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RESEARCH ARTICLE **OJBR0301002-288**

IMPACT OF SOLVENT POLARITIES ON ANTIOXIDANT CAPACITY OF SOME MEDICINAL PLANTS

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ABSTRACT

It is well established that various extraction factors, including the method, temperature, time, and solvent system, significantly influence the antioxidant quality of plant-derived products. Extraction conditions are an important factor in the process of obtaining bioactive compounds from plant matrix. Previously, extraction of the plant materials was carried out with Polar (methanol and ethanol) solvents via maceration and thereafter assayed for its antioxidant capacity. In this study, solvents of gradient polarity were used to fractionate the crude extracts of ten (10) plant materials and thereafter assayed using an in-vitro DPPH assay for their antioxidant capacity. Findings revealed that one (1) of the ten (10) plant materials gave fractions that were very prominent in scavenging free radicals across the non-polar and mid-polar solvents which was supported by the IC50 values obtained (ASH= 0.77 µg/ml; ASC=0.05 µg/ml; ASE=0.62 µg/ml: CNNB= 0.98 µg/ml; PRRA=0.99 µg/ml). However, the standard ascorbic acid showed better activity (Vit. C = 0.04).

Keywords: *Antioxidant capacity, Gradient solvents, In-vitro DPPH assay, Oxidative stress, Polarity*

INTRODUCTION

The human body responds to oxidative stress with antioxidant defense, but in certain cases, it may be insufficient, triggering different physiological and physiopathological processes. Currently, many processes are identified related to the production of free radicals. Among them are mutagenesis, cell transformation, cancer, arteriosclerosis, myocardial infarction, diabetes, inflammatory diseases, central nervous system disorders, and cell ageing (Valko *et al.*, 2007). The naturally occurring antioxidant compounds have been proven effective in preventing oxidative damage to living and non-living systems (Fukumoto and Mazza, 2000). These substances are either synthesized endogenously or taken from exogenous natural sources such as plants. Naturally occurring antioxidants include some enzymes such as glutathione peroxidase, catalase, superoxide dismutase and some non-enzymatic phytochemicals compounds including phenolic acids, polyphenols, flavonoids, anthocyanins, ascorbic acid, tocopherols, and β-carotenes (Salazar, 2008). In the literature, several techniques are available for the determination of the anti-oxidative potential of plant samples. However, a more efficient and simplistic method amongst them is the *In-vitro* DPPH assay. This method can be used with both polar and non-polar organic solvents to evaluate hydrophilic and lipophilic antioxidants (Mac-Donald, 2006; Nimse and Pal, 2015). In a previous study by Yesufu *et al.* (2018) the antioxidant capacity of crude extracts of the plants mentioned below was evaluated, this research work was designed to evaluate the scavenging potentials of the plants' fractions from solvents of different polarity.

MATERIALS AND METHODS

The crude extracts of ten (10) previously extracted plant materials which include *Adansonia digitata leave (Malvaceae), Annona squamosa leaves, (Annonaceae), Boswellia dalzielii stem bark (Burseraceae), Cola acuminata leaves (sterculiaceae), Parkia biglobosa leaves, (Fabaceae), Piliostigma reticulatum leaves (leaves), Securidaca longepedunculata (root bark), Senna singueana (leaves), Tamarindus indica (stem bark), Vitellaria paradoxa (stem bark)* were extracted by cold maceration and then fractionated with gradient solvents which include *n*-hexane, chloroform, ethyl acetate, *n*-butanol and water. The fractions were concentrated at room temperature in beakers and allowed to dry in a fume cupboard and their weights were recorded.

DPPH ASSAY (*IN-VITRO*)

The radical scavenging potential was evaluated using DPPH assay (Mentor *et al.*, 2001). 3 mL of 0.004% DPPH working solution (prepared using DPPH stock solution and methanol in correct proportions to give 0.899 abs) was added per every 100 µL of different concentrations (6.25, 12.5, 25, 50 and 100 µg/ml) of the extract and incubated at 37^0C for 30 minutes in the dark. Absorbance was taken at 517nm wavelength in UV spectrophotometer. The negative control contained 100 µL of methanol in place of the sample solution. The percentage antioxidant inhibition was obtained by the equation:

% AOA = Control (Abs) -Sample (Abs) / Control (Abs) * 100.

Ascorbic acid was used as a positive control. Inhibition curves were made and IC_{50} value per sample was calculated by regression analysis (Sebaugh, 2011).

RESULTS

The results for the *in-vitro* scavenging assay are shown in Figures 1-5. The various fractions obtained from solvents of different polarities (n-hexane, chloroform, ethylacetate, n-butanol and aqueous residue) were analyzed using the assay spectrometrically and results were compared across all the plant materials. Also, the fractions have been symbolically represented as follows; PRH- *P. reticulatum* hexane fraction; TMH- *T. indica* hexane fraction; ADH- *A. digitata* hexane fraction; PBH- *P. biglobosa* hexane fraction; SLTH- *S. longepedunculata* hexane fraction; VPH-*V. paradoxa* hexane fraction; SSH- *S. singueana* hexane fraction; ASH- *A. squamosa* hexane fraction; CNH- *C. nitida* hexane fraction; BDH- *B. dalzielii* hexane fraction

PRC- *P. reticulatum* hexane fraction; TMH- *T. indica* hexane fraction; ADH- *A. digitata* hexane fraction; PBH- *P. biglobosa* hexane fraction; SLTH- *S. longepedunculata* hexane fraction; VPH-*V. paradoxa* hexane fraction; SSH- *S. singueana* hexane fraction; ASH- *A. squamosa* hexane fraction; CNH- *C. nitida* hexane fraction; BDH- *B. dalzielii* hexane fraction

The results from Fig 1 which was obtained at 6.25 µg/ml showed the following.

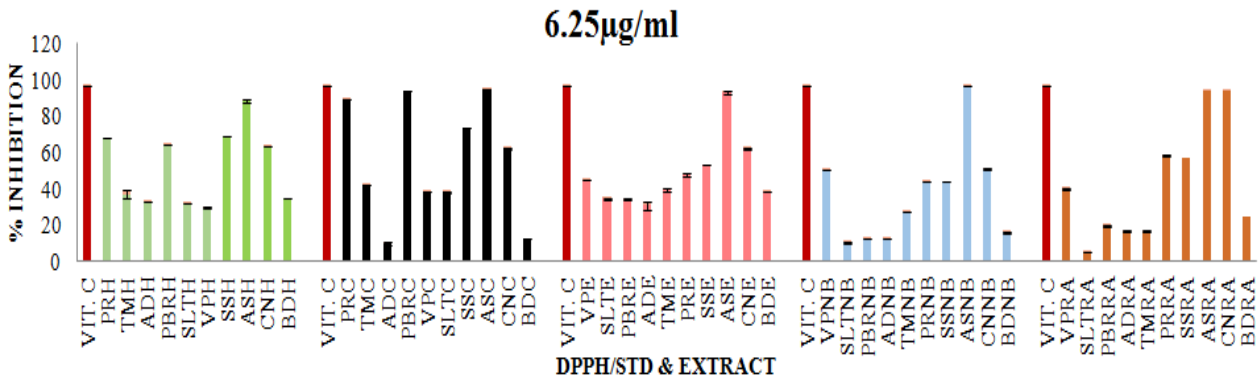

Figure 1: Percent Inhibition of DPPH free radicals at Conc. 6.25µg/ml

In fig.1, the n-hexane, chloroform, ethyl acetate and n-butanol fractions revealed *Annona squamosa* (ASH, ASC, ASE, ASND) showed the highest percent inhibition. *Vitellaria paradoxa* gave the least for n-hexane (VPH), *Adansonia digitata* showed the least for chloroform (ADC) and ethylacetate (ADE) while *Securidaca longepedunculata* showed the least for n-butanol (SLTNB). In residual aqueous fraction, findings showed improved inhibition for *C. acuminata* (CNRA) like *A. squamosa* (ASRA) while *S. longepedunculata* (SLTRA) gave the least inhibition.

The result from figure 2 obtained at 12 µg/ml shows the following.

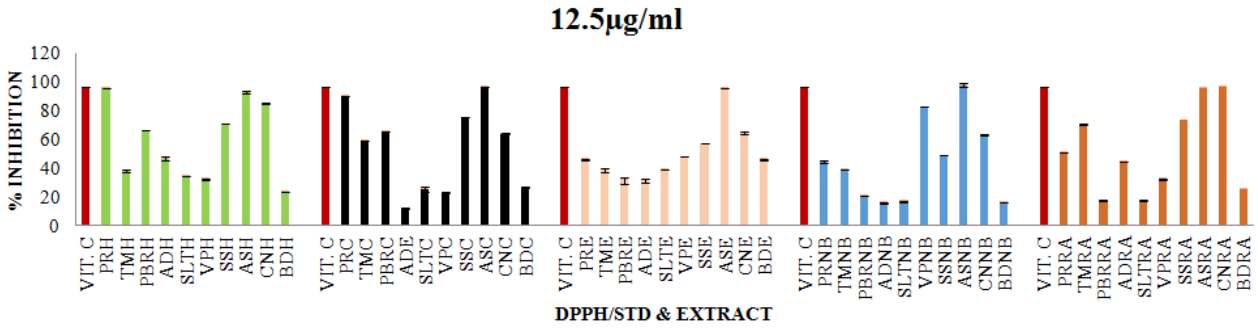

Figure 2: Percent Inhibition of DPPH free radicals at Conc. 12.5µg/ml

In figure 2, n-hexane fraction of *Piliostigma reticulatum* (PRH) gave the highest inhibition though closely followed by *A. squamosa* (ASH) while *B. dalzielii* (BDH) gave the least inhibition. With the mid polar chloroform, ethylacetate and n-butanol fractions, A. squamosa (ASC, ASE, ADNB) gave the highest. *A. digitata* (ADH) showed the least for chloroform, *P. biglobosa* declined to give the least for ethylacetate, while *B. dalzielii* showed the least for n-butanol. In the residual aqueous fraction, *C. accuminata* (CNRA) competed favourably with *A. squamosa* (ASRA) to show the highest inhibition while S. longepedunculata gave the least inhibition.

The result from fig 3 obtained at 25µg/ml showed the following.

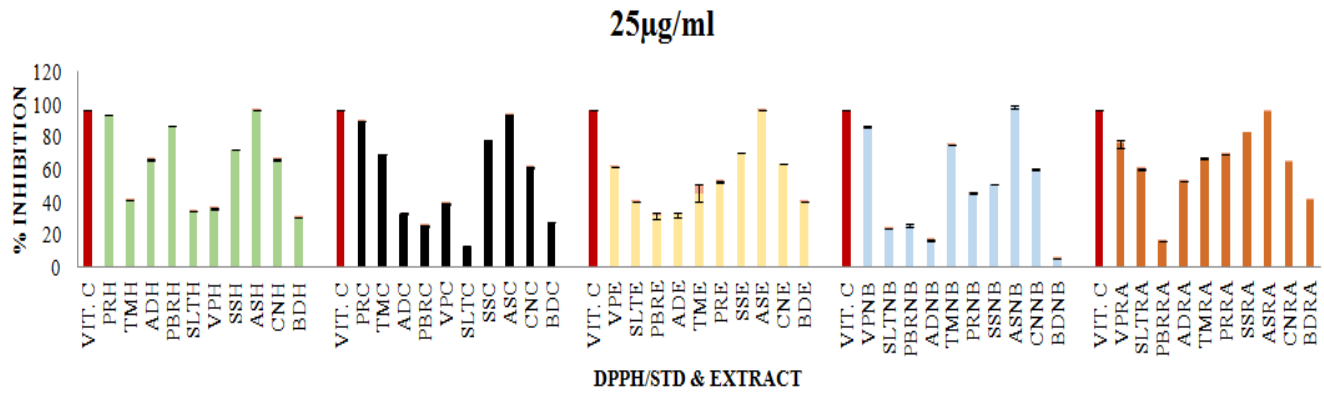

Figure 3: Percent Inhibition of DPPH free radicals at Conc. 25µg/ml

In figure 3 All the fractions; n-hexane, chloroform, ethylacetate, n-butanol and residual aqueous showed *A. squamosa* (ASH, ASC, ASE, ASNB, ASRA) gave the highest inhibition. while *B. dalzielii* (BDH) gave the least for n-hexane, S. *longepedunculata* (SLC) gave the least inhibition for chloroform. *P. biglobosa* (PBRE) and *A. digitata* (ADE) showed very close inhibition to give the least for ethylacetate, *B. dalzielii* (BDNB) gave the least for n-butanol while *P. biglobosa* (PBRRA) gave the least inhibition for the residual aqueous fraction.

The result from figure 4 obtained at 50µg/ml showed the following.

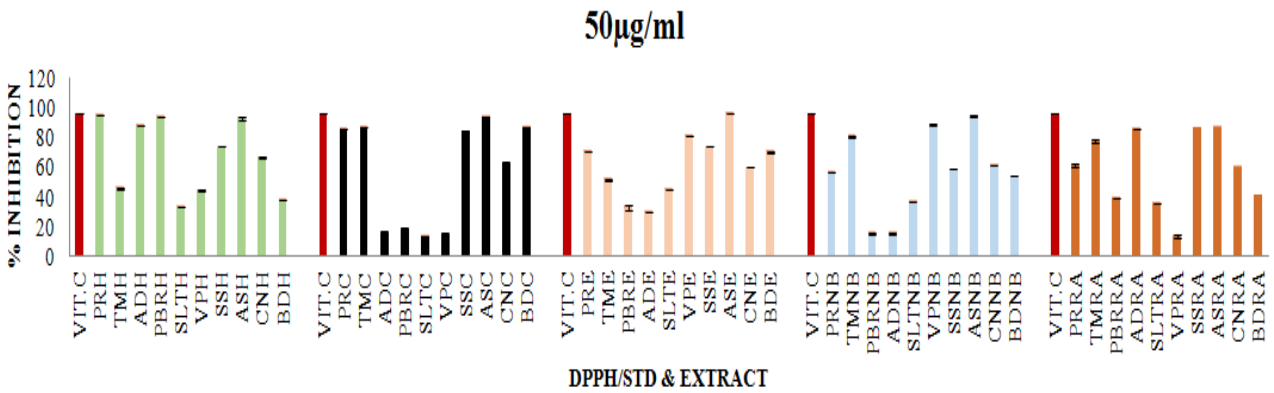

Figure 4: Percent Inhibition of DPPH free radicals at Conc. 50µg/ml

In figure 4, the n-hexane fraction showed closely elevated inhibition for *P. reticulatum* (PRH), *P. biglobosa* (PBRH) and *A. squamosa* (ASH) with PRH having the highest while *S. longepedunculata* (SLTH) gave the least. However, chloroform, ethylacetate, n-butanol and residual aqueous fractions, revealed *A. squamosa* (ASC, ASE, ASNB, ASRA) had the highest inhibition with *S. longepedunculata* (SLTC), as the least for chloroform. *A. digitata* showed the least for ethylacetate (ADE) and n-butanol (ADNB) while *V. paradoxa* (VPRA) gave the least inhibition for residual aqueous.

The result from figure 5 obtained at 100µg/ml showed the following.

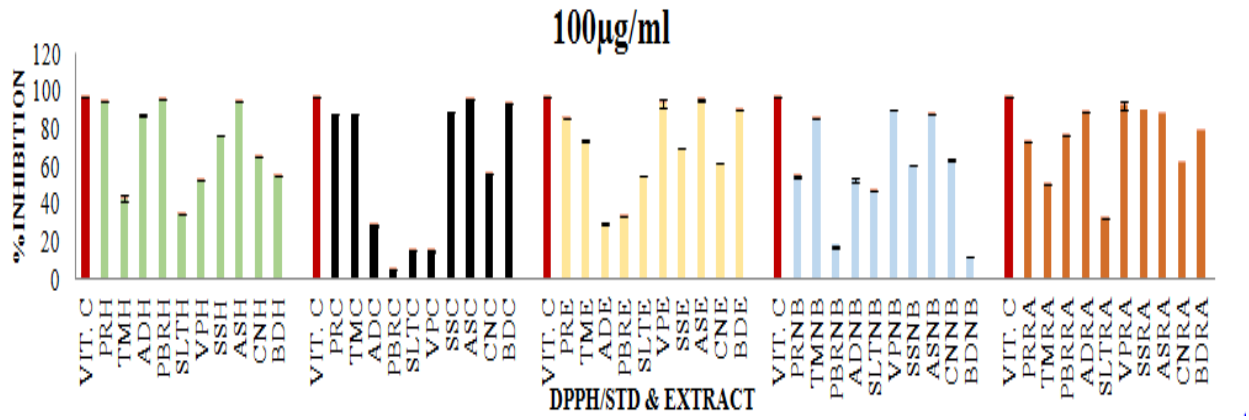

Figure 5: Percent Inhibition of DPPH free radicals at Conc. 100µg/ml

In figure 5, the *n*-hexane fraction showed closely elevated activity for *P reticulatum* (PRH), *P. biglobosa* (PBRH) and *A.squamosa* (ASH) with *P reticulatum* (PRH) having the highest inhibition while *S. longepedunculata* (SLTH) gave the least. *A. squamosa* maintained higher inhibition for chloroform (ASC) and ethylacetate (ASE) with *P. biglobosa* chloroform (PBRC) and *A.digitata* ethylacetate (ADE) having least inhibition *V. paradoxa* was prominent for n-butanol (VPNB) and residual aqueous (VPRA) while the least was *B. dalzielii* for n-butanol (BDNB) and *S. longepedunculata* for residual aqueous (SLTRA).

The results for IC_{50} calculated for the fractions are shown in Table 1 as follows;

Table 1: IC_{50} values of Plant Fractions obtained from Gradient Solvents.

Plant Material (µg/ml)	Hexane	Chloroform	Ethylacetate	n-Butanol	Residual Aqueous
A. digitata	1.01	1.09	0.62	1.06	1.02
A. squamosa	0.77	0.05	0.62	1.32	1.26
B. dalzielii	2.99	3.15	3.08	2.84	3.11
C. acuminata	0.85	1.13	1.51	0.98	1.03
P. biglobosa	0.99	0.99	1.61	1.45	1.04
P. reticulatum	0.97	1.91	1.01	1.02	0.99
S. longepedunculata	1.89	0.98	1.04	1.05	1.04
S. singuaena	0.91	0.95	0.99	1.02	0.99
T. indica	1.07	1.01	1.02	1.02	1.02
V. Paradoxa	1.04	1.02	1.01	0.99	1.02

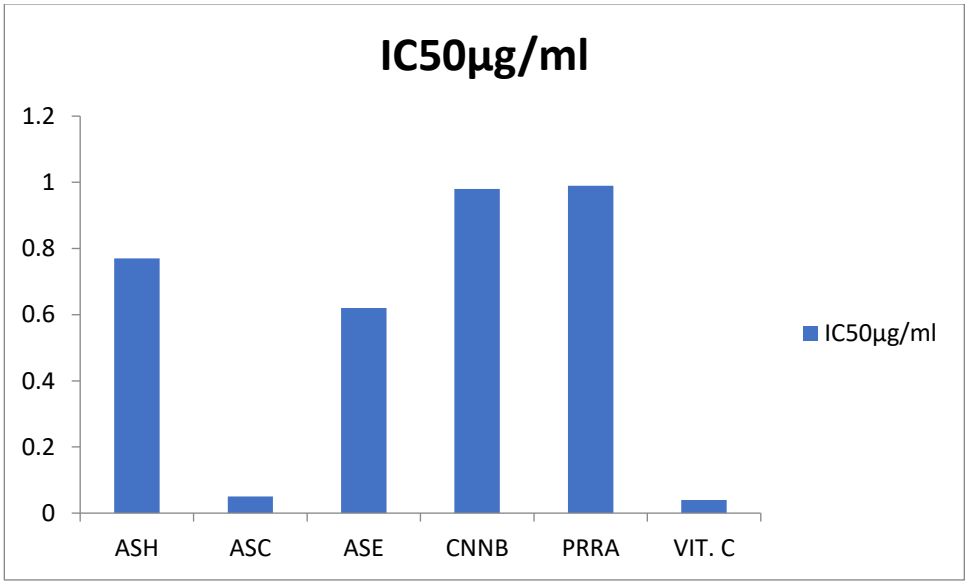

Figure 6: IC_{50}- Inhibition based on Solvent Selection (ASH= *A. squamosa* hexane; ASC = *A. squamosa* chloroform; *A. squamosa* ethylacetate; CNNB= *C. acuminata*; *n*-butanol; PRRA = *P. reticulatum* aqueous)

DISCUSSION

Several *in-vitro* chemical assays with different reaction principles have frequently been used to determine the antioxidant potential of plant extracts. DPPH radical assay is simple and the most common by which we can quickly understand the antioxidant efficiency of any compound, including plant extracts in a short time (Huang *et al.*, 2005; Kadare and Singh, 2011). When a solution of DPPH• is in contact with a substance that can donate a hydrogen atom

or with another radical (R•), the reduced form DPPH-H or DPPH-R is produced with the consequent loss of colour and therefore the decrease or loss of absorbance. Consequently, the reduction of DPPH• provides an index to estimate the ability of the test compound to trap radicals (Gramza *et al.,* 2004; Sandra *et al.,* 2006). The experimental model uses the percentage of DPPH• remaining to obtain the quantities that are required to reduce the initial concentration to 50% (IC_{50}). Low values of IC_{50} indicate a high antioxidant strength, and a rapid decrease in absorption is observed during the reaction (Vertuani *et al.,* 2004).

The results obtained across the different concentrations gave an in-cite into the role solvents can play in the bioactivity of the plant material. At 6.25µg/ml, *A. squamosa, C. acuminata S. singuaena* showed prominent scavenging potential in non-polar, mid polar and polar solvent. *P. biglobosa* and *P. reticulatum,* were prominent inhibitors in non-polar and mid-polar solvent while *V. paradoxa* showed increase potential in polar solvent. The degree of activity is as follows; AS> SS> CN> PR> PB> VP> TM> SL> BD> AD. At 12.5µg/ml, Similar findings occurred except that *S. singuaena* showed increased potential in the non-polar solvent than in polar solvent. The degree of activity is as follows; AS> SS> CN> BD> PR> PB> VP> TM> BD> AD> SL. At 25µg/ml, findings were similar except for the increased potential of *T. indica* in the mid-polar solvent. The degree of activity is as follows; AS>SS>CN>PR>TM>VP>PB>AD>SL>BD. At 50µg/ml, findings showed increased potential in the mid polar for *B. dalzielii* with degree as follows; AS> SS> CN> PR> BD> TM> VP> PB> AD> SL. At 100µg/ml, findings showed V. paradoxa had increased potential in polar solvents with degree as follows; AS> BD> SS> CN> VP> PR> TMC> AD> PB> SL.

The IC_{50} value is defined as the amount of sample to elicit 50% reduction of initial DPPH concentration. It was calculated from the linear regression of the plot of concentration of test compound (µg/ml) against % of inhibition. This was obtained based on the scavenging potential of the plant material in different solvents as shown in Table 1 above. For the non-polar *n*-hexane, *A. squamosa* (ASH) demonstrated the highest antioxidant capacity with IC_{50}= 0.77. The degree of IC_{50} are as follows; AS> CN> SS> PR> PB> AD> VP> TM> SL> BD. For the mid-polar chloroform, *A. squamosa* demonstrated the highest antioxidant capacity with IC_{50}= 0.05. The degree of IC_{50} is as follows; AS> SS> SL> PB> TM> VP> AD> CN> PR> BD. For mid- polar ethylacetate, *A. squamosa* demonstrated the highest antioxidant potential with IC_{50}= 0.62. The degree of IC_{50} is as follows; AS> AD> SS> VP> PR> TM> SL> CN> PB> BD. For the near polar n-butanol, C. accuminata demonstrated the highest antioxidant potential with IC_{50}= 0.98. The degree of IC_{50} are as follows; CN> VP> SS> PR> TM> SL> AD> AS> PB> BD. For the Polar aqueous residue, P. reticulatum demonstrated the highest antioxidant capacity with IC_{50}= 0.99. The degree of IC_{50} are as follows; PR> SS> AD> VP> TM> CN> SL> PB> AS> BD.

Kafuti *et al.* (2019) earlier investigated crude and solvent fractions of *B. dalzielii* stem bark and reported it to possess antioxidant activity in DPPH assay (IC_{50}= 50.11) for hexane and (IC_{50}= 3.15) for ethylacetate. Although, there is contrast to our findings in n-hexane (IC_{50}= 2.99) it corroborates the findings for mid-polar chloroform (IC_{50}= 3.15) and ethylacetate (IC_{50}= 3.08). Muanda *et al.* (2010) reported high antioxidant activity from the root bark of *S. longepedunculata* (IC_{50}= 5.5 µg/ml). While that reported in the study was lower (IC_{50}= 0.98-1.89 µg/ml). Aderogba *et al.* (2005) reported antioxidant activities of compounds isolated from ethylacetate leaves extract of P. reticulatum to range between 4.68-50.7µg/ml. while the study reported its ethylacetate extract at IC_{50}= 1.01µg/ml. Talla *et al.*

(2016) has reported scavenging activities in crude and isolated compound of stem bark of *V. paradoxa*, where it was observed that the crude methanol extract (CME) exhibited more scavenging property (IC_{50} =12.28 µg.mL^{-1}) than the isolated (-)-epicatechin (IC_{50}= 22.18µg.mL^{-1}). In the study, all fractions of *V. paradoxa* showed lower values (IC_{50}= 0.99- 1.04 µg/ml) thus supporting its potency. Baska *et al.* (2007) earlier reported antioxidant activity of three annona species; *A. squamosa* (IC_{50}= 0.94 µg/ml), *A. reticulata* (IC_{50}= 0.98 µg/ml) and *A. muricata* (IC_{50}= 0.94 µg/ml). Whereas findings in the study were or had a close agreement (IC_{50}= 0.05- 1.26µg/ml) to that reported. In accordance with the criteria for evaluation of *in-vitro* antioxidant activities of natural products, some studies (Omisore *et al.*, 2005; Katalinic *et al.,* 2006; Ndhala *et al.,* 2010; Lawal *et al.,* 2016) have proposed the following; i- crude extracts and compounds are considered to have high or significant inhibition or capacity at (IC_{50} < 10 µg/mL for extract and IC_{50} < 1 µg/mL for compounds), promising activity (IC_{50} = 10–50 µg/mL for extract and IC50 = 5–10 µg/mL for compounds), moderate activity (IC_{50} = 50–100 µg/mL for extract and IC50 = 5–10 µg/mL for compounds), while samples with IC50 > 100 µg/mL for extract and > 10 µg/mL for compounds were considered to have low antioxidant capacity.

CONCLUSION

The study focused on the antioxidant potential of gradient solvent fractions of some medicinal plants. The solvents ranged from non-polar to polar solvents. One (1) of the Plant materials (*A. squamosa*) seemed to have inhibited consistently in all concentrations across all the solvents. This was supported by their IC_{50} results as shown in fig 6 above.

ACKNOWLEDGMENTS

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AUTHORS' DECLARATION

The authors hereby declare that the work presented in this article is original and has not been previously submitted in any journal.

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