S. scabrum* and *V. amygdalina* leaves is strongly dependents on the non-enzymatic antioxidants present in them.

Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary anti-oxidants (Yen and Chen, 1995). The presence of the reductants in the extracts cause the reduction of the Fe^{2+}/Ferricynanide complex to the ferrous form (Amarowicz *et al.,* 2004). The higher reducing power of the aqueous methanol extracts of *A.wilkesiana* and *S.scabrum* leaves compared to their water extracts showed that the aqueous methanol extracts are better electron donors and can reduce oxidants than their corresponding water extracts. This also suggests that the reductants are more of non polar compounds. However, the higher reducing power of the water extracts of *C.aconitifolius* and *V.amygdalina* leaves showed that the water extracts were better electron donors and could reduce oxidants compared to their corresponding aqueous methanol extracts. It also suggests that the reductants are more of polar compounds.

The correlation analysis showed a significant ($p<0.05$) moderate relationship (r=0.515) with TPC, poor relationship with TFC (r=0.288) and weak relationship (r=0.412) with DPPH. The moderate correlation between reducing power and TPC could be as a result of 1:1 of the response of the water and aqueous methanol extracts of the four plant leaves since *A. wilkesiana* and *S.scabrum* showed higher reducing power for their aqueous methanol extracts while *C.aconitifolius* and *V.amygdalina* showed higher reducing power for their water extracts. The poor correlation between TFC and reducing power showed that the flavonoids present in the plants may not be responsible for the reducing power of the plants. The weak correlation between reducing power and DPPH suggests that the compounds responsible for the scavenging activities of the plant leaves may not be responsible for their reducing potentials.

5.0.CONCLUSION

In conclusion, this study showed that aqueous methanol has a higher capacity to extract more phenols, flavonoids and increase the free radical scavenging activities of the plants leaves studied.

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