

## Effect of Extraction Solvents on Phenolic, Flavonoid and Antioxidant activities of Three Nigerian Medicinal Plants

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**Abstract:** The effect of extracting solvents (absolute methanol, ethanol, acetone and ethyl acetate) on the phenolic, flavonoid contents and antioxidant activities of the bark of *Azadirachta indica*, leaves of *Acalypha wilkesiana*, and *Solanum scabrum* 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 inhibition of lipid peroxidation. Acetone extract of *S. scabrum* recorded the highest phenolic content (34.2g GAE/100g) while methanol extract of *A. indica* recorded the lowest (3.77g GAE/100g). Ethanol extract of *A. indica* recorded the highest flavonoid content (8.7 g QE/100g) while acetone extract of *A. wilkesiana* recorded the lowest (3.41 g QE/100g). Methanol extract of *A. wilkesiana* recorded the highest DPPH free radical scavenging activity (85.65%) while ethyl acetate extract of *A. indica* recorded the highest inhibition of lipid peroxidation (41.57%). The result of this study showed that the activity of antioxidant of different plants is dependent on extracting solvents. Nature and Science 2011;9(7):53-61]. (ISSN: 1545-0740). <http://www.sciencepub.net>.

**Keywords:** Polyphenols, Antioxidant activity, Medicinal plants, Extracts, solvents

### INTRODUCTION

Plants have been a source of medicine in the past centuries and today scientists and the general public recognize their value as a source of new or complimentary medicinal products (Premanath & Lakshmidivi, 2010). The medicinal value of these plants lies in some chemical active substances that produce definite physiological action on the human body (Aiyelaagbe & Osamudiamen, 2009). Medicinal plants are used by 80% of the world population as the only available medicines especially in developing countries (EL-Kamali & EL-amir, 2010). Nigeria have a great variety of natural vegetation, which is used in tradi-medicine to cure various ailments, some plants are also useful for ornamental purposes, while many due to their odoriferous nature are used in flavoring or as food additives and preservatives (Egwaikhinde & Gimba, 2007). Practically, every part of the *A. indica* (leaves, bark, fruit, flowers, oil and gum) have been reported to be associated with various remedial properties such as the treatment of general body pain after child delivery, pyorrhea, intestinal worms, antimicrobial effects, storage behavior, *in vitro* antiviral activity and antibacterial agent (Biu *et al.*, 2009; Taha *et al.*, 2008). *Acalypha* species are popularly used for the treatment of malaria, dermatological and gastro-intestinal disorders. Seeds

from *A. wilkesiana* (Euphorbiaceae) are essential components of a complex plant mixture used by traditional healers in southwest Nigeria in the treatment of breast tumors and inflammation (Udoband *et al.*, 2010).

Most of the physiological impairment, tissues damages, pathological events or diseases affecting humans have been attributed by recent scientific studies to be caused by unstable and extremely reactive chemical species called free radicals and/or reactive oxygen species (Tawaha *et al.*, 2007; Sathishkumar *et al.*, 2009; Subhasree *et al.*, 2009; Jang *et al.*, 2006). The imbalance between the production of bodily antioxidant defense system and free radical formation results in oxidative stress. Oxidative stress has been implicated in the alteration genetic material, inducing oxidation that causes membrane lipid peroxidation, decreased membrane fluidity and inducing metabolic injury and death. This may lead to accelerated aging, cancer, cardiovascular diseases, neurodegenerative diseases, and inflammation (Neergheen *et al.*, 2006; Prakash *et al.*, 2007; Wong *et al.*, 2006; Kubola & Siriamornpun, 2008; Subhasree *et al.*, 2009).

Lipid peroxidation is one of the major causes of deterioration in foods that results in the formation of potentially toxic compounds. This has led to the use of synthetic antioxidants, such as butylated

hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butyl hydroquinone (TBHQ) and propylgallate (PG) as food additives to preserve against deterioration; however, their use is increasingly restricted, due to their potential health risks and toxicity (Tawaha *et al.*, 2007). Hence, scientists are now searching for naturally occurring antioxidants in plant sources for food or medicinal materials as alternative for synthetic antioxidants (D'Abrosca *et al.*, 2007; Loo *et al.*, 2007; Stanojevi *et al.*, 2009). Antioxidants protective effect against lipoperoxidative damage depends on the hydroxyl group in each molecule; however, the effectiveness of antioxidants has been found to be related also to their incorporation rate into cells and their orientation in the biomembranes (Saija *et al.*, 1994). Natural antioxidants endogenous to food of plant origin can scavenge reactive oxygen and nitrogen species (RONS); evidence suggests that these may be of great importance in preventing the onset of oxidative diseases in the human body (Amarowicz *et al.*, 2010). Plants are a major source of phenolic compounds, which are synthesized as secondary metabolites during normal development in response to stress conditions, such as wounding and UV radiation among others.

Plants may contain simple phenolics, phenolic acids, coumarins, flavonoids, stilbenes, hydrolysable and condensed tannins, lignins and lignans. Distribution of phenolics in plants at the tissue, cellular and subcellular levels is not uniform. Insoluble phenolics are found in cell walls, while soluble phenolics are present within the plant cell vacuoles. Cell wall phenolics may be linked to various cell components such as sugars. Therefore, the nature of polyphenol compounds in plants is complex (Maisuthisakul *et al.*, 2008). The beneficial effects of plant phenolics are related to their antioxidant activity, particularly their ability to scavenge free radicals, to donate hydrogen atoms or electrons, or to chelate metal cations. Besides, phenolic compounds contribute largely to the colour and sensory characteristics of fruits and vegetables. In addition, phenols participate in growth and reproduction processes, and provide protection against pathogens and predators. At the cellular level, it participate in cell protection against the harmful action of reactive oxygen species (ROS), mainly oxygen free radicals, produced in response to environmental stresses such as salinity, drought, high light intensity or mineral nutrient deficiency, because of the imbalance between the production and scavenging of ROS in chloroplasts. These cytotoxic-activated oxygen species can seriously disrupt normal metabolism through oxidative damage to lipids, proteins and nucleic acids. Accordingly, plants

containing high concentrations of antioxidants show considerable resistance to the oxidative damage caused by the ROS, as shown in the case of salt stressed plants (Meot-Duros & Magne, 2009).

Recovery of antioxidant compounds from plant materials is typically accomplished through different extraction techniques taking into account their chemistry and uneven distribution in the plant matrix. Solvent extraction is most frequently used technique for isolation of plant antioxidant compounds (Sultana *et al.*, 2009). However, the extract yields, polyphenolic contents, and resulting antioxidant activities of the plant materials are strongly dependent on the nature of extracting solvent and method, due to the presence of different antioxidant compounds of varied chemical characteristics and polarities that may or may not be soluble in a particular solvent (Sultana *et al.*, 2009; Jakopic *et al.*, 2009). Polar solvents are frequently employed for the recovery of polyphenols from a plant matrix. The most suitable of these solvents are (hot or cold) aqueous mixtures containing ethanol, methanol, acetone, and ethyl acetate (Sultana *et al.*, 2009).

Sultana *et al.* (2009) reported that aqueous, ethanolic and methanolic extracts of barks of *Azadirachta indica*, *Acacia nilotica*, *Eugenia jambolana*, *Terminalia arjuna*, leaves and roots of *Moringa oleifera*, fruit of *Ficus religiosa*, and leaves of *Aloe barbadensis* exhibited better antioxidant activities and phenolic contents compared to absolute methanol and ethanol. Fifty percent (50%) aqueous solvent extracts from black tea at 2, 8 and 18 h gave markedly higher amounts of total polyphenol and antioxidant activity as compared to absolute ones (Turkmen *et al.*, 2007). Among aqueous solvents, acetone or N, N-dimethylformamide (DMF) was the most efficient solvent with respect to the three parameters measured. In the case of absolute solvent extracts, DMF and methanol were much more efficient than ethanol and acetone. Jakopic *et al.*, (2009) reported that methanol extract of walnut fruits extracts yielded higher total phenolic contents compared to the ethanolic extract. According to koffi *et al.* (2010) ethanolic extract of Ivorian plants extracted higher phenolics compared with acetone, water, and methanol. Therefore, the present study was aimed to determine the effect of different extracting solvents on the total phenolic, flavonoid content, and antioxidant activities of the plants under study.

## MATERIALS AND METHODS

### Chemicals

Methanol, ethanol, ethyl acetate and acetone were Analar grade (Sigma-Aldrich). Thiobarbituric acid (TBA), FeCl<sub>3</sub>, 2,2-diphenyl-1-picrylhydrazyl

(DPPH), Folin–Ciocalteu reagent, aluminium chloride, quercetin, were obtained from Sigma–Aldrich S.p.A. (Milan, Italy).

### Preparation of plant samples

*Acalypha wilkesiana* leaf and *Azadirachta indica* stem bark were obtained from the horticultural garden in December 2009 at Babcock University, Ilisan Remo, Ogun State, Nigeria. *Solanum scabrum* leaf was purchased in Ilisan Remo market. The fresh samples were washed and air-dried for one week. All dried plant materials were ground to powder and sieved to obtain fine particles. 20 g of each sample was soaked with methanol, ethanol, ethylacetate and acetone for 72 h. The filtrates were concentrated using rotary evaporator at 40°C. The dried extracts were weighed and store at -4°C.

### Determination of plant yield

The percentage yield was obtained using this formula  $W_2 - W_1 / W_0 \times 100$ . Where  $W_2$  is the weight of the extract and the container,  $W_1$  the weight of the container alone and  $W_0$  the weight of the initial dried sample

### Determination of Total Phenolic Content (TPC)

This was estimated as described by Singleton and Rossi (1965) and modified by Gulcin *et al.* (2003). One ml aliquot of extracts or standard solution of gallic acid (10, 20, 30, 40 and 50 µg/ml) was added in a volumetric flask containing 9 ml 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 min at room temperature. After incubation the absorbance against the reagent blank was determined at 750 nm. A reagent blank was prepared using distilled water instead of the plant extract. The amount of phenolic compound in the extract was determined from the standard curve produced with varying concentrations (10, 20, 30, 40, 50 µg/ml) of gallic acid ( $R^2=0.9986$ ). The total phenolic content of the plant was then calculated as shown in the equation below and expressed as mg Gallic acid equivalent (GAE)/g fresh weight. All samples were analyzed in triplicates

### Determination of Total Flavonoid Content (TFC)

The TFC was measured following a spectrophotometric method by Dewanto *et al.* (2002). Briefly, extract of each plant material (1 ml containing 100 µg/ml) were diluted with water (4 ml) in a 10 ml volumetric flask. Initially, 5% NaNO<sub>2</sub> solution (0.3 ml) was added to each volumetric flask at 5 min, 10%

AlCl<sub>3</sub> (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 (g/100g of dry weight). Triplicate reading were taken for each sample and the result averaged.

### Determination of DPPH Radical Scavenging Activity

This was carried out according to the 2,2-diphenyl-2-picrylhydrazyl (DPPH) assay system by Mensor *et al.* (2001). One ml of a 0.3 mM DPPH methanol solution was added to 2.5 ml solution of the extract or standard (100 µg/ml, 200 µg/ml, 300 µg/ml) and allowed to react at room temperature for 30 min. The absorbance of resulting mixture was measured at 518 nm and converted to percentage antioxidant activity (AA %), using the formula:

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

Abs = Absorbance.

Methanol (1.0 ml) plus extract solution (2.5 ml) was used as blank. 1ml of 0.3 mM DPPH plus methanol (2.5 ml) was used as a negative control. Solution of gallic acid served as positive control. This assay was carried out in triplicates for each concentration.

### Determination of Inhibition of Lipid Peroxidation

A modified thiobarbituric acid reactive substances assay was used to measure the lipid peroxide formed, using egg yolk homogenate as lipid-rich media (Ruberto *et al.*, 2000). Egg homogenate (0.5 ml, 10 % v/v) and 0.1 ml of each extract were added to a test tube and made up to 1 ml with distilled water. 0.05 ml of FeSO<sub>4</sub> (0.07 M) was added to induce lipid peroxidation and incubated for 30 min. Then 1.5 ml of 20% acetic acid (pH adjusted to 3.5 with NaOH) and 1.5 ml of 0.8% (w/v) TBA in 1.1% sodium dodecyl sulphate and 20% TCA were added and the resulting mixtures were vortexed and then heated at 95°C for 60 min. After cooling, 5.0 ml of butan-1-ol was added to each tube and centrifuged at 3000 rpm for 10 min. The absorbance of the organic layer was measured at 532 nm. Percentage inhibition of lipid peroxide formed by the extracts were calculated according to  $(1 - E/C) \times 100$ .

Where C = is the absorbance value of the fully oxidized control and E absorbance in the presence of extract.  $(Abs_{532+TBA} - Abs_{532-TBA})$ .

## RESULTS

Table 1 showed the percentage yield of extracts of *A. wilkesiana* leaf, *A. indica* bark, and *S. scabrum* leaf in acetone, ethanol, ethylacetate and methanol in solvent.

In *A. wilkesiana*, the methanolic extract gave the highest yield (14.67%) while the ethylacetate extract gave the least yield (2.73%). In *A. indica*, acetone extract gave the highest yield (11.7%) while ethylacetate extract gave the lowest yield (3.43%). In *S. scabrum*, methanolic extract gave the highest yield (17.23%) while ethyl acetate extract gave the lowest yield (4.13) (Table 1).

Table 2 showed the total phenolic content of *A. wilkesiana* leaf, *A. indica* stem bark and *S. scabrum* leaf in four different solvents. The total phenolic content of the three samples were determined as gallic acid equivalent (GAE) in g per 100 g dry weight. In *A. wilkesiana*, the methanolic extract recorded the highest TPC (13.77 g GAE/100g) followed by ethylacetate (12.83 g GAE/100g), acetone (10.8 g GAE/100g) and ethanol (5.87g GAE/100g) in *A. indica*, acetone recorded the highest TPC (15.1g GAE/100g), followed by ethylacetate (13.5 g GAE/100g), ethanol (7.47 g GAE/100g) and methanol extract (3.77 g GAE/100g). in *S. scabrum*, acetone extract recorded the highest TPC (34.2 g GAE/100g), followed by ethanol extract (29 g GAE/100g), ethylacetate extract (13.5 g GAE/100g) and methanol (13.4 g GAE/100g).

Results also showed an increased magnitude in TFC in the order of methanol extract (4.05 g QE/100 g) > ethanol extract (3.69 g QE/100 g) > ethylacetate extract (3.67 g QE/100 g) > acetone extract (3.41 g QE/100 g) of *A. wilkesiana*. *A. indica* bark extracts indicated ethanol extract (8.7 g QE/ 100 g) > ethylacetate extract (5.32g QE/100g) > acetone extract (5.15g QE/100g) > methanol extract (5.14g QE/100g).

In *S. scabrum* extract indicated ethylacetate extract (5.26g QE/100g) > methanol extract (5.20g QE/100g) > ethanol extract (3.81g QE/100g) > acetone (3.51g QE/100g) (Table 2).

#### DPPH scavenging activity

The magnitude of DPPH scavenging power of different solvents of *A. wilkesiana* was in the order of methanol extract (85.65%) > acetone extract (84.90%) > ethylacetate extract (83.99%) > ethanol extract (70.17%), *A. indica* showed acetone extract (85.52%) > methanol extract (84.13%) > ethylacetate extract (82.10%) > ethanol (81.49%), *S. scabrum* showed ethanol extract (72.33%) > methanol extract (70.39%) > ethylacetate extract (68.94%) > acetone extract (52.84%) (Figure 1).

#### Inhibition of lipid peroxidation

Result of the percentage inhibition of lipid peroxidation of egg homogenate showed *A. wilkesiana*, *A. indica* and *S. scabrum* in acetone, ethanol, ethylacetate and methanol are in the range of 24.1% to 40.27%. In *A. wilkesiana*, acetone extract (40.27%) and methanol extract (25%) recorded the highest and lowest inhibitions respectively. In *A. indica* stem bark, ethylacetate extract (41.57%) and acetone (32.27%) extract recorded the highest and lowest inhibition respectively. *S. scabrum* acetone (27.6%) and ethylacetate (27.6%) extracts recorded the highest inhibition while methanol extract (24.1%) showed the lowest inhibition of lipid peroxide (Figure 2).

#### Percentage yield

Table 1: Percentage yield of plant extracts in different solvents

Plant used	Yield (%)			
	Acetone	Ethanol	Ethylacetate	Methanol
<i>A. wilkesiana</i>	4.34 ± 0.23 <sup>ab</sup>	5.37 ± 0.19 <sup>ab</sup>	2.73 ± 0.26 <sup>b</sup>	14.67 ± 0.88 <sup>a</sup>
<i>A. indica</i>	11.7 ± 0.12 <sup>a</sup>	6.8 ± 0.12 <sup>ac</sup>	3.43 ± 0.19 <sup>b</sup>	9.77 ± 0.12 <sup>ab</sup>
<i>S. scabrum</i>	15.47 ± 0.18 <sup>ab</sup>	4.77 ± 0.15 <sup>ac</sup>	4.13 ± 0.15 <sup>b</sup>	17.23 ± 0.18 <sup>a</sup>

a-Highest yield and significantly different (p <0.05) from other yields (ab, ac & b) of each row.

ab- significantly different (p <0.05) from b only.

ac- significantly different (p <0.05) from b only.

Extract yields with identical alphabets show no significant difference (p <0.05).

**Total Phenolic Content (TPC)**

Table 2: Total phenolic content (g GAE/100 gdw) and total flavonoid contents (g QE/100 gdw)

Extract	TPC			TFC		
	AWL	AIB	SSL	AWL	AIB	SSL
Acetone	10.80±0.4 <sup>a</sup>	15.10±0.04 <sup>a</sup>	34.2±0.26 <sup>a</sup>	3.41±0.03 <sup>e</sup>	5.15±0.03 <sup>e</sup>	3.51±0.01 <sup>e</sup>
Ethanol	5.87±0.12 <sup>b</sup>	7.47±0.07 <sup>b</sup>	29±1.51 <sup>b</sup>	3.69±0.03 <sup>f</sup>	8.70±0.04 <sup>f</sup>	3.81±0.02 <sup>f</sup>
Ethylacetate	12.83±0.82 <sup>c</sup>	13.5±0.1 <sup>c</sup>	13.37±0.12 <sup>c</sup>	3.67±0.01 <sup>f</sup>	5.32±0.06 <sup>g</sup>	5.26±0.02 <sup>g</sup>
Methanol	13.77±0.01 <sup>c</sup>	3.77±0.03 <sup>d</sup>	13.40±0.2 <sup>c</sup>	4.05±0.06 <sup>g</sup>	5.14±0.02 <sup>e</sup>	5.20±0.03 <sup>g</sup>

Data are expressed as the average of three determinations ± S.E

Data with different lower case letters on individual solvent extracts of each plant are significantly different (p<0.05)

AWL: *acalypha wilkesiana* leaf

AIB: *azadiractha indica* stem bark

SSL: *solanum scabrum* leaf

Dw- dry weight

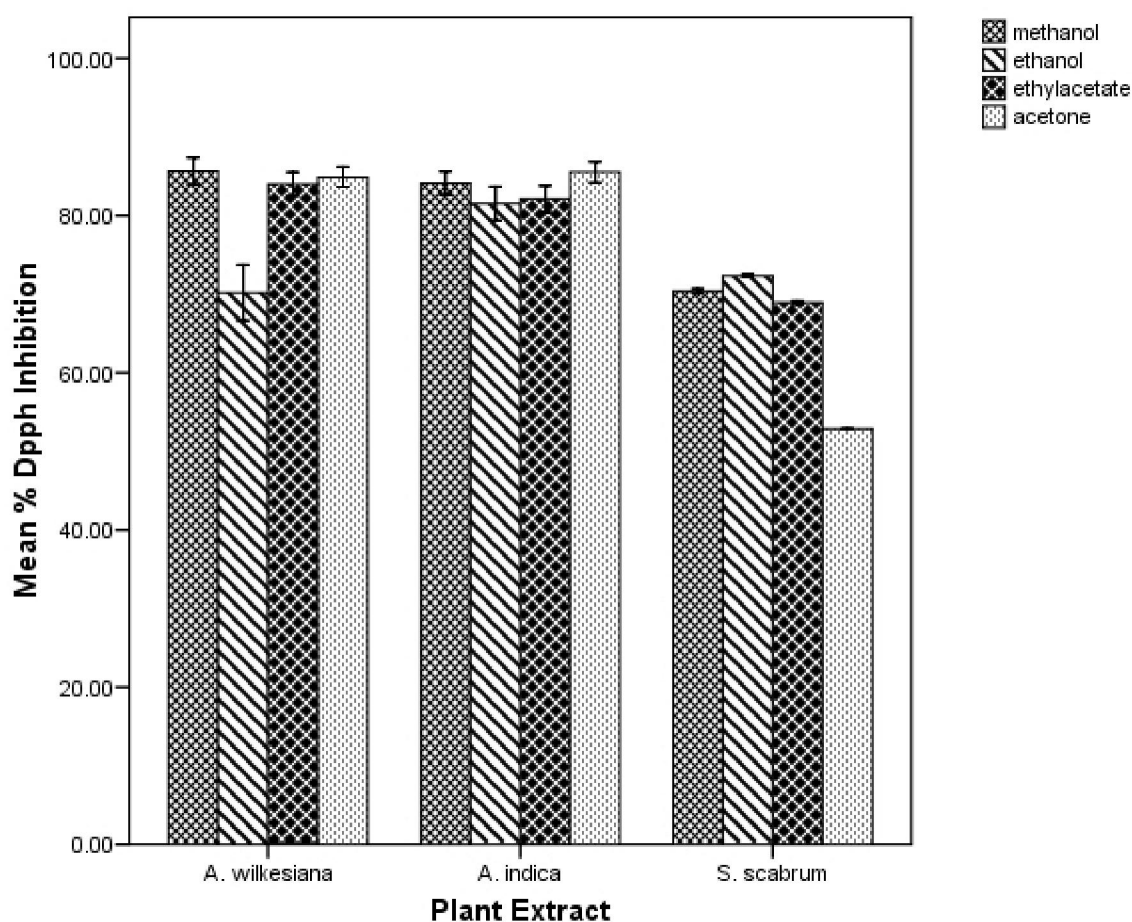


Figure 1: Graph of percentage inhibition of DPPH free radical of the plant extracts.

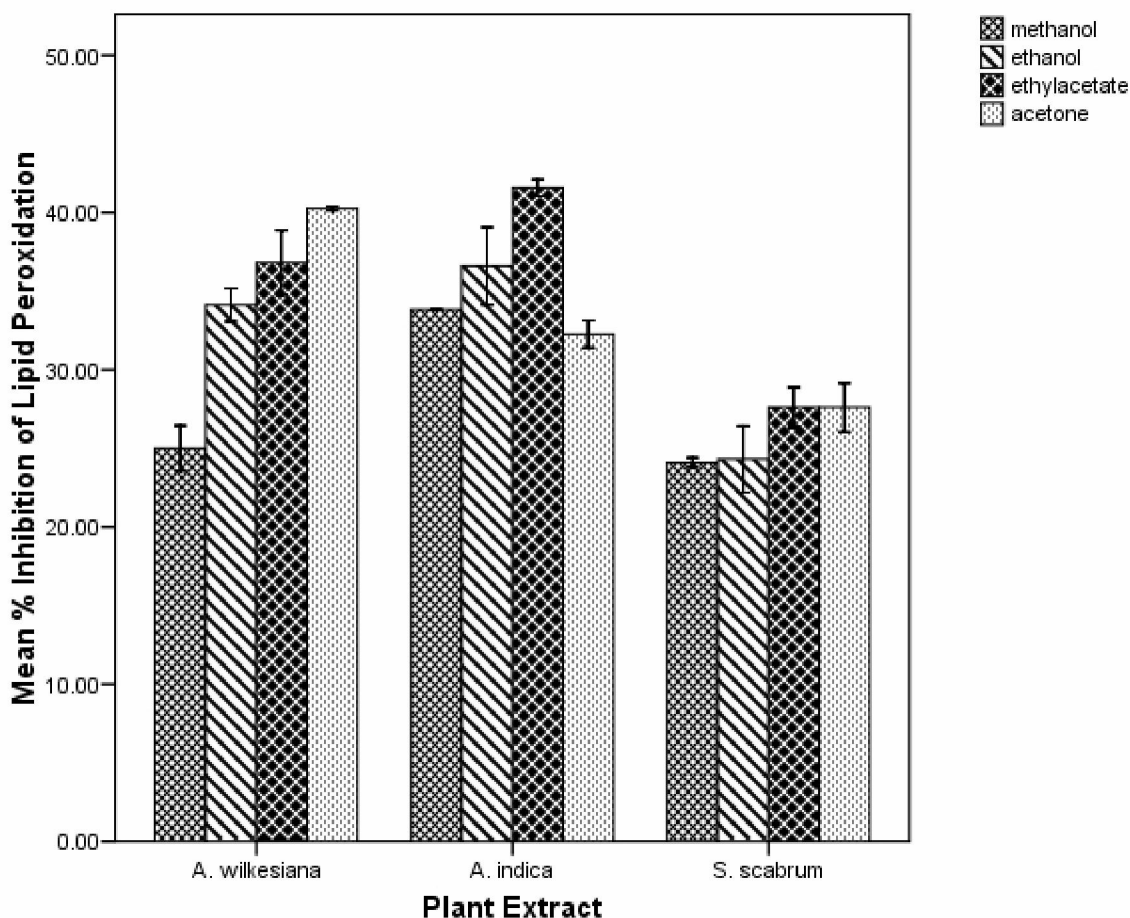


Figure 2: Graph of percentage inhibition of lipid peroxidation of three plant extracts in four different solvents.

### Discussion

The result of the percentage yield suggested that absolute methanol was a better solvent for the extraction of *A. wilkesiana* and *S. scabrum*, while acetone was a better solvent for the extraction of *A. indica*.

In a study by Nahak and Sahu (2010) on antioxidant activity of *A. indica* leaf, methanol gave the least yield compared to water and ethanol. In another study of phenolic compounds and antioxidant activity of Henna leaves extract (*Lawsonia inermis*) by Hosien and Zinab (2007), water gave higher yield compared to methanol. Stanojevic *et al.* (2009) showed that the magnitude of extract yield of *Hieracium pilosella* leaf was ethanol>methanol>water. Sultana *et al.* (2009), reported that absolute ethanol gave the highest yield of *A. indica* bark compared to absolute methanol, aqueous methanol and ethanol. Although acetone and ethyl

acetate were not used in that study, the values of the extract yields were higher than the values in this study.

Methanol extract of *A. wilkesiana* had high total phenolic content while acetone extract of *A. indica* and *S. scabrum* showed high total phenolic content suggesting that methanol and acetone could serve as better solvents for phenolic compound extraction. Result also showed that the *A. wilkesiana* and *A. indica* with the highest extract yield also had the highest phenolic content (Tables 1 & 2). Previous findings had shown that the efficiency of the phenolics extraction depends on the type of the plant and kind of solvent used (Jang *et al.*, 2007; Jakopic *et al.* 2009). The study conducted by Koffi *et al.* (2010) concluded that ethanol was found to be the best solvent for the extraction of phenolics of 26 Ivorian plants. The phenolic content of methanol and ethyl acetate extract of *A. indica* bark in this study was lower than the phenolic content of methanol and ethyl acetate of *A. indica* bark reported

by Ghimeray *et al.*, 2009. This trend could be as a result of the different geographical location of the plants.

Many flavonoids are found to be strong antioxidants capable of effectively scavenging the reactive oxygen species because of their phenolic hydroxyl groups (Cao *et al.*, 1997; Miller & Ruiz-Larrea, 2002; Subhasree *et al.*, 2009). The study showed methanol, ethyl acetate and ethanol had a significantly high ( $P < 0.05$ ) flavonoid content in *A. wilkesiana*, *S. scabrum* and *A. indica* respectively than other extracts of each plant. This indicates that methanol was the best solvent for the extraction of flavonoid in *A. wilkesiana*, ethyl acetate for the extraction of flavonoid in *S. scabrum* and ethanol for flavonoid extraction in *A. indica*.

Comparing the flavonoid content of *A. wilkesiana* with the phenolic content, methanol also extracted the highest phenol. However, this trend did not result into a strong linear correlation ( $r = 0.4$ ) at  $p < 0.01$  between phenolic contents and flavonoid contents of *A. wilkesiana*. In *A. indica*, ethanol extracts showed significantly high flavonoid content than the other extracts (Table 2). In *S. scabrum*, ethyl acetate extract gave the highest flavonoid content which was higher than acetone and ethanol extract. This suggests that ethyl acetate and methanol are better solvents for the extraction of flavonoids compared to acetone and ethanol in *S. scabrum*. In general, *A. indica* contains more flavonoids compared to *A. wilkesiana* and *S. scabrum*, since the least flavonoid content in *A. indica* is higher than the highest flavonoid content in *A. wilkesiana* and close to the highest flavonoid content in *S. scabrum*. This could be because the *A. indica* bark sample was woody and is the external part of the plant compared to the leaves of the other samples. According to Miller & Ruize-Larrea (2002) flavonoids are found particularly at the woody and external parts of plants. This result showed that *A. indica* had highest content of flavonoids in ethanol extract than all the extracts of *A. wilkesiana* and *S. scabrum*. The flavonoid content of methanol and ethyl acetate extract of *A. indica* bark in this study was higher than the flavonoid content of methanol and ethyl acetate of *A. indica* bark reported by Ghimeray *et al.*, 2009. The difference in the geographical location could be responsible for this observation.

The strong negative correlation ( $r = -0.998$ ) between TPC and TFC of *S. scabrum* suggests that the extract that gave the highest phenolic content gave the lowest flavonoid content. Contrary to the relationship between phenolics and extract yield, there were strong correlation between extract yield and flavonoid for *A.*

*wilkesiana* ( $r = 0.801$ ), and *S. scabrum* ( $r = 0.981$ ) but a negative correlation for *A. indica* ( $r = -0.229$ ).

Study showed that methanol, acetone and ethanol exhibited high free radical inhibition in *A. wilkesiana*, *S. scabrum* and *A. indica* respectively than other extracts (Figure 1). This suggests that these solvents are better for the extraction of plant antioxidants in the studied plants. Study had also shown that antioxidant activity of extracts is strongly dependents on the extraction solvent (Jang *et al.*, 2007). The linear correlation between TPC and percentage inhibition of DPPH radical showed that phenolics in various extracts had a strong correlation with DPPH radical scavenging activity. The phenolic contents of *A. indica* and *S. scabrum* did not show any correlation with inhibition of DPPH free radicals. Several studies have shown a positive correlation between the total content of phenolic compounds and the antioxidant activity in plants (Kim *et al.*, 2003; Deridane *et al.*, 2006; Bouayed *et al.*, 2007; Lim & Quah, 2007; Tawaha *et al.*, 2007).

Lipid peroxidative activity showed that acetone extract was significantly high compared to methanol, ethanol and ethylacetate extracts of *A. wilkesiana* leaf. In *A. indica* stem bark, ethylacetate extract was significantly high compared to methanol, ethanol and acetone extract while there was no significant difference in all extracts in *S. scabrum*. Generally, the inhibition of lipid peroxidation of all extracts was lower than their respective antioxidant activity against DPPH free radicals. There was a strong negative correlation ( $r = -0.980$ ) that was significantly different at  $p < 0.05$  between inhibition of lipid peroxidation and total flavonoid content of *A. wilkesiana*. This suggests that the extract with the least flavonoid content exerted the highest inhibition of lipid peroxidation. There was also linear correlation ( $r = 0.6$ ) at  $p < 0.05$  between inhibition of lipid peroxidation and flavonoid content of *A. indica*. There was no correlation between inhibition of lipid peroxidation and total phenolic content of all the plants under study.

## Conclusion

The result of this study showed that acetone extract of *S. scabrum* gave the highest TPC while ethanol extract of *A. indica* gave the highest TFC. Although, methanol extract of *A. wilkesiana* gave the highest inhibition of DPPH free radicals activity while ethyl acetate extract of *A. indica* gave the highest inhibition of lipid peroxidation. This present study supports the view that the amount of flavonoid, phenols and extent of antioxidant activity is dependent on the type of plant part and solvent used for extraction.

## REFERENCES

1. Aiyelaagbe O.O, Osamudiamen PM. Phytochemical screening of active compounds in *Mangifera indica* Leaves from Ibadan, Oyo State. *Plant Science Research* 2009; 2(1):11-13.
2. Amarowicz R, Estrella I, Hernández T, Robredo S, Troszyn´ska A, Kosin´ska A, Pegg RB. Free radical-scavenging capacity, antioxidant activity, and phenolic composition of green lentil (*Lens culinaris*). *Food Chemistry* 2010; 121: 705-711.
3. Biu AA, Yusufu SD, Rabo JS. Phytochemical screening of *Azadirachta indica* (Neem) (*maliaceae*) in Maiduguri, Nigeria. *Bioscience research communications* 2009; 21(6): 281-283.
4. Bouayed J, Khosro PB, Rammal H, Dicko A, Desor F, Younos C, Soulimani R. Comparative evaluation of the antioxidant potential of some Iranian medicinal plants. *Food Chemistry* 2007; 104: 364-368.
5. Cao G, Sofic E, Prior RL. Antioxidant and pro-oxidant behaviour of flavonoids: Structure activity relationships. *Free Radical Biology and Medicine* 1997; 22: 749-760.
6. D'Abrosca B, Pacifico S, Cefarelli G, Mastellone C, Fiorentino A. Limoncella' apple, an Italian apple cultivar: Phenolic and flavonoid contents and antioxidant activity. *Food Chemistry* 2007; 104:1333-1337.
7. Dewanto V, Wu X, Adom KK, Liu RH. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of Agriculture and Food Chemistry* 2002; 50:3010-3014.
8. Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chemistry* 2006; 97:654-660.
9. Egwaikhide PA, Gimba CE. Analysis of the phytochemical content and anti-microbial activity of *Plectranthus glandulosus* whole plant. *Middle-East Journal of Scientific Research* 2007; 2: 135-138.
10. EL-Kamali HH, EL-amir MY. Antibacterial activity and Phytochemical screening of ethanol extracts obtained from selected Sudanese medicinal plants. *Current research Journal of Biological Sciences* 2010; 2(2):143-146.
11. Ghimeray AK, Jin C, Ghimine BK, Cho DH. Antioxidant activity and quantitative estimation of azadirachtin and nimbin in *azadirachta indica* A. Juss grown in foothills of Nepal. *African Journal of Biotechnology* 2009; 8 (13): 3084-3091.
12. Hosein HKM, Zinab D. Phenolic compounds and antioxidant activity of *Henna* leaves extract (*Lawsonia inermis*). *World Journal of Dairy and Food Science* 2007; 2: 38-41.
13. Jakopic J., Veberic R, Stampar F. Extraction of phenolic compounds from green walnuts fruits in different solvents. *Acta Agriculturae Slovenica* 2009; 93(1): 11-15.
14. Jang HD, Chang KS, Huang YS, Hsu CL, Lee SH, Su MS. Principal phenolic phytochemicals and antioxidant activities of three Chinese medicinal plants. *Food Chemistry* 2007; 103:749-756.
15. Kim DO, Jeong SW, Lee CY. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chemistry* 2003; 81, 321-326.
16. Koffi E, Sea T, Dodehe Y, Soro S. Effect of solvent type on extraction of polyphenols from twenty three Ivorian plants. *Journal of Animal and Plant Science* 2010; 5 (3): 550-558.
17. Kubola J, Siriamornpurn. Phenolic content and antioxidant activities of bitter melon (*Momordica charantia* L.) leaf, stem and fruit fraction extracts in vitro. *Food Chemistry* 2008; 110:881-890.
18. Lim YY, Quah EPL. Antioxidant properties of different cultivars of *Portulaca oleracea*. *Food Chemistry* 2007;103: 734-740.
19. Loo AY, Jain K, Darah I. Antioxidant and radical scavenging activities of the Pyroligneous acid from a mangrove plant, *Rhizophora apiculata*. *Food Chemistry* 2007;104: 300-307.
20. Maisuthisakul P, Pasuk S, Ritthiruangdej P. Relationship between antioxidant properties and chemical composition of some Thai plants. *Journal of Food Composition and Analysis*. 2008; 21: 229-240.
21. Mensor LL, Fabio SM, Gildor GL, Alexander SR, Tereza CD, Cintia SC. and Suzane GL. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical methods. *Phytotherapy Research*. 2001;15: 127-130.



22. Meot-Duros L, Magne C. Antioxidant activity and phenol content of *Crithmum maritimum* L. leaves. *Plant Physiology and Biochemistry*. 2009; 47: 37-41.
23. Miller NJ, Ruiz-Larrea M.B. Flavonoid and other plant phenols in diet: Their significance as antioxidants. *Journal of Nutrition and Environmental Medicine*. 2002; 12: 39-51.
24. Nahak G, Sahu RK. *In vitro* antioxidative activity of *Azadirachta indica* and *Melia azedarach* Leaves by DPPH scavenging assay. *Nature & Science* 2010; 8(4): 77-82.
25. Neergheen VS, Soobrattee MA, Bahorun T, Aruoma OI. Characterization of the phenolic constituents in Mauritian endemic plants as determinants of their antioxidant activities *in vitro*. *Journal of Plant Physiology* 2006; 166: 787-799.
26. Prakash D, Upadhyay G, Singh BN, Singh HB. Antioxidant and free radical-scavenging activities of seeds and agro-wastes of some varieties of soybean (*Glycine Max*). *Food Chemistry* 2007;104: 783-790.
27. Premanath R, Lakshmidheri N. Studies on antioxidant activity of *Tinospora cordifolia* (Miers) leaves using *in vitro* models. *Journal of American Science*. 2010; 6(10): 736-743.
28. Ruberto G, Baratta MT, Deans SG, Dorman HJ. Antioxidant and antimicrobial activity of *Foeniculum vulgare* and *Crithmum maritimum* essential oils. *Planta Medica*. 2000; 66, 687-693.
29. Saija A, Scalese M, Lanza M, Marzullo D. Flavonoids as antioxidant agents: Importance of their interaction with biomembranes. *Free Radical Biology and Medicine* 1994;19(4): 481-486.
30. Sathishkumar R, Lakshmi PTV, Annamalai A. Effect of drying treatment on the content of antioxidants in *Encostemma littorale* blume. *Research Journal of Medicinal Plant* 2009; 3(3): 93-101.
31. Singleton VL, Rossi JA. Colorimetry of total phenolic with phosphomolybdic-phosphotungstic acid reagent. *Am. J. Enol. Viticult.*, 1965;16:144-158.
32. Subhasree B, Baskar R, Keerthana RL, Susan RL, Rajasekaran P. Evaluation of antioxidant potential in selected green leafy vegetables. *Food Chemistry* 2009;115:1213-1220
33. Sultana B, Anwar F, Ashraff M. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules* 2009; 14:2167-2180.
34. Stanojevi L, Stankovi M, Nikoli V, Nikoli L, Risti D, anadanovic-Brunet J, Tumbas V. Antioxidant activity and total phenolic and flavonoid contents of *Hieracium pilosella* L. extract. *Sensor*. 2009; 9:5702-5714.
35. Taha MME, Wahab SIA, Othman F, Hanachi P, Abdul AB, Al-Zubairi AS. Chemopreventive properties of *Azadirachta indica* aqueous extract on DEN-AAF Hepatocarcinogenized rats. *International Journal of Molecular Medicine & Advance Science* 2008; 4(2):50-54.
36. Tawaha K, Alali FQ, Gharaibeh M, Mohammad M, EL-Elimat T. Antioxidant activity and total phenolic content of selected Jordanian plant species. *Food Chemistry* 2007; 104: 1372-1378.
37. Turkmen N, Velioglu YS, Sari F, Polat G. Effect of extraction conditions on measured total polyphenol contents and antioxidant and antibacterial activity of black tea. *Molecules* 2007; 12: 484-496.
38. Udobang J, Nwafor PA, Okonkon JE. Analgesic and antimalaria activity of crude leaf extract and fractions of *acalypha wilkesiana*. *Journal of Ethnopharmacology* 2010; 127: 373-378.
39. Wong SP, Leong LP, Koh JHW. Antioxidant activities of aqueous extracts of selected plants. *Food Chemistry* 2006; 99: 775-783.

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