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DETERMINATION OF THE ANTIOXIDANT CAPACITY OF THE CRUDE EXTRACTS OF SOME MEDICINAL PLANTS

Hassan B. Yesufu*[1], Ibrahim Iliya[1], Fanna I. Abdulrahman[2]

[1*]Department of Pharmaceutical Chemistry, University of Maiduguri, P.M.B 1069, Maiduguri, Nigeria.

[2]Department of Chemistry, University of Maiduguri, P.M.B 1069, Maiduguri, Nigeria.

ABSTRACT

A total of Ten (10) plant species from some States in Nigeria were analyzed for their antioxidant activities using the DPPH, H_2O_2 and FRAP assay methods. The data obtained showed that *Piliostigma reticulatum* gave the highest scavenging property at the least concentration (6.25µg/ml) while C. accuminata gave the highest scavenging property at the highest concentration (100µg/ml) in DPPH assay. The trend at the least concentration is as follows; *P. reticulatum*> *A. digitata*> *T. indica*> *C. accuminata*> *V. paradoxa*> *B. diezelli*> *S. longipediculata*> *A. squamosa*> *P. biglobosa*> *S. singueana.* While the trend at the highest concentration is as follows; C. accuminata> B. diezelli> T indica> P. biglobosa> A. digitata> V. paradoxa> A. squamosa> P. reticulatum> S. longipediculata> S. singueana. The H_2O_2 assay showed that B diezelli gave the highest scavenging property both at the least (6.25) and highest (100). Also the trend is as follows; B. diezelli> A. squamosa> S. longipediculata> S. singueana> V. paradoxa> P. bigloosa> C. accuminata> A. digitata> T. indica> P. reticulatum at the least concentration. While at the highest, the trend is as follows; B. diezelli> S. longipediculata> S. singueana> V. paradoxa> P. biglobosa> P. reticulatum> C. accuminata> A. digitata> T. indica. The FRAP assay showed that S. longipediculata gave the highest scavenging activity at the least concentration (6.25) while C. accuminata gave the highest activity at the highest concentration. The trend at the least concentration is as follows; S. longipediculata> C. accuminata> T. indica> A. digitata> V. paradoxa> A. squamosa> P. reticulatum> P. biglobosa> S. singueana> B. diezelli. While at the highest concentration the trend is; C. accuminata> S. longipediculata> T. indica> A. digitata> A. squamosa> V. paradoxa> P. reticulatum> P. biglobosa> S. singueana> B. diezelli. In the FRAP assay, five (5) crude extracts (*S. longepedicunlata, T. indica, A. digitata, A .squamosa C. accuminata*) were more active than the standard drug (ascorbic acid and butylated hydroxyl anisole) at higher concentrations.

KEYWORDS

Medicinal Plants, DPPH, H_2O_2, FRAP and *In-vitro assays*.

Author for Correspondence:
Hassan B. Yesufu,
Department of Pharmaceutical chemistry,
University of Maiduguri, Borno State, Nigeria.

Email: hbyesufu@unimaid.edu.ng

INTRODUCTON

The protective action of medicinal plants has been attributed to the presence of antioxidants, especially polyphenolic compounds and antioxidant vitamins including ascorbic acid, tocopherol, β-carotene, flavonoids, tannins, anthocyanins and other phenolic constituents[1-4]. Many diseases, cataracts,

arterosclerosis, diabetes, arthritis, immune deficiency diseases and aging are associated with oxidative damage[4-5]. Many lants contain antioxidant compounds that may function as free radical scavengers, complexers of pro-oxidant metals reducing agents and quenchers of singlet oxygen formation[6]. Occuring naturally in plants, phenolic compounds including flavonoids, tannins and phenolic acids are currently of growing interest due to their biological effect in human health[7-9]. Food rich in antioxidants play an essential role in the prevention of cardiovascular diseases, cancer[10-11] and neurodegenerative diseases including parkinson and alzheimer diseases[12]. During the past decades, extensive analytical research has been carried out on the extracts of various plant resources and products. The techniques often used include UV - Vis spectrophotometry, thin - layer chromatography[13-14] gas-liquid chromatography[15], HPLC[16-17] and capillary electrophoresis[18] in order to determine the antioxidant activity of medicinal plants. The study was conducted to determine the scavenging capacity of some medicinal plant.

MATERIAL AND METHODS

Plant Material

Ten (10) Plants material which include *Adansonia digitata (leaves), Annona squamosa (leaves), Boswella diezelli (stem bark), Cola accuminata (leaves), Parkia biglobosa (stem bark), Piliostigma reticulatum (stem bark), Securidaca longipediculata (stem bark), Senna singueana (leaves) Tamarindus indica (stem bark), Vitelleria paradoxa (stem bark)* were obtained from within and outside Maiduguri.

Extraction

Cold maceration technique was employed for extraction using ethanol and methanol as solvents. After filtration and evaporation to dryness the filtrate obtained were stored

Determination of *In-vitro* Antioxidant Activity

The plants material were subjected to *In-vitro* antioxidant screening employing three assay procedures which are; DPPH *In vitro* assay, Hydrogen peroxide assay and Ferric reducing antioxidant power (FRAP) assay.

DPPH *In-vitro* Assay

The DPPH radical-scavenging assay was carried out according to the method described[19]. One mililiter of a 3mM DPPH (2, 2-diphenyl-1-picrylhidrazyl) methanol solution was added to a solution of the extract or standard (6.25µg/ml-100 µg/ml) and allowed to react at room temperature for 30 mins. The absorbance of the resulting mixture is measured at 518 nm after 30 min of reaction in the dark and converted to percentage antioxidant activity (AA%). Solution of L- ascorbic acid served as positive control. The absorbance was determined using the SP8001 ultraviolet-visible spectrophotometer and the antioxidant activity calculated using the equation below;

$$\% \text{ Antioxidant activity} = \frac{\text{Absorbance of DPPH} - \text{Absorbance of vit C/extract}}{\text{Absorbance of DPPH}} \times 100\%$$

Hydrogen Peroxide Scavenging Assay

This activity was determined according to the method described[20]. An aliquot of H_2O_2 (2 mM) and various concentrations (10-80µg/ml) of samples were mixed (1:1 v/v) and incubated for 10 min at room temperature. After incubation, the absorbance of hydrogen peroxide at 230 nm was determined against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for background subtraction. Solution of L- ascorbic acid and Butylated hydroxyl anisole served as positive control. The absorbance was determined using the SP8001 ultraviolet-visible spectrophotometer and the antioxidant activity calculated using the equation below;

$$\% \text{ Antioxidant activity} = \frac{\text{Absorbance of } H_2O_2 - \text{Absorbance of vit C/extract}}{\text{Absorbance of } H_2O_2} \times 100\%$$

Ferric Reducing Anti-oxidant Power (FRAP) Assay

The reducing power of the extracts was determined according to the method[21]. 10 mg of extract was dissolved in one (1ml) of distilled water. One (1ml) of the reaction mixture containing varying concentration (6.25-200µg/ml) of the text extract and standard were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 ml, 1%). The mixtures were incubated at 50 ^{0}C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture,

then centrifuged at 3000 r.p.m for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%) and the absorbance measured at 700 nm. L-ascorbic acid and BHA served as the reference material. All the tests were performed in triplicate and graph plotted with an average of three observations.

RESULTS

The results obtained using the various assay were as follows;

Summary of Results

The *In-vitro* DPPH assay of Ten (10) medicinal plants showed promising results for the plant materials. At concentration of 6.25 µg/ml, *P. reticulatum* showed better scavenging effect than all other plant when compared to L-ascorbic acid and Butylated hydroxyl anisole which were use as standard antioxidants. At a higher dose of 12.5µg/ml, *T. indica and A. digitata* alongside *P. reticulatum* showed better scavenging effect while at 50g, *B. diezelli, V. paradoxa* and *A. squamosa* showed increase scavenging effect. However, *S. longepediculata, P. biglobosa and S. singueana* showed lower scavenging activity across the different concentrations.

The Hydrogen peroxide assay of the Ten (10) plants showed closely related scavenging effect for A. *squamosa and B. diezelli* when compared to the standard (L-ascorbic acid and BHA). *S. singueana and S. longipediculata* also showed very good inhibition of peroxy radicals. The plants scavenging effect was consistent across the various concentrations (10-80µg/ml).

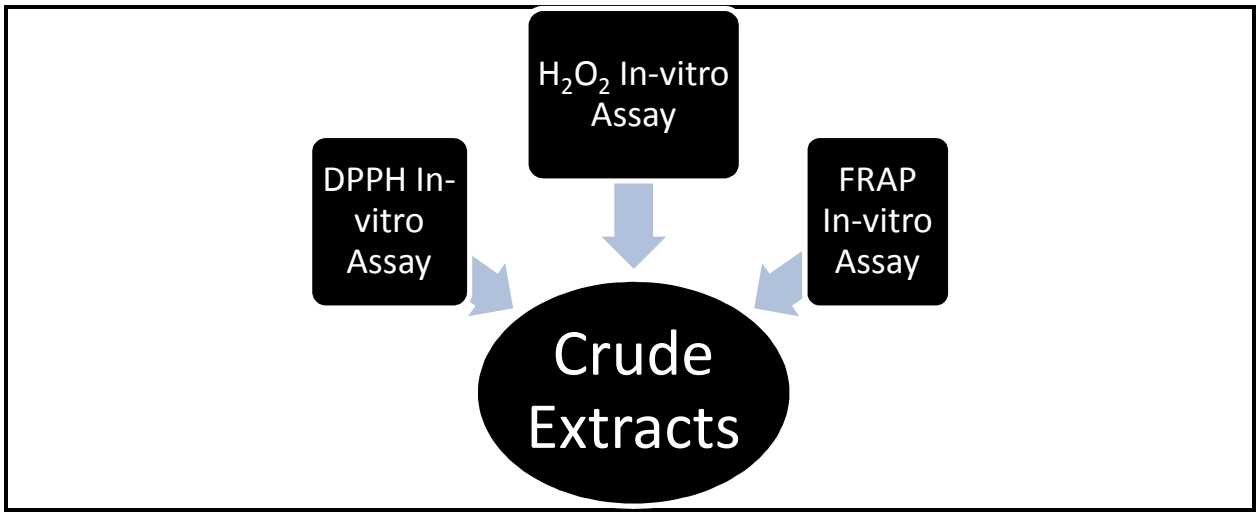
The Ferric reducing antioxidant Power (FRAP) assay of the Ten (10) plants showed better reduction potential for *S. longipediculata, T. indica, A. digitata, A. squamosa, V. paradoxa and C. accuminata* at lower concentration. This was consistent as the concentrations increased. At a concentration of 50µg/ml, all five (5) extracts surpassed L-ascorbic acid and BHA in reducing potential. However, *B. diezelli* showed lower reductive potential across the various concentrations. It should be noted that the stronger the reducing power, the stronger the antioxidant capacity.

DISCUSSION

The DPPH assay revealed three plant material to be very active antioxidants, having capacities that were comparable to the standard drugs. In an earlier study conducted by Alexandre[22], it was observed that decoctions of *Piliostigma reticulatum* barks may help to prevent oxidative damage and infections such as diarrhea and dysentery in the human body and may contribute to food preservation. These show that the bark of *Piliostigma recticulatum* could be used as a potential natural antioxidants and antibacterial agent. Also, the stem bark possess antibacterial and antioxidants properties[23]. *Tamarindus indica* was reported to possess hypolipomic and antioxidant activities[24-25]. Vertuani *et al*[26], and Besco *et al*[27]. reported a comparative study of the in-vitro antioxidant capacity (IAC) of A. digitata (fruit, leaves, seed an fibre). Better activity was found in the fruit fibre. However, other plant parts showed signficant IAC. These studies are in support of our findings that *P. reticulatum, T. indica and A. digitata* possess significant scavenging activity.

The Hydrogen peroxide assay recorded significant inhibition of peroxyl radicals by most of the extracts. Interestingly, plant such as *S. singueana* and *S. longepeducunlata* showed inhibition of the H_2O_2 radicals. However, *A. squamosa* and *B. diezelli* were foremost as they showed comparable inhibition to the Standard drug (Vit C and BHA). Studies have shown that the North Ethiopian species of Senna scavenge free radicals an inhibit erythrocyte hemolysis[28]. While in Nigeria, several studies reported the antioxidant activities and furthermore, anti diabetic activities of the acetone fraction of the stem back and these activities were attributed to resorcinol and dibenzofuran[29,30]. Pandey *et al*[31], reported that the ethanol bark extract of Annona squamosa showed significant antioxidant activity using *in-vitro* antioxidant models like DPPH radical scavenging, Hydroxyl radical scavenging and superoxide radical savenging. Yesufu *et al*[32], reported that the leaf extract of *C.*

accuminata possess antioxidant activity which occurred more in the hexane fraction. *V. paradoxa* was also reported to possess antioxidant activity. Shea butter contains plant antioxidants such as vitamins A and E, as well as catechins. The vitamins A and E protect the cells from free radicals and environmental damage. The cinnamic acid esters in shea fat helps in preventing skin damage from ultraviolet radiation[33]. The optimum antioxidant activity of methanol leaf extract of P. biglobosa was reported in a previous study as 250µg/ml[34].



DPPH *In-vitro* Assay

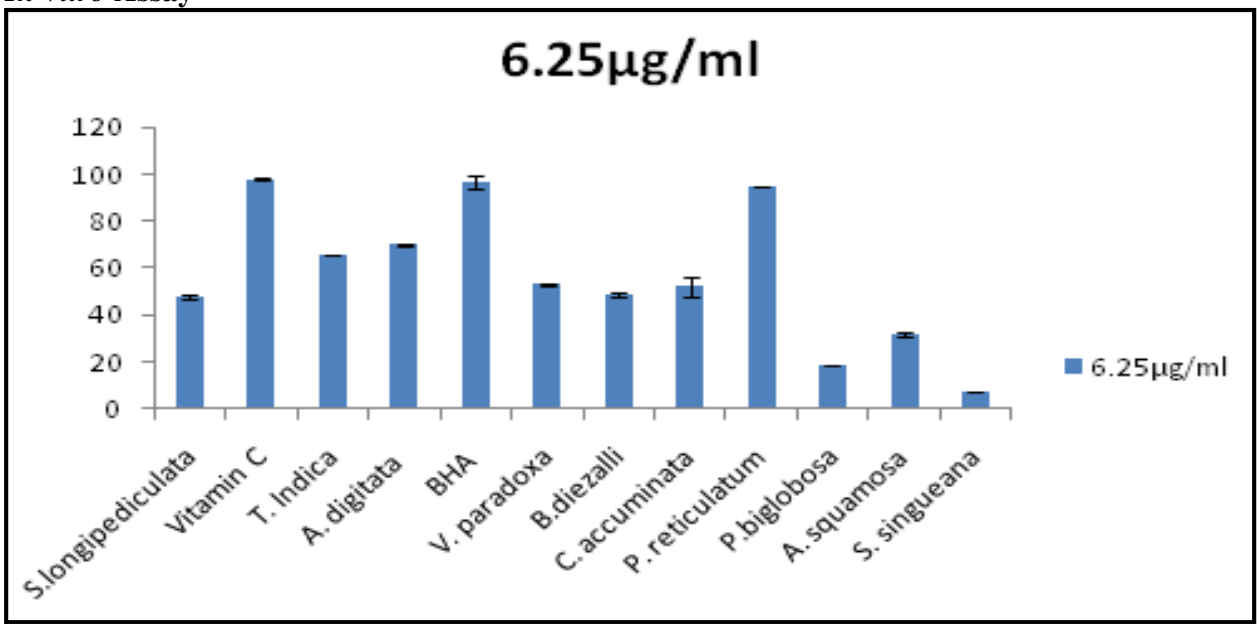


Figure No.1: Antioxidant Capacity of Ten (10) extracts /standards at 6.25µg/ml

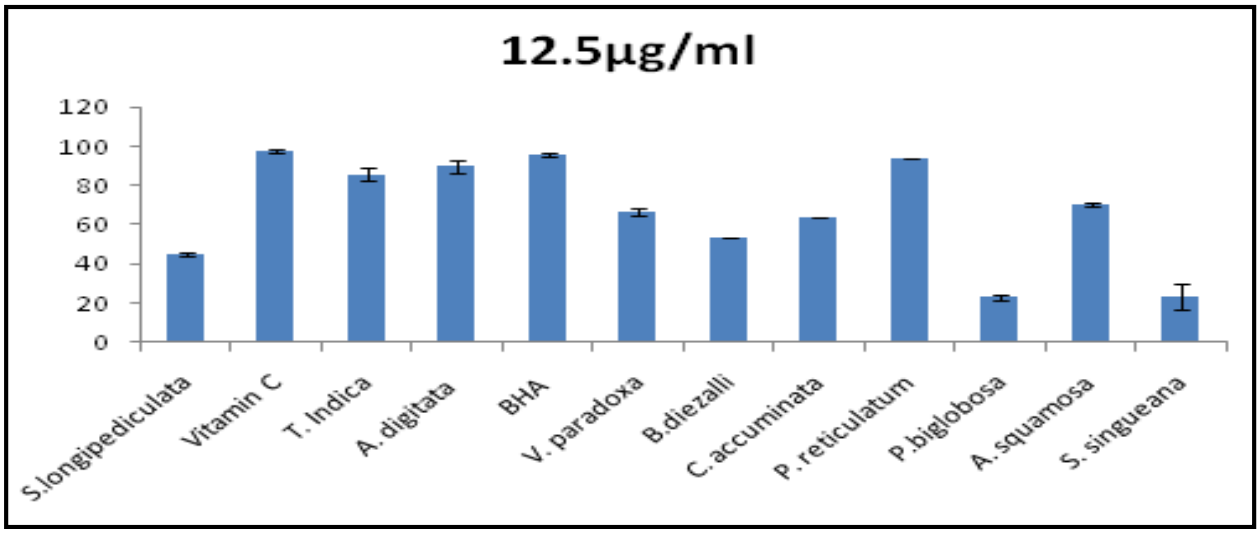


Figure No.2: Antioxidant Capacity of Ten (10) extracts /standards at 12.5µg/ml

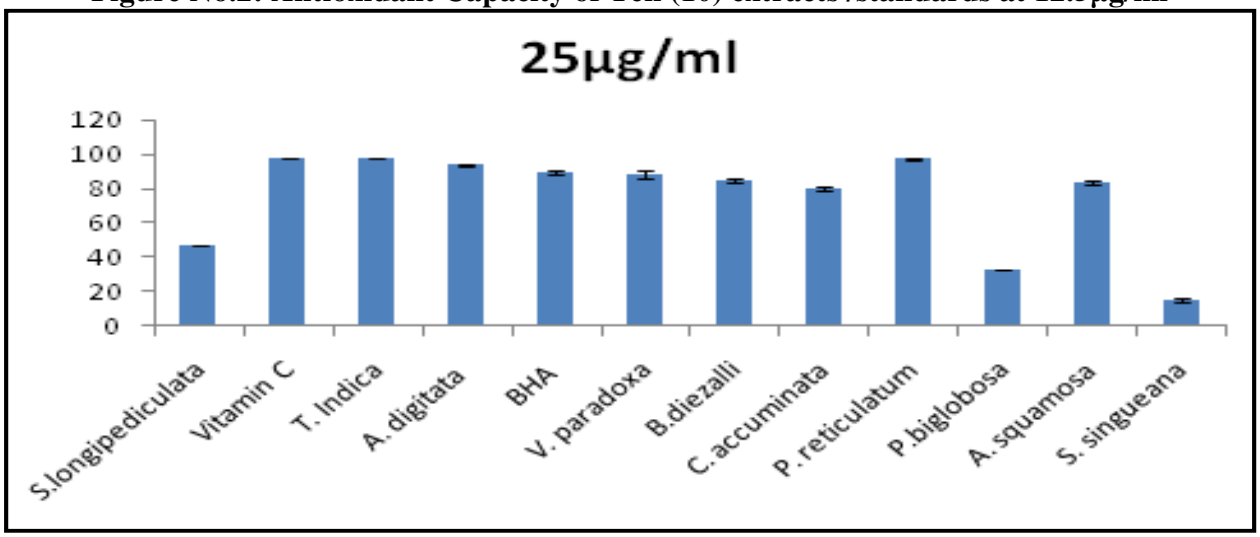


Figure No.3: Antioxidant Capacity of Ten (10) extracts /standards at 25µg/ml

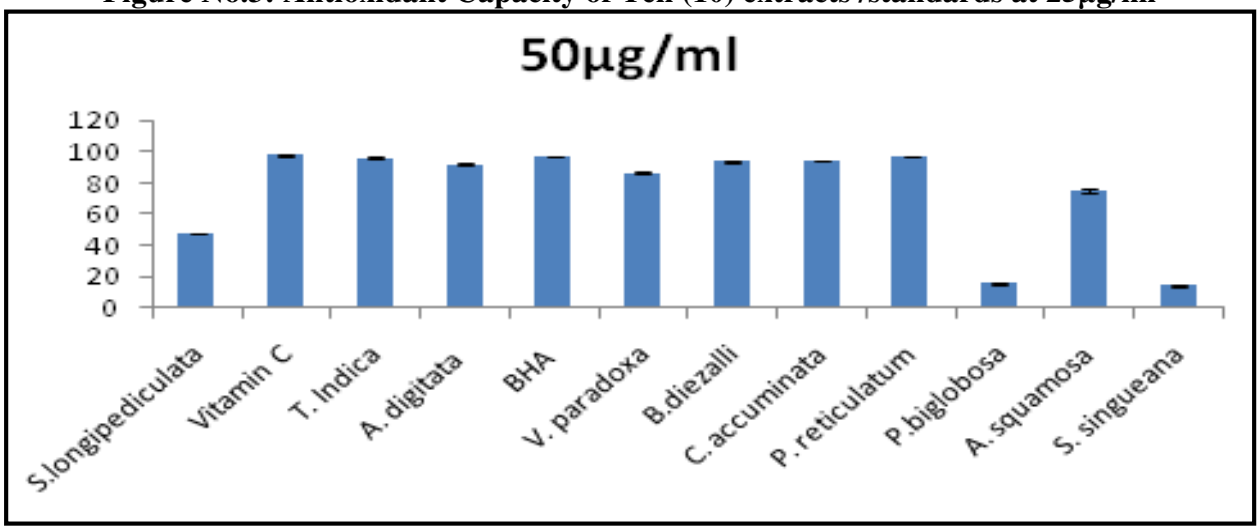


Figure No.4: Antioxidant Capacity of Ten (10) extracts /standards at 50µg/ml

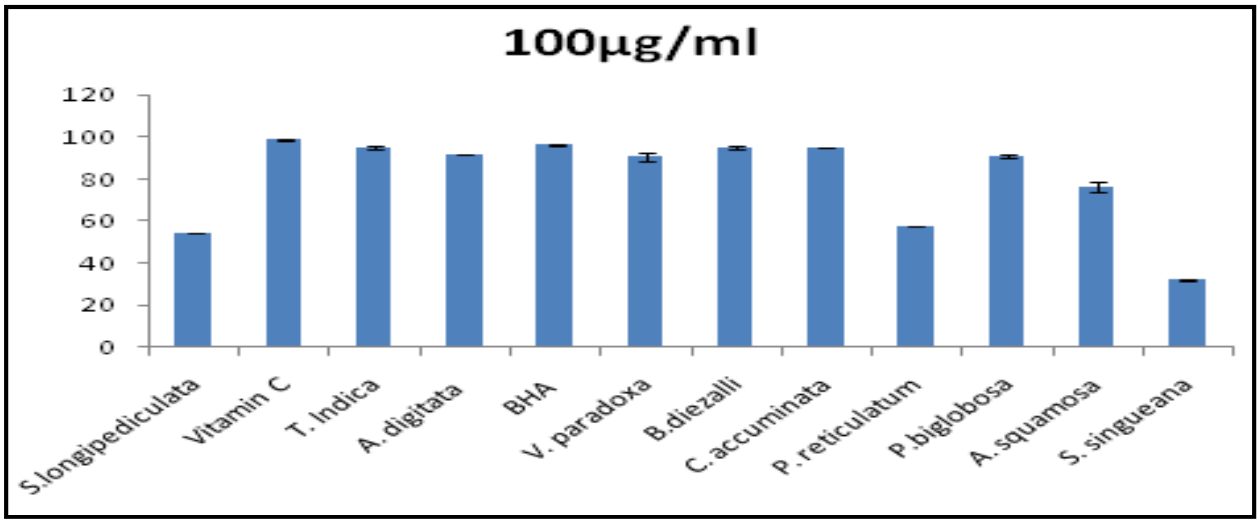


Figure No.5: Antioxidant Capacity of Ten (10) extracts /standards at 100µg/ml

Hydrogen Peroxide (H_2O_2) Assay

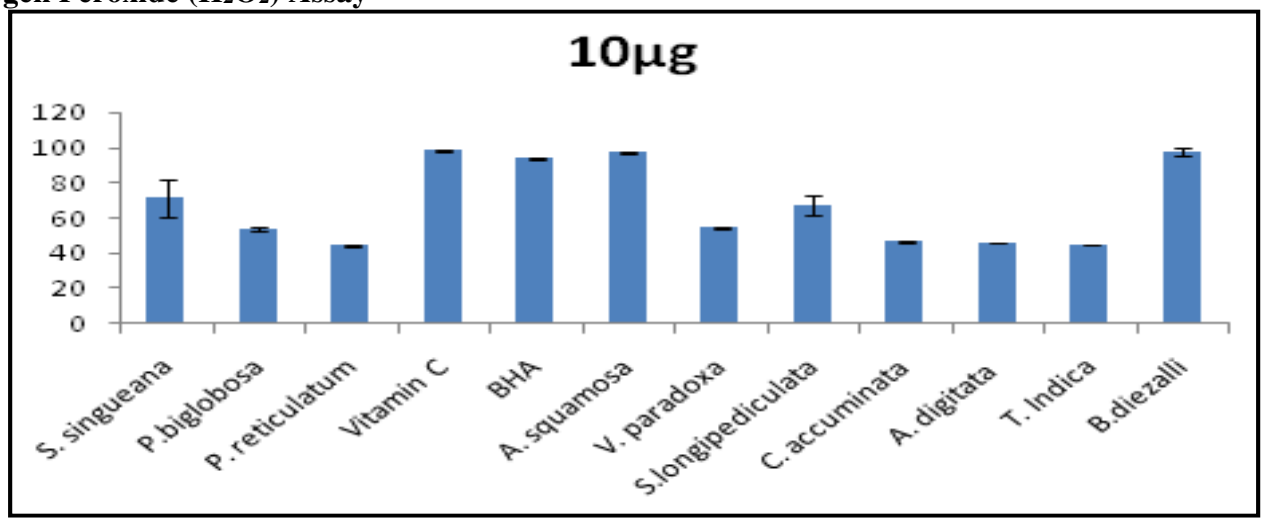


Figure No.6: Antioxidant Capacity of Ten (10) extracts /standards against H_2O_2 radicals at 10µg/ml

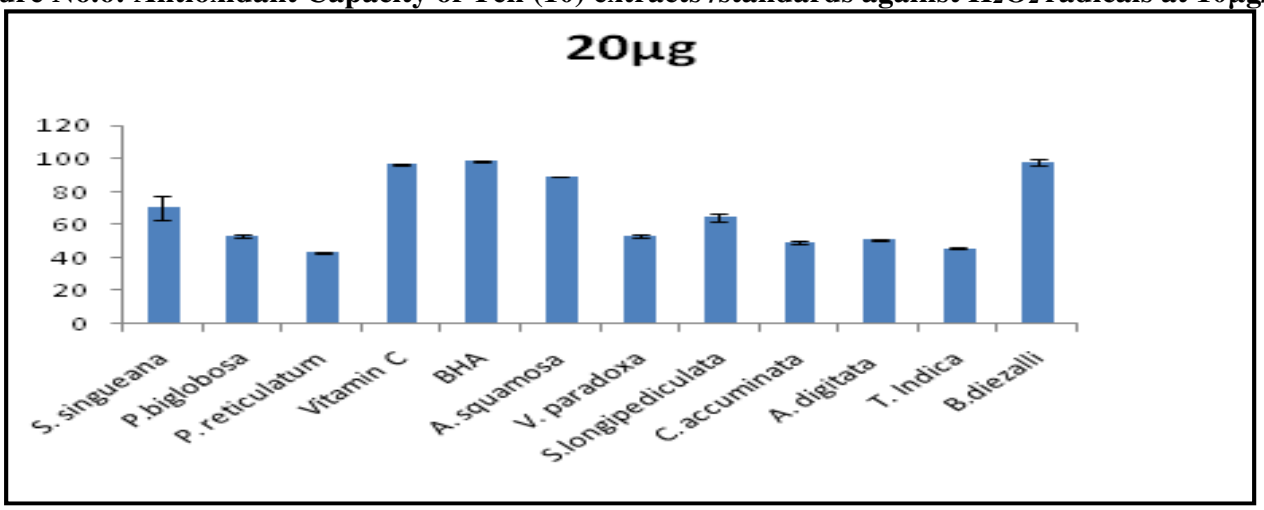


Figure No.7: Antioxidant Capacity of Ten (10) extracts /standards against H_2O_2 radicals at 20µg/ml

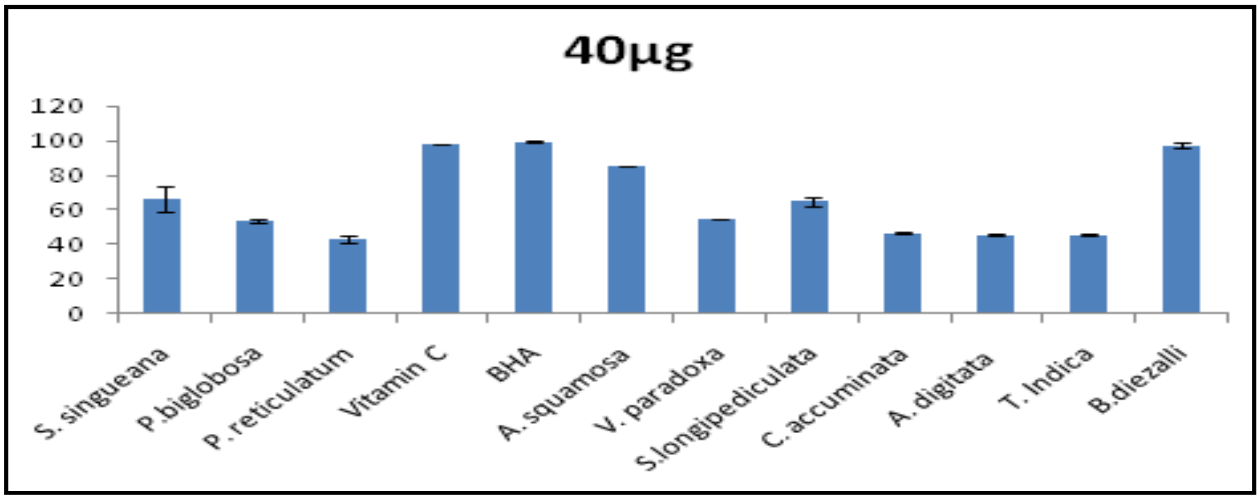


Figure No.8: Antioxidant Capacity of Ten (10) extracts /standards against H_2O_2 radicals at 40µg/ml

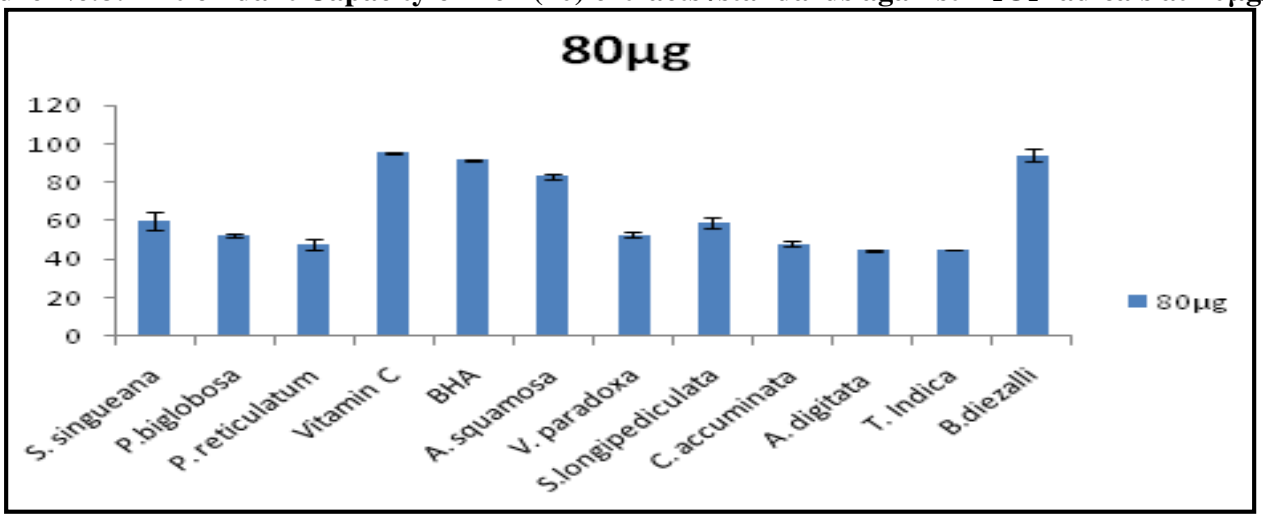


Figure No.9: Antioxidant Capacity of Ten (10) extracts /standards against H_2O_2 radicals at 80µg/ml

Ferric Reducing antioxidant Power (FRAP) Assay

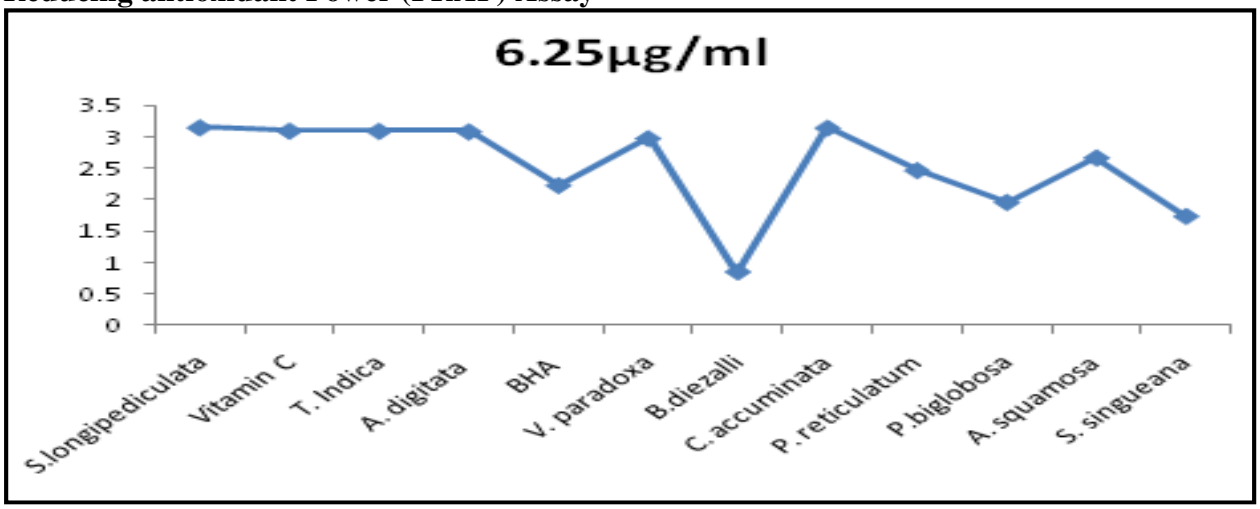


Figure No.10: Antioxidant Capacity of Ten (10) extracts /standards against singlet (0) radicals at 6.25µg/ml

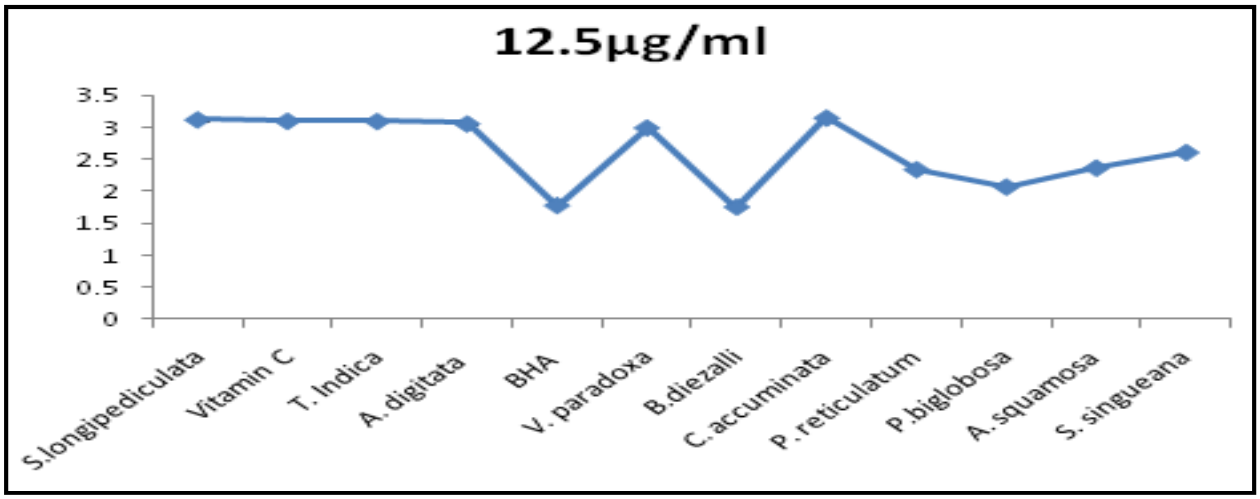


Figure No.11: Antioxidant Capacity of Ten (10) extracts /standards against singlet (0^-) radicals at 12.5µg/ml

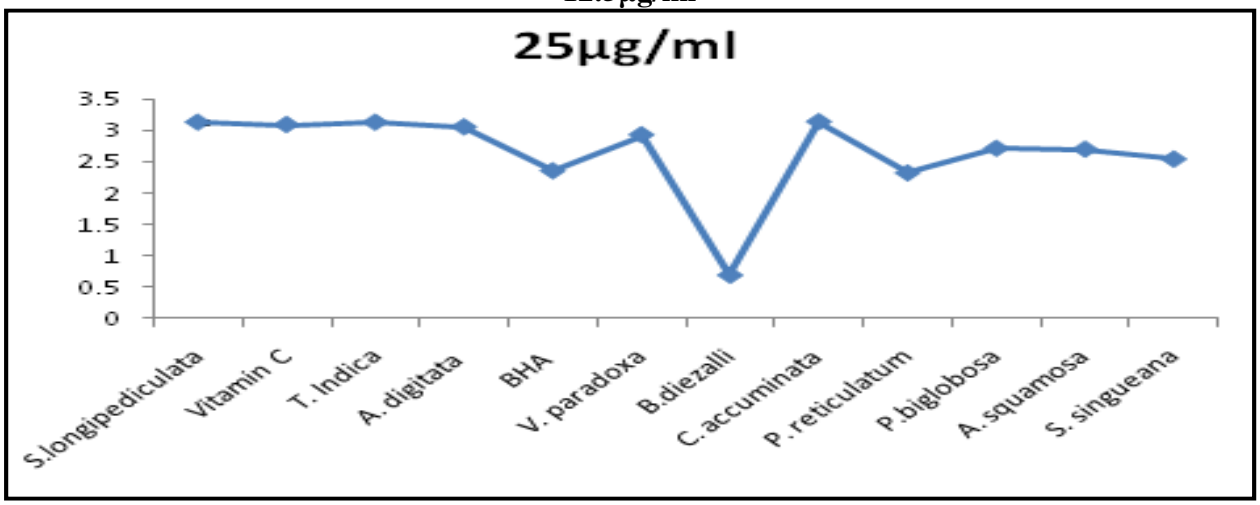


Figure No.12: Antioxidant Capacity of Ten (10) extracts /standards against singlet (0^-) radicals at 25µg/ml

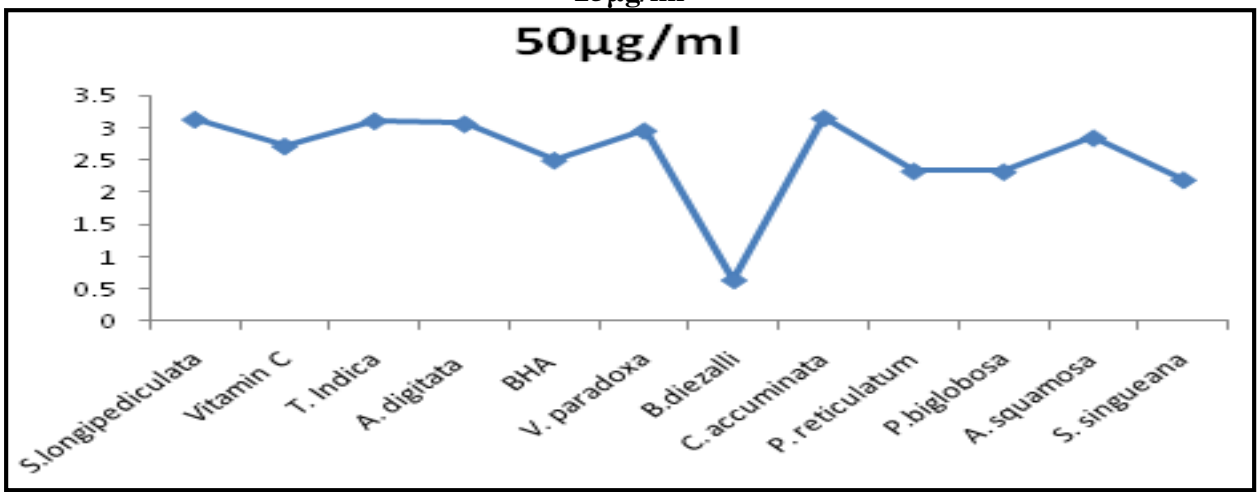


Figure No.13: Antioxidant Capacity of Ten (10) extracts /standards against singlet (0^-) radicals at 50 µg/ml

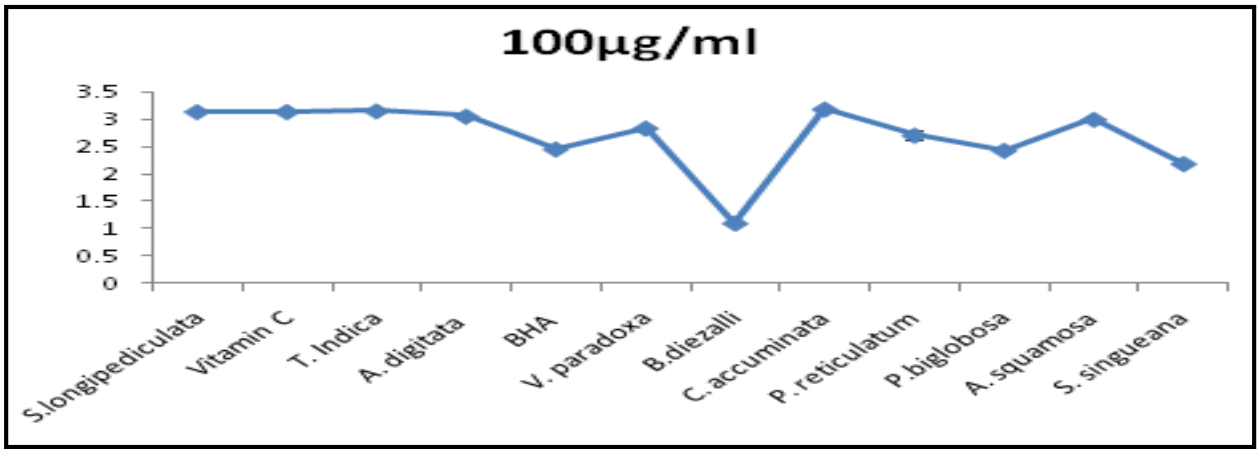


Figure No.14: Antioxidant Capacity of Ten (10) extracts /standards against singlet (0^{-}) radicals at 100 µg/ml

CONCLUSION

Ten medicinal plants (10) were analysed for their antioxidant contents and activities respectively. The methanol crude extract of *Piliostigma reticulatum* was more active in the DPPH assay than all other plants material. *Annona squamosa* and *Parkia biglobosa* methanol crude extracts were most active than the other extracts in the hydrogen peroxide assay. The FRAP assay showed that five (5) crude extracts (*S. longepedicunlata, T. indica, A. digitata, A .squamosa C. accuminata*) were more active than the standard drug (ascorbic acid and butylated hydroxyl anisole) at higher concentrations.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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