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Evaluation of antioxidant potentials of the methanolic leaf extracts of vegetables, fruits and medicinal plants commonly consumed in Kaduna state, Nigeria

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Abstract

The leaves of commonly consumed plants classified into vegetables, fruits and medicinal plants were extracted using methanol and investigated for antioxidant potential. Free radical scavenging activity was evaluated using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) with ascorbic acid as standard. The plant leaves exhibited lower antioxidant activity compared to ascorbic acid with 97.47% inhibition and IC₅₀ value of 8.06 ± 0.02 µg/mL. Among the plant extracts, *Azadirachta indica* (Neem plant) showed the highest antioxidant potential with IC₅₀ value of 14.03 ± 0.06 µg/mL, followed by extract of *Vernonia amygdalina* (Bitter leaf), *Moringa oleifera* (Drumstick tree) and *Ocimum gratissimum* (Clove basil) with IC₅₀ values of 14.53 ± 0.12 µg/mL, 14.88 ± 0.12 µg/mL and 16.89 ± 0.15 µg/mL respectively. The high antioxidant activity exhibited by these plant leaves validates their use in traditional medicine and source of nutrient in food; acting as a potential antioxidant for the prevention of free radical related diseases.

Keywords: Antioxidants, 2, 2-diphenyl-1-picrylhydrazyl, free radicals, vegetables, fruits, medicinal plants

1. Introduction

The current increase in the knowledge of free radicals and reactive oxygen species (ROS) in life sciences has continued to promise a new age of health and disease management^[1]. Living organisms generate free radicals as part of the body's normal metabolic product. Free radicals or oxidative injury now appears to be the primary cause of a number of human disorders^[2]. Consequently, therapy using antioxidants (free radical scavengers) has potentials to prevent or ameliorate many of these disorders^[3]. When there is an imbalance between ROS production and antioxidant defense, it results to oxidative stress which impairs cellular functions through series of events leading to various pathological conditions^[4]. Free radicals are causative agents for several inflammatory diseases which poses a threat to man^[5, 6]. Natural antioxidants may be inefficient so for this reason dietary intake becomes important.

The inconsiderate use of synthetic antioxidants is posing a great challenge to the human environment. It is necessary to develop effective and natural methods for combating these ailments by consuming foods rich in antioxidants. Plants have received attention of researches as potential bioactive compound to combat oxidative stress as a result of free radicals and other ROS. The use of synthetic antioxidants is posing a lot of threat to human environment which may lead to negative health consequences. Besides this, there may be disadvantages in the use of synthetic compounds in humans. Synthetic antioxidants in foods, though usually used in small concentrations, are considered as one of the main factors causing allergies to users. Therefore, the growing demand for a more natural food supplement has called for the replacement of synthetic antioxidants with plants of antioxidant properties.

There is need to control the occurrence of cancer, cardiovascular dysfunction, autoimmune diseases and various inflammatory diseases arising from the accumulation of ROS. So it is important to adopt an alternative strategy to prevent these effects. Hence, various plants are being tested for their antioxidant activity.

Also, use of synthetic antioxidants may lead to the generation of more radical species resulting to treatment failure, increased treatment costs as well as the rate of fatalities and create even broader control problems.

Therefore, there has been increasing interest to replace synthetic antioxidants with natural, effective and non-toxic compounds. The growing interest in alternative natural antioxidants has made scientists to find new uses and applications of these plants [1]. Some natural substances of plant origin have good antioxidant properties and have been used as anti-aging agents for centuries.

Recent research into free radicals has confirmed that food rich in antioxidants play an important role in the prevention of cardiovascular diseases, cancers and neurodegenerative diseases [7, 8, 9]. Antioxidant from natural sources increases the antioxidative capacity of the plasma and decreases the risk of many diseases. The increase in dietary antioxidant intake may help to support the limiting antioxidant concentrate and also promote the normal functioning of the physiological systems [10]. For this reason, antioxidants derived from plants are now receiving so much attention.

Spices and herbs are recognized as sources of natural antioxidants that can protect humans from oxidative stress and as a result play an important role in the prevention of diseases whose origin is related to ROS [2]. There are many synthetic antioxidants in use. However, it is reported that they are carcinogenic [11]. There is therefore a need for a more effective, less toxic and cost effective antioxidants. Plants appear to have these desired comparative advantages, hence the growing interest in natural antioxidants from plants [12].

The aim of this study is to evaluate the antioxidant potentials of the methanolic leaf extracts of Vegetables, Fruits and Herbs commonly consumed in Kaduna using DPPH radical scavenging assay.

2. Materials and Methods

2.1 Plant Material

Fresh leaves of the various plants were collected from different locations as most of the edible leaves were purchased from the vegetable market at Kaduna state, Nigeria. The plants were identified by a Botanist at the Ahmadu Bello University, Zaria.

2.2 Chemicals and Apparatus

All the chemicals used are of analytical reagent grade. 2, 2-diphenyl-1-picryl-hydrazyl radical (Sigma-Aldrich, Germany; M.W. 394.32), ascorbic acid (Sigma-Aldrich GmbH, Sternheim, Germany; M.W. 176.13), methanol (Merck Darmstadt, Germany), deionized water (Milli Q), pipettes, 100 mL and 1000 mL measuring cylinder; analytical balance (Sartorius) and UV-Visible spectrophotometer (PerkinElmer, Lambda 35).

2.3 Preparation of Methanolic Leaf Extracts

The preparation of the plant extracts was carried out at the National Agency for Food and Drug Administration and Control (NAFDAC), Kaduna Area Laboratory. The leaves of the various plants were separated from undesirable plant parts. They were washed, air dried at room temperature and ground to fine powder. About 25g of the leaf powder was taken in a clean flat-bottomed glass container and soaked in 100 mL of methanol. The container with its content was sealed and kept for 72 hours accompanied with occasional shaking and stirring. The extract was centrifuged (3000xg) thrice and the clear supernatant was filtered over Whatman No. 1 filter paper. The extract was evaporated to dryness by rotary flash evaporator (Buchi type, Switzerland) under

reduced pressure at 45°C. Different concentrations were prepared from the resultant crude extract to determine the *in vitro* antioxidant capacity.

2.4 DPPH Radical Scavenging Assay

This assay was also carried out at the National Agency for Food and Drug Administration and Control (NAFDAC), Kaduna Area Laboratory. The method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H. This transformation results in a color change from purple to yellow, which is measured spectrophotometrically [12, 13]. The free radical scavenging activity of the methanolic leaf extracts was measured using 2, 2-diphenyl-1-picryl-hydrazyl according to the method described by McCune and Johns [14] with slight modifications. The reaction mixture (3.5 mL) consist of 1 mL of 0.004% methanolic solution of DPPH, 1 mL of the extract at various concentrations (15, 30, 45 and 60 µg/ml) and 1.5 mL of methanol. These solutions were vortexed thoroughly and incubated for 30 min in dark. The absorbance was then measured at 517 nm with methanol serving as blank. The control solution, without the extract was prepared by mixing 1mL of DPPH solution and 2.5mL of methanol. Ascorbic acid was taken as a reference standard. The percentage inhibition of DPPH was calculated by comparing the results of the test with the control using the formula:

$$\text{Inhibition (\%)} = \frac{A_o - A_1}{A_o} \times 100$$

Where: A_o was absorbance of control and A_1 was absorbance of test sample. The results were expressed as IC_{50} , which means the concentration at which DPPH radical was quenched by 50%. The concentration of extract providing 50% inhibition (IC_{50}) was calculated from the plotted graph of % inhibition versus concentration curve.

2.5 Statistical Analysis

Statistical analysis was carried out using GraphPad Prism (Version 6.0). The experiment was done in triplicates for each substance. Linear regression analysis was used to calculate the 50% inhibition concentration (IC_{50}) values. The results were expressed as mean \pm SD increase in IC_{50} with respect to ascorbic acid and compared with One-way analysis of variance (ANOVA). The p values less than 0.05 was considered to be statistically significant.

3. Results

3.1 DPPH Radical Scavenging Activity

The % inhibition of DPPH radical by the plant extracts at different concentration increased with increase in concentration of the extract for all samples. The plant leaves exhibited lower antioxidant activity compared to ascorbic acid with 97.47% inhibition and IC_{50} value of 8.06 ± 0.02 µg/mL. Among the plants tested, *Azadirachta indica* showed the highest antioxidant potential with IC_{50} value of 14.03 ± 0.06 µg/mL, followed by extract of *Vernonia amygdalina*, *Moringa oleifera* and *Ocimum gratissimum* with IC_{50} values of 14.53 ± 0.12 µg/mL, 14.88 ± 0.12 µg/mL and 16.89 ± 0.15 µg/mL respectively. Lower IC_{50} values indicate higher antioxidant activity of the extracts [15].

Percentage Scavenging Activity of Medicinal Plants at Various Concentration ($\mu\text{g/ml}$).

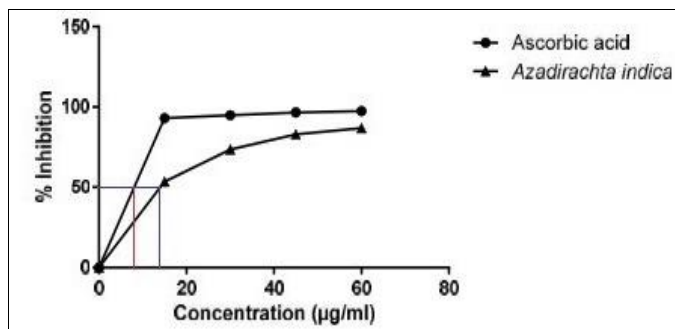


Fig 1: DPPH radical Scavenging activity of *Azadirachta indica*

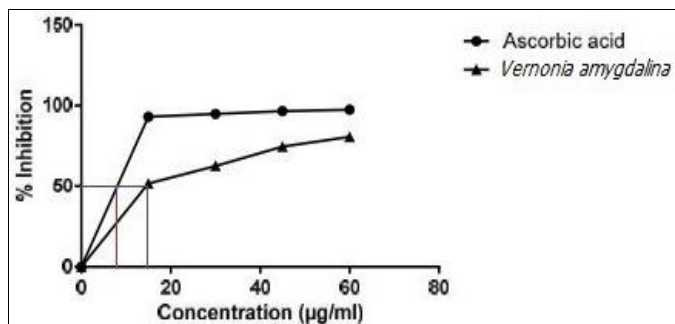


Fig 2: DPPH radical Scavenging activity of *Vernonia amygdalina*

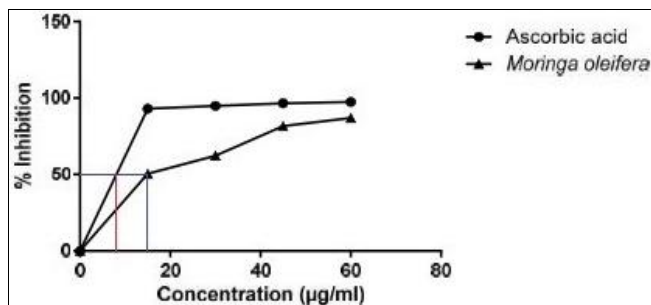


Fig 3: DPPH radical Scavenging activity of *Moringa oleifera*

Percentage Scavenging Activity of Vegetables at Various Concentration ($\mu\text{g/ml}$).

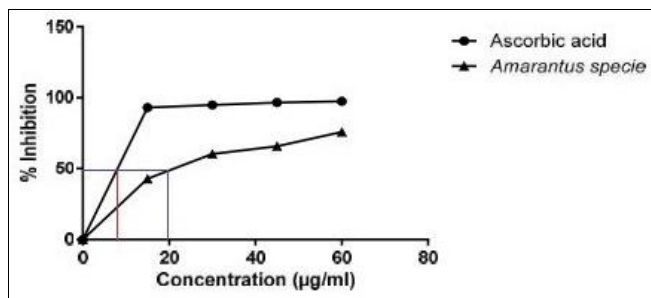


Fig 4: DPPH radical Scavenging activity of *Amaranthus specie*

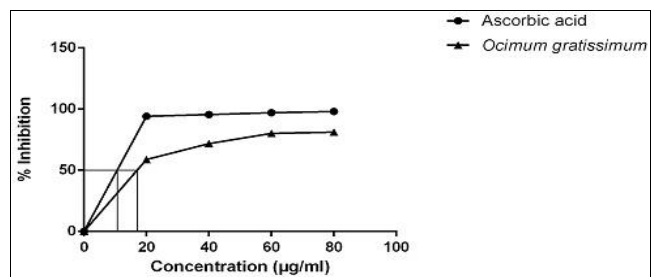


Fig 5: DPPH radical Scavenging activity of *Ocimum gratissimum*

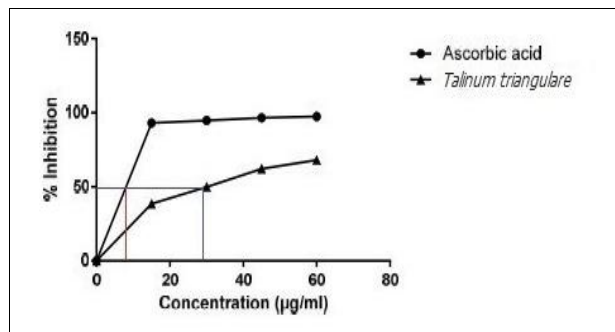


Fig 6: DPPH radical Scavenging activity of *Talinum triangulare*

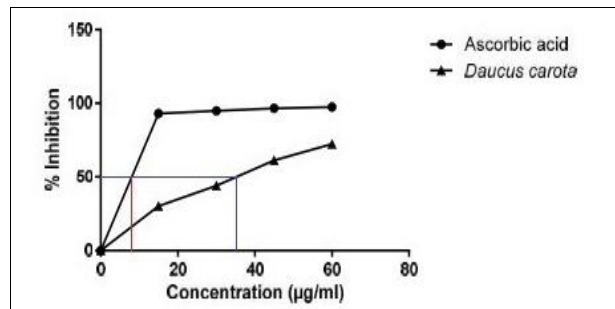


Fig 7: DPPH radical Scavenging activity of *Daucus carota*

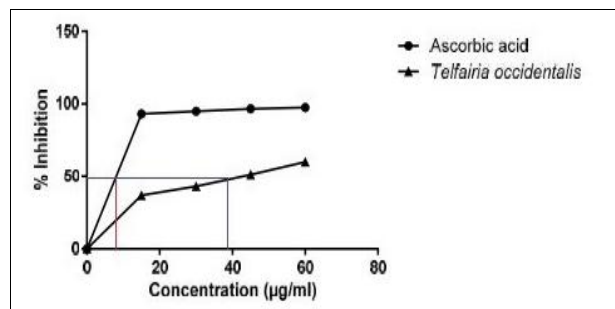


Fig 8: DPPH radical Scavenging activity of *Telfaira occidentalis*

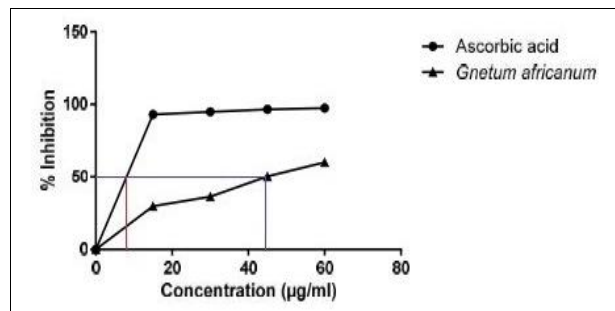


Fig 9: DPPH radical Scavenging activity of *Gnetum africanum*

Percentage Scavenging Activity of Fruit Leaves at Various Concentration ($\mu\text{g/ml}$).

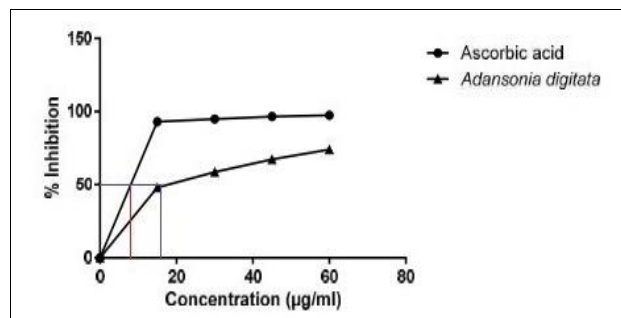


Fig 10: DPPH radical Scavenging activity of *Adansonia digitata*

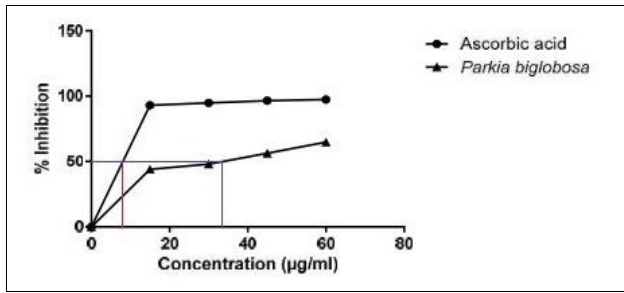


Fig 11: DPPH radical Scavenging activity of *Parkia biglobosa*

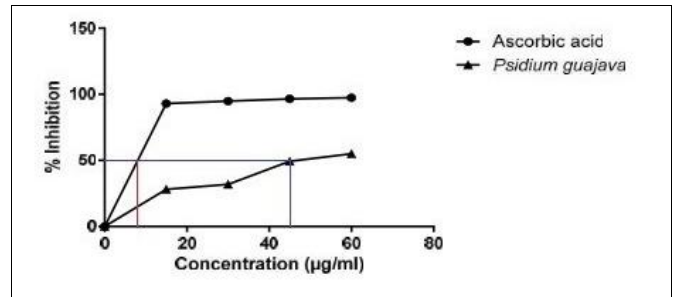


Fig 15: DPPH radical Scavenging activity of *Psidium guajava*

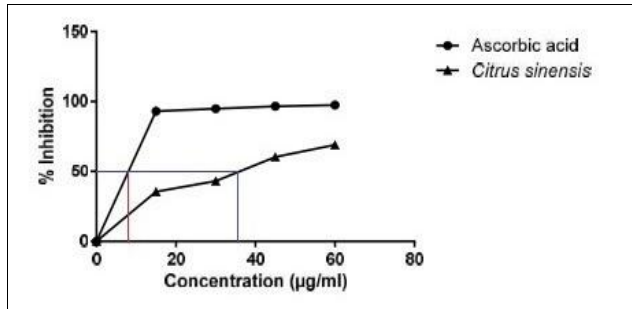


Fig 12: DPPH radical Scavenging activity of *Citrus sinensis*

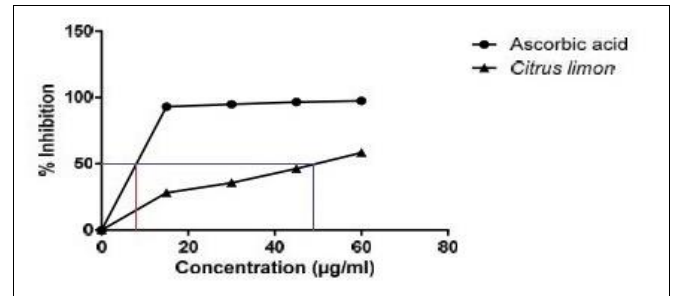


Fig 16: DPPH radical Scavenging activity of *Citrus limon*

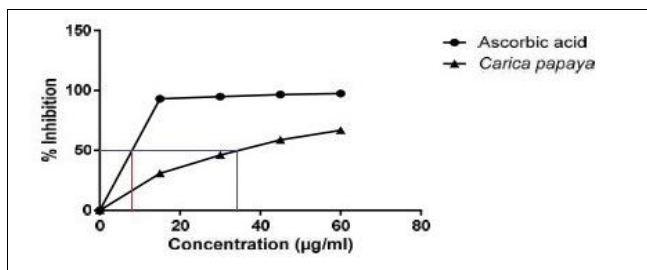


Fig 13: DPPH radical Scavenging activity of *Carica papaya*

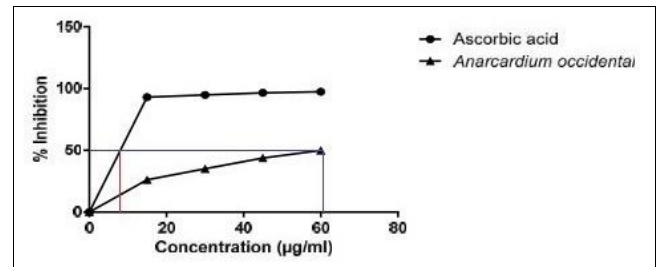


Fig 17: DPPH radical Scavenging activity of *Anacardium occidentale*

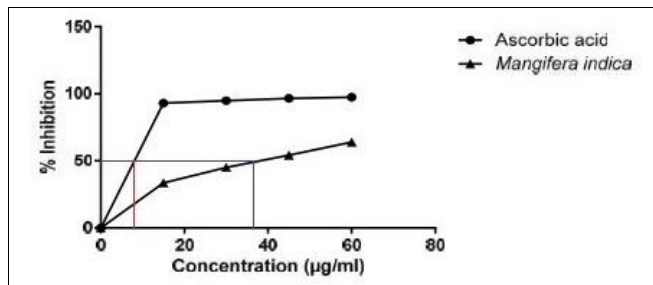


Fig 14: DPPH radical Scavenging activity of *Mangifera Indica*

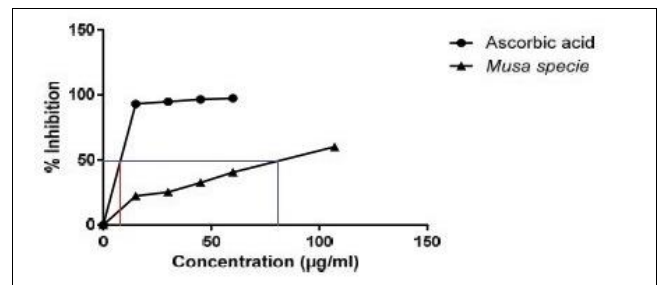


Fig 18: PPH radical Scavenging activity of *Musa specie*

Table 1: IC₅₀ value (µg extract/ml) of methanolic leaf extract of 20 plants.

| Classification | Common name | Scientific name | Family | IC ₅₀ |
|------------------|-----------------------------|-------------------------------|---------------|------------------|
| Standard | Ascorbic acid | | | 8.06 ± 0.02 |
| Medicinal plants | Neem plant | <i>Azadirachta indica</i> | Meliaceae | 14.03 ± 0.06 |
| | Bitter leaf | <i>Vernonia amygdalina</i> | Asteraceae | 14.53 ± 0.12 |
| | Drumstick tree | <i>Moringa oleifera</i> | Moringaceae | 14.88 ± 0.12 |
| Vegetables | Clove basil (African basil) | <i>Ocimum gratissimum</i> | Lamiaceae | 16.89 ± 0.15 |
| | Spinach | <i>Amarantus specie</i> | Amaranthaceae | 19.56 ± 0.42 |
| | Water leaf | <i>Talinum triangulare</i> | Talinaceae | 32.33 ± 0.07 |
| | Carrot | <i>Daucus carota</i> | Apiaceae | 34.82 ± 0.12 |
| | Fluted pumpkin | <i>Telfairia occidentalis</i> | Cucurbitaceae | 37.99 ± 0.23 |
| | African jointfir | <i>Gnetum africanum</i> | Gnetaceae | 45.61 ± 0.15 |
| Fruits | Baobab | <i>Adansonia digitata</i> | Malvaceae | 15.60 ± 0.21 |
| | African locust bean | <i>Parkia biglobosa</i> | Fabaceae | 33.99 ± 0.14 |
| | Orange | <i>Citrus sinensis</i> | Rutaceae | 34.76 ± 0.22 |
| | Pawpaw | <i>Carica papaya</i> | Caricaceae | 35.09 ± 0.41 |
| | Mango | <i>Mangifera indica</i> | Anacardiaceae | 36.72 ± 0.23 |
| | Guava | <i>Psidium guajava</i> | Myrtaceae | 43.75 ± 0.16 |
| | Lemon | <i>Citrus limon</i> | Rutaceae | 48.98 ± 0.73 |
| | Cashew | <i>Anacardium occidentale</i> | Anacardiaceae | 61.12 ± 0.99 |
| | Banana | <i>Musa specie</i> | Musaceae | 83.74 ± 1.06 |

Each value in the table was obtained by calculating the average of three experiments ± standard deviation (n=3).

4. Discussion

In the DPPH assay, the antioxidants were able to reduce the violet DPPH radical to the yellow coloured diphenylpicrylhydrazine. This is due to the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant leading to the formation of the non-radical form DPPH-H, a stable diamagnetic molecule ^[13]. The resulting colour change from purple to yellow with the decrease in absorbance at 517 nm indicates the formation of DPPH-H in the reaction mixture. Lower absorbance of the reaction mixture indicates higher DPPH radical scavenging activity. The DPPH radical scavenging activity of the plant extracts increased with an increasing concentration which was found to be statistically similar to that of the ascorbic acid standard. Similar findings have been observed by authors ^[16]. The varying results depend on the extraction solvent for the particular antioxidant assay. For the DPPH radical assay, plant leaves extracted with water showed lower antioxidant activities as compared to ethanol and methanol. Methanol extracts exhibited higher antioxidant activities due to its polar nature and also that of DPPH. A pattern of increasing antioxidant activity with increasing polarity has been reported ^[17]. Because like dissolves like, the polar methanol solvent tends to extract polyphenols and phenolic compounds which are also polar. Polyphenols and phenolic compounds are good antioxidants.

The result from the investigation of antioxidant activities of plant leaves validates their use as natural antioxidants. The leaves of medicinal plants generally exhibited higher antioxidant activities than that of vegetables and fruits. However, the leaves of fruits have also been reported to exhibit antioxidant properties than the edible plant parts ^[18]. Phenolic compounds, tannins and alkaloids are the important bioactive constituents in plant leaves which exerts certain therapeutic properties on a variety of ailments ^[19]. This study only focused on the leaves of plants and not on other plant parts. Most of the plant leaves may not have similar antioxidant potential as the edible plant part. The low antioxidant activities observed by some plant leaves does not reflect that the edible plant parts are also low in antioxidants. Higher antioxidant activities exhibited by fruits ^[20] is as a result of higher concentration of polyphenols and vitamins in the fruits compared to their leaves.

5. Conclusion

The assessment of the antioxidant potential of plant leaves serve as the baseline for the chemical identification of active molecules which may be used as anticancer, antioxidant or antimicrobial compounds. They could also be chemically manipulated into effective drugs. These drugs could be used to manage inflammatory ailments; consequently, contributing positively to the economy of the country and the world at large. Obtaining antioxidant compounds from plants will be a great move towards reducing the side effects associated with current treatment methods as natural products are considered safer than synthetic drugs ^[21].

In addition, these plants could be included in the primary health care, as encouraged by the World Health Organization ^[22]. Though there are plants in clinical testing at the moment, the search for new products is of outmost importance as diseases are constantly developing resistance to existing drugs. Screening plants for other biologically active compounds such as antifungal and antibacterial activities and not just antioxidants will also help in the implementation of conservation measures for medicinally useful plants.

6. Competing Interests

Authors have declared that no competing interests exist.

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