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Ethnobotanical study, phytochemical composition and *in vitro* antioxidant activity of the methanol extracts of thirty-two medicinal plants from Southern Nigeria

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The prevalent disease conditions globally, the detrimental effects, and the resistance of microorganisms to synthetic drugs are really worrisome. Measures to checkmate these situations include researches on the role of medicinal plants in health care delivery. This study is aimed at assessing the antioxidant activities of some medicinal plants normally used for the treatment of various ailments in southern Nigeria and searching for new sources of environmentally benign antioxidants. Thirty-eight medicinal plant extracts were screened for phytochemicals and *in vitro* antioxidant properties by the diphenyl-1-picrylhydrazyl, nitric oxide, and ferric-reducing power assays. The leaf extract of *Chrysophyllum albidum* exhibited the highest total phenolics of 348.98±0.941 mgGAE/g, while the lowest concentration was obtained in the fruit exocarp extract of *Persea Americana* (19.00±1.191 mgGAE/g). The highest and the lowest total flavonoids were observed in the leaf extract of *Icacina trichanta* (109.59±0.481 mgCE/g) and the seed extract of *Persea Americana* (1.46±0.000 mgCE/g). Total flavonols were highest in the whole-plant extract of *Cleome ciliata* (933.90±0.186 mgQUE/g) and lowest in the root extract of *Combretum racemosum* (63.97±0.121 mgQUE/g). Nine extracts gave the best antioxidant scavenging activity with a percentage DPPH >70.00% and an IC₅₀ <0.5000 mg/ml. These results suggest that some medicinal plants in southern Nigeria have strong antioxidant scavenging abilities. Further investigation to determine the antioxidant activity of the nine active extracts by *in vivo* methods, as well as isolation and characterization of these active antioxidant compounds, may enhance the development of new drugs for the treatment of oxidative-stress-related illnesses.

Key words: Thirty-eight plant extracts; total phenolics, flavonoids, flavonols, and antioxidant activity.

INTRODUCTION

Ethnobotanical survey of medicinal plants used in various regions of Nigeria has been considered by several authors (Ajibesin et al., 2008; Adebayo and Krettli, 2011; Kankara et al., 2015; Mowobi et al., 2016; Odoh et al., 2018; Segun et al., 2018). Some of these studies only

provide extensive lists of plant species, with indications of their various parts and how they are used by folks for the treatment of different ailments. However, few reports exist on the extensive studies of the effects of extracts of these medicinal plants on various microorganisms and

endogenous factors such as superoxide anion radical, hydroxyl radical, hydrogen peroxide radical, nitric oxide radical, singlet oxygen, etc, that are the major causes of oxidative stress in humans and other organisms. Antimicrobial, anti-plasmodial, and antiviral activities of methanol extract of medicinal plants used by community dwellers in the Western region of Nigeria for treatment of various diseases have been reported (Ogbole et al., 2018).

It is therefore not surprising that the use of these medicinal plants is still limited to the rural communities because extensive research into the phytochemical profile, dosage, and synergistic effect of the mixture of different medicinal plants extracts on some micro-organisms and reduction in the incidents of oxidative-stress related diseases due to beneficial health functionality of phenolic antioxidants present in various parts of these medicinal plants have not yet been thoroughly investigated. Minerals and anti-minerals components in *Gongronema latifolium* (utasi) leaf have been reported (Etesin et al., 2018). The Southern region of Nigeria is endowed with various medicinal plants whose medicinal efficacy needs to be properly established.

Some plants have been used for the treatment of practically all types of illnesses ranging from infectious agents such as bacteria, fungi and viruses to metabolic and neurological disorders etc., as well as primary sources of chemical diversity for biologically active molecules that enhance pharmaceutical discovery over the past several decades (Bernstein et al., 2018; Kandanur et al., 2019). In fact, many of the initial drugs developed in modern western medicine were inspired by natural plant products. For example, one of the first plant-inspired pharmaceuticals, aspirin, the semi-synthetic acetylsalicylate, is based on the naturally occurring salicylic acid found in willow bark and used traditionally for the treatment of fever and pain (Taylor et al., 2001). Inflammation which can be acute or chronic is a physiological response that can be induced by various stimuli such as microbial infection (pathogenic) and mechanical (physical) or chemical tissue damage which normally acts as a defense mechanism by signaling proteins at the site of infected tissues or cells (Choudhari et al., 2013; Pompermaier et al., 2018). Chronic inflammation results in disorders such as arthritis, asthma, colitis, dermatitis, and even neuro-degenerative diseases including Alzheimer's and Parkinson's disease (Medzhitov, 2008). Nevertheless, when it exhibits fulminant or becomes chronic, therapeutic measures are often necessary (Freissmuth et al., 2012).

Oxidation is an essentially biological process for many

living things for the production of energy (Wu et al., 2014). This reaction results in the formation of reactive oxygen species such as free radicals in the human body, which are removed by antioxidant defenses. If these free radicals are allowed to remain in the body and their concentrations are over physiological limits, it will lead to damage to the body (Liu and Jiang, 2012). Free radicals are usually unstable, highly reactive species that lose an electron as a result of this activity and result in a dangerous chain reaction called free radical damage. Reactive oxygen species (ROS) are widely believed to be involved in the etiology of many diseases including inflammation usually indicated by the signs of oxidative stress seen in those diseases (Battu et al., 2011). Other chronic diseases associated with ROS include cancer, diabetes, aging, atherosclerosis, hypertension, and heart attack (Basma et al., 2011; Perumal et al., 2012).

Traditionally, medicinal plants are used for the treatment of more than one disease. They may possess very high bioactivity against common targets. Therefore, the antioxidant property has significance because it can target ROS implicated in many disease conditions (Mayakrishnan et al., 2012). In view of the prevailing Coronavirus pandemic and the historical Spanish flu of 1918 that killed millions of people globally as well as other global health-related issues, it is therefore of immense scientific interest to explore and exploit the health potentials of the available medicinal plants in our communities with the aim of finding remedies to these global health pandemics and proposing solutions to future challenges in the global health care delivery. The present study has therefore been carried out to investigate the phytochemical and *in vitro* antioxidant potentials of the methanol extracts of some selected medicinal plants commonly used in our local community to manage various diseases.

MATERIALS AND METHODS

Field survey, plant collection, and identification

The ethno-botanical survey was carried out in Oct to Dec. 2018, and Jan. 2019 in selected Local Government Areas of Akwa Ibom State, Nigeria (Abak, Etinan, Mkpato Enin, Nsit Ibom, and Uyo local government areas). The pieces of information collected on various data such as local names, plant parts used, ailments treated, therapeutic effect, methods of administration, methods of preparation of the plant parts used, duration of treatment, and doses, were obtained through personal interactions with the traditional medical practitioners, village heads, community elders, patients, and youths. Information was collected based on the list of surveys (Sofowora, 2012). In the process, the plant materials used for various therapeutic purposes gathered from the users were

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collected, identified, and authenticated by the use of the flora of Nigeria and West Africa, a guide to the identification of some arable land weeds of West Africa and the use of other publications on medicinal plants and weeds (Keay et al., 1964; Hutchinson and Dalziel, 1968; Unamma, 1988; Akobundu and Agyakwa, 1989; Etukudo, 2003; Ajibesin et al., 2008; Iwu, 2014). The voucher specimens were subsequently preserved and stored in the herbarium of the Natural Products Chemistry (NPH) unit of the Department of Chemistry, Akwa Ibom State University, Nigeria. The different plants' parts for analysis were collected in the field for laboratory analysis between November 2018 and Jan. 2019.

Preparation and extraction of plant materials

Fresh leaves of each medicinal plant were shade dried at ambient temperature, while the seeds, fruits, stem bark, and roots were oven-dried at 60°C and pulverized. The dried powdered specimen of each plant part was extracted with methanol following the procedure described elsewhere (Ogbole et al., 2018). The dried crude extracts were kept in a refrigerator until needed. Although the traditional users of these medicinal plants prefer water as the solvent for the preparation of medicinal plant extracts to other solvents, the choice of methanol in this research was based on the reasons provided in the literature of Ogbole et al., (2018). Among the reasons include but are not limited to the following amphiphilicity of methanol and its ability to dissolve a wide range of compounds than water, including polar and to a large extent some non-polar compounds. Also, methanol is volatile and evaporates easily in order to separate it from the extract compared to water.

Determination of total phenolic content (TPC)

The total phenolic contents in the methanol extracts were determined using the method of Adaramoye and Akanni (2016) and were expressed as gallic acid equivalent per gram of dry weight (mg GAE/g) of extracts. The total phenolic content in the extract was calculated using this formula: Total phenolic content = $GAE \times V/m$, where GAE is the gallic acid equivalence (mg/ml) determined from the calibration curve ($Y = 0.1209X + 0.0456$; $r^2 = 0.9497$); V is the volume of extract (ml) and m is the weight (g) of the pure plant extract.

Determination of total flavonoid content (TFC)

The aluminium trichloride colorimetric method (Basma et al., 2011) was used in the determination of total flavonoid content in the methanolic plant extracts. Total flavonoid compounds in the plant extracts were calculated using the following formula:

$$\text{Total flavonoid content} = CE \times V/m,$$

where CE is the catechol equivalent (mg/ml) of catechin solution established from the calibration curve ($Y = 0.77X + 0.0185$; $r^2 = 0.9602$), V is the volume of extract (ml) and m is the weight (g) of the pure plant extract and the results were expressed as milligrams of catechol equivalent per gram of dry weight of extracts (mg CE/g). The data were recorded as mean \pm SD for three replicates samples.

Determination of total flavonols (TF)

The procedure reported by Kumaran and Karunakaran (2007) with slight changes was adopted to estimate the total flavonols in the methanol extracts of the medicinal plants. 2.0 ml of 5 g/250 ml $AlCl_3$ ethanol solution and 3.0 ml (12.5 g/250 ml) sodium acetate solution were added to 1.0 ml of sample (standard). The solution was made

up to 10 ml with distilled water and mixed thoroughly. The mixture was allowed to stand for 2.5 h at 20°C. The absorbance of the yellowish color mixture was measured at 440 nm after 2.5 h. The extract samples were evaluated in triplicate at a final concentration of 1 mg/ml. The flavonol content was calculated as milligrams of quercetin equivalent per gram of dry weight of extract (mg QUE/g) using the following equation based on the calibration curve:

$$Y = 0.684X + 0.1013; r^2 = 0.9858.$$

$$\text{Total flavonols} = QUE \times V/m,$$

where QUE is the quercetin equivalent (mg/ml) established from the calibration curve; V is the volume of extract (ml) and m is the weight (g) of the pure plant extract.

2,2-Diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging assay

The radical scavenging of the extracts was measured based on the method described elsewhere (Basma et al., 2011; Adaramoye and Akanni, 2016) using the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH). Ascorbic acid and Catechin were used as references. The ability to scavenge DPPH radical was calculated by the following equation:

$$\text{DPPH radical scavenging activity (\%)} = [(Abs \text{ control} - Abs \text{ sample}) / (Abs \text{ control})] \times 100$$

where Abs control is the absorbance of DPPH radical + methanol; Abs sample is the absorbance of DPPH radical + sample extract/standard.

Nitric oxide (NO) scavenging activity

The NO was generated by sodium nitroprusside and the quantity was determined using the Griess reagent (Perumal et al., 2012). 2 ml of 10 mM sodium nitroprusside dissolved in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of the extract and standard at various concentrations (0.2 – 0.8 mg/ml). The mixture was incubated at 25°C for 150 min. After incubation, 0.5 ml of the incubated solution was withdrawn and mixed with Griess reagent as follows: 1.0 ml sulfanilic acid reagent (0.33 g/100 ml glacial acetic acid) at room temperature for 5 min, followed by the addition of 1 ml naphthyl ethylenediamine dichloride (NED) (0.1% w/v). The mixture was again incubated at ambient temperature for 30 min. The pink chromophore generated during diazotization of nitrite ions with sulphanilamide and subsequent coupling with NED was measured spectrophotometrically at 540 nm against a blank (Perumal et al., 2012). Ascorbic acid and catechin were used as standard references. The ability to scavenge NO radical was calculated using the following equation:

$$\text{Nitric oxide radical scavenging activity (\%)} = [(Abs \text{ control} - Abs \text{ sample}) / (Abs \text{ control})] \times 100,$$

where Abs control is the absorbance of NO radical and Abs sample is the absorbance of NO radical + sample extract/standard.

Ferric reducing-antioxidant power (FRAP) assay

The methods reported in the literature (Jimoh et al., 2010; Chaves et al., 2020) were adopted for the FRAP assay with slight modifications. The stock solutions included 300 mM sodium acetate trihydrate buffer (3.1 g $C_2H_3NaO_2 \cdot 3H_2O$ and 16 ml glacial acetic

acid, $C_2H_4O_2$), pH 3.6, 10 mM TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ, and 2.5 ml $FeCl_3 \cdot 6H_2O$. The temperature of the solution was increased to 37°C before use. Plant extracts (150 μ l) were allowed to react with 2850 μ l of the FRAP solution for 30 min in the dark condition. The absorbance of the colored product (ferrous tripyridyltriazine complex) was measured spectrophotometrically at 593 nm. The results are expressed in μ M Fe (II)/mg dry mass and compared with that of ascorbic acid using the following equation:

$$FRAP = FE \times V/m;$$

where FE is the milligram equivalents of $FeSO_4$, V is the volume of the extract in μ l and m is the mass of the sample in milligram. The calibration curve ($Y = 0.17X + 0.283$; $r^2 = 0.9414$) was established using various concentrations of $FeSO_4$.

RESULTS

Ethnobotanical survey

The information on the plants used in the ethno-medicine among the five local government areas of Akwa Ibom State, namely: Abak, Etinan, Mkpato Enin, Nsit Ibom, and Uyo where the field survey was carried out is given in Table 1. The various plants belonging to 22 families have been arranged in alphabetical order of their families. Local names are given in the Ibibio language, the language commonly spoken by all the ethnic groups of Akwa Ibom State. The plant parts used by the people and the various ailments treated as well as the plant parts used in this investigation are also provided in Table 1.

Percentage yield and polyphenols content

The percentage yield, as well as total phenolics, flavonoids, and flavonols contents of the methanol extracts of the different medicinal plant parts, are given in table 2.

Percentage yield

The highest yield of 39.40% was observed in the fruit extract of *Chrysophyllum albidum* G. Don with voucher No. NPH 36. The next was the fruit extract of *Massularia acuminata* (NPH 34) with a yield of 35.18%. The lowest yield of 3.08% was obtained for the root extract of *Combretum micranthum* G. Don with voucher No. NPH 17. The percentage yields for the root extracts were generally low and fall within 3.08 and 7.26%. Those of leaf extracts fall within 3.92 and 16.58%. Those of the whole-plant extracts were between 4.32 and 12.18% yields. The yields in the seed extracts were observed to be within 9.74 and 13.62% and percentage yields of others which include fruit exocarps, tuber, whole fruit with seed and barks, etc., fall within 8.10 and 26.90% (table 2).

Total phenolic content

The total phenolic contents of the methanol extracts of the selected medicinal plants are reported as gallic acid equivalents by reference to a standard calibration curve ($Y = 0.1209 + 0.0456x$; $r^2 = 0.9497$). The highest total phenolic content of 348.98 ± 0.941 mg GAE/g was obtained in the leaf extract of *C. albidum* G. Don. (NPH 37), a sapotaceae. The consideration of the extracts from other parts of *C. albidum* such as fruit exocarp (NPH 35), fruit (NPH 36), and seed (NPH 38) revealed that the phenolic content varies as 156.10 ± 0.141 , 65.44 ± 0.170 , and 60.88 ± 0.290 mg GAE/g, respectively (Table 2). The phenolic content in the fruit exocarp was greater than that of the fruit. For plant samples in which more than one part was collected, the total phenolics obtained are as follows: fruit exocarp (NPH 28) of *Persea americana*, the content was 19.00 ± 1.191 mg GAE/g and this was the lowest phenolic content among the 32 plant samples collected. The seed extract content was 39.37 ± 0.778 mg GAE/g. The leaf extract of *Neptunia oleracea* (NPH 31) had a content of 27.21 ± 0.106 mg GAE/g, while that of the root extract (NPH 32) was 299.26 ± 0.583 mg GAE/g.

Total flavonoid content

The total flavonoids content is reported as catechin equivalents by reference to standard curve ($Y = 0.77X + 0.0185$; $r^2 = 0.9602$). The highest flavonoid content of 109.59 ± 0.481 mg CE/g was obtained in the leaf extract of *Icacina trichantha* (NPH 26) belonging to Icacinaceae family. The lowest concentration of 1.46 ± 0.000 mg CE/g was found in the seed extract of *P. americana* Mill of Lauraceae family. The fruit exocarp extract of the same *P. americana* Mill (NPH 28) contained total flavonoids of 46.13 ± 0.188 mg CE/g. In other plant samples in which different parts were examined such as *C. albidum*, the flavonoid content was still highest in the leaf (leaf extract (NPH 37) – 49.80 ± 0.047 mg CE/g) and lowest in the seed (seed extract (NPH 38) – 6.01 ± 0.001 mg CE/g). The flavonoid content in the fruit exocarp extract (NPH 35) was 42.70 ± 0.000 mg CE/g and that of fruit extract (NPH 36) was 17.08 ± 0.054 mg CE/g. The leaf extract of *Combretum racemosum* (NPH 16) had a content of 23.38 ± 0.233 mg CE/g, while the root extract of the same plant (NPH 17) had a content of 9.59 ± 0.000 mg CE/g (Table 2). There was no significant difference between those of *N. oleracea* leaf (NPH 31) (3.45 ± 0.049 mg CE/g) and root extract (NPH 32) of 4.48 ± 0.140 mg CE/g.

Total flavonols

The total flavonols content is reported as quercetin equivalents by reference to standard curve ($Y = 0.684X + 0.1013$; $r^2 = 0.9858$). The whole-plant extract of *Cleome*

Table 1. Ethnobotanical information on plants used in this study.

| Family | Botanical name | local name (Ibibio) | Part ethno-botanically used | Part used in this study | Voucher number | Ailment treated |
|----------------|---|---------------------|-----------------------------|--------------------------|----------------|--|
| Acanthaceae | <i>Acanthus montanus</i> (Nees) T. Anders | Mbara ekpe | Root | Root | NPH 1 | Boil, inflammatory disease |
| Acanthaceae | <i>Asystasia gangetica</i> (Linn.) T. Anders | Eka mmeme | Stem bark | Whole-plant | NPH 2 | Skin cancer |
| Acanthaceae | <i>Eramomastax polysperma</i> (Benth.) Dandy | Edem ididuot | Leaves | Leaves | NPH 3 | Internal heat, malaria |
| Acanthaceae | <i>Hypoestes verticillaries</i> (Linn. F) | Ayara memme | Leaves | Leaves | NPH 4 | Asthma |
| Acanthaceae | <i>Justicia secunda</i> Vahl | Iyip ikong | Leaves | Leaves | NPH 5 | Blood cleanser, immune booster |
| Agavaceae | <i>Dracaena arborea</i> (Wild.) Link Enum. Hort | Okono | Root, leaves | Root | NPH 6 | Gonorrhoea, boils, burns |
| Amaranthaceae | <i>Cyathula prostrata</i> (L.) Blume | Nkimubut | Leaves | Whole-plant | NPH 7 | Cancer |
| Apocynaceae | <i>Alstonia boonei</i> De Wild. | Ukpo | Root, leaves | Stem bark | NPH 8 | Asthma, malaria, stomach ache |
| Asteraceae | <i>Aspilia africana</i> (Pers.) C.D. Adams | Ndiduen inuene | Leaves | Leaves | NPH 9 | Dysentery |
| Asteraceae | <i>Emilia praetermissa</i> | Utimense | Leaves, stem | Whole-plant | NPH 10 | Fever, malaria |
| Asteraceae | <i>Mikania micrantha</i> (L.) Kunth | Nyaha udia | Whole plant | Whole-plant | NPH 11 | Fever, malaria |
| Asteraceae | <i>Synedrella nodiflora</i> Gaertn | Mbiod udo inyang | Leaves | Leaves | NPH 12 | Malaria, immune booster |
| Cesalpiniaceae | <i>Cassia aleta</i> L. | Adaya okon | Leaves | Leaves | NPH 13 | Malaria, ring worm |
| Capparidaceae | <i>Cleome ciliata</i> Schum & Thonn | Ikpat unen | Leaves | Whole-plant | NPH 14 | Stomach ache, malaria |
| Caricaceae | <i>Carica papaya</i> L. | Akpoood/Udia edi | Leaves, fruits, root, seed | Unripe fruits with seeds | NPH 15 | Malaria, regulate blood pressure |
| Combretaceae | <i>Combretum racemosum</i> | Asaka | Root | Leaves | NPH 16 | Pile, cancer |
| Combretaceae | <i>Combretum micranthum</i> | Asaka | Root | Root | NPH 17 | Pile, cancer |
| Combretaceae | <i>Terminalia catappa</i> L. | Mmansang mbakara | Leaves | Leaves | NPH 18 | Malaria, fever |
| Compositae | <i>Vernonia conferta</i> Benth | Ikpo mfang | Leaves | Leaves | NPH 19 | Malaria, internal heat |
| Cucurbitaceae | <i>Lagenaria siceraria</i> (Molina stanal) | Mfang ikang | Leaves | Leaves | NPH 20 | Burns |
| Dioscoreaceae | <i>Diocorea villosa</i> L. | Udia adung | Stem tuber | Stem tuber | NPH 21 | Fibroid |
| Euphorbiaceae | <i>Euphorbia heterophylla</i> Linn. | Adia ke gari | Leaves | whole-plant | NPH 22 | Purgative |
| Euphorbiaceae | <i>Euphorbia hirta</i> Linn. | Etikene ekpo | Leaves | whole-plant | NPH 23 | Stomach ache |
| Euphorbiaceae | <i>Cnidioscolus aconitifolius</i> | Nnun ition | Leaves | Leaves | NPH 24 | Malaria, immune booster |
| Fabaceae | <i>Mucuna sloanei</i> | Ibaba | Leaves, seed | Leaves | NPH 25 | Boils |
| Icacinaeae | <i>Icacina trichantha</i> Oliv. | Efik ison, okpokpo | Leaves, seed | Leaves | NPH 26 | Cough, asthma, hypertension |
| Lamiaceae | <i>Solenostemon monostachyus</i> (P. Beauv.) | Ntodikwot | Leaves | Whole-plant | NPH 27 | Malaria, fever |
| Lauraceae | <i>Persea americana</i> Mill | Eben mbakara | Seed, fruit | Fruit exocarp | NPH 28 | |
| Lauraceae | <i>Persea americana</i> Mill | Eben mbakara | Seed, fruit | Seed | NPH 29 | Cardiovascular pains, hypertension |
| Loranthaceae | <i>Mistletoe Viscum album</i> | Ndoro enyong | Leaves | Leaves | NPH 30 | Cancer, high blood pressure |
| Mimosaceae | <i>Neptunia oleracea</i> Lour. | Afia mbabak iko | Root | Leaves | NPH 31 | Hypertension, stomach ulcer and diabetes |
| Mimosaceae | <i>Neptunia oleracea</i> Lour. | Afia mbabak iko | Root | Root | NPH 32 | Spleen enlargement |
| Moraceae | <i>Ficus exasperata</i> Vahl. | Ukwok | Leaves | Leaves | NPH 33 | Hypertension |
| Rubiaceae | <i>Massularia acuminata</i> (G. Don) | Okok | Leaves, root, stem | Fruits | NPH 34 | Malaria, tooth decay, internal heat |
| Sapotaceae | <i>Chrysophyllum albidum</i> G. Don | Udara | Leaves | Fruit exocarp | NPH 35 | Dysmenorrhoea |
| Sapotaceae | <i>Chrysophyllum albidum</i> G. Don | Udara | Leaves | Fruit | NPH 36 | Dysmenorrhoea |
| Sapotaceae | <i>Chrysophyllum albidum</i> G. Don | Udara | Leaves | Leaves | NPH 37 | Dysmenorrhoea |
| Sapotaceae | <i>Chrysophyllum albidum</i> G. Don | Udara | Leaves | Seed | NPH 38 | Dysmenorrhoea |

Table 2. Percentage yield and phytochemical composition of the extracts.

| Voucher number | Yield (%) | Total phenolic content (mg GAE/g) | Total flavonoids (mg CE/g) | Total flavonols (mg QUE/g) |
|----------------|-----------|-----------------------------------|----------------------------|----------------------------|
| NPH 1 | 4.70 | 213.15±0.580 | 160.89±0.016 | 256.87±0.000 |
| NPH 2 | 8.64 | 130.99±0.199 | 11.53±0.000 | 195.39±0.107 |
| NPH 3 | 3.92 | 47.11±0.884 | 26.41±0.078 | 293.48±0.078 |
| NPH 4 | 16.58 | 144.73±0.148 | 50.01±0.236 | 302.27±0.112 |
| NPH 5 | 7.20 | 66.80±0.262 | 57.85±0.163 | 535.97±0.196 |
| NPH 6 | 7.26 | 59.65±0.332 | 12.78±0.063 | 119.32±0.170 |
| NPH 7 | 4.36 | 50.28±0.113 | 9.61±0.039 | 94.58±0.018 |
| NPH 8 | 15.24 | 167.58±0.389 | 42.70±0.000 | 188.24±0.109 |
| NPH 9 | 10.08 | 206.28±0.000 | 58.57±0.042 | 308.04±0.000 |
| NPH 10 | 11.26 | 88.72±0.332 | 43.36±0.481 | 337.35±0.093 |
| NPH 11 | 4.32 | 112.33±0.389 | 36.04±0.229 | 273.77±0.139 |
| NPH 12 | 5.86 | 49.33±0.191 | 19.64±0.000 | 486.40±0.000 |
| NPH 13 | 7.62 | 207.26±1.669 | 107.57±0.084 | 527.40±0.080 |
| NPH 14 | 12.18 | 345.85±0.000 | 72.08±0.229 | 933.90±0.186 |
| NPH 15 | 26.90 | 44.61±0.127 | 8.29±0.015 | 251.06±0.054 |
| NPH 16 | 9.74 | 280.89±0.778 | 23.38±0.233 | 247.84±0.368 |
| NPH 17 | 3.08 | 314.24±1.039 | 9.59±0.000 | 63.97±0.121 |
| NPH 18 | 9.38 | 179.93±0.127 | 18.91±0.080 | 480.56±0.000 |
| NPH 19 | 4.68 | 21.47±0.290 | 6.22±0.156 | 319.76±0.026 |
| NPH 20 | 4.70 | 14.29±0.389 | 17.46±0.127 | 325.67±0.117 |
| NPH 21 | 8.10 | 68.49±0.636 | 27.60±0.226 | 153.11±0.053 |
| NPH 22 | 5.34 | 107.45±0.587 | 22.58±0.014 | 335.09±1.034 |
| NPH 23 | 11.46 | 207.14±0.339 | 30.41±0.074 | 465.98±0.067 |
| NPH 24 | 9.36 | 70.25±1.556 | 8.58±0.014 | 334.44±0.118 |
| NPH 25 | 10.40 | 122.75±0.332 | 43.56±0.152 | 272.91±0.057 |
| NPH 26 | 7.50 | 110.90±0.000 | 109.59±0.481 | 574.22±0.136 |
| NPH 27 | 5.15 | 33.87±0.297 | 8.74±0.127 | 223.98±1.033 |
| NPH 28 | 18.70 | 19.00±1.191 | 46.13±0.188 | 87.31±0.043 |
| NPH 29 | 13.62 | 39.37±0.778 | 1.46±0.000 | 137.01±0.023 |
| NPH 30 | 5.96 | 123.74±0.778 | 9.16±0.133 | 401.61±0.000 |
| NPH 31 | 7.52 | 27.21±0.106 | 3.45±0.049 | 145.71±0.076 |
| NPH 32 | 7.04 | 299.26±0.583 | 4.48±0.140 | 175.73±1.034 |
| NPH 33 | 8.82 | 53.66±0.134 | 35.48±0.097 | 486.40±0.000 |
| NPH 34 | 35.18 | 20.85±0.262 | 8.62±0.478 | 182.35±0.053 |
| NPH 35 | 25.46 | 156.10±0.141 | 42.70±0.000 | 609.31±0.134 |
| NPH 36 | 39.40 | 65.44±0.170 | 17.08±0.054 | 312.23±0.052 |
| NPH 37 | 8.94 | 348.98±0.941 | 49.80±0.047 | 746.71±0.105 |
| NPH 38 | 9.74 | 60.88±0.290 | 6.01±0.001 | 170.09±.745 |

Source: Authors 2022

ciliata (NPH 14) of Capparidaceae family had the highest level of flavonol (933.90 ± 0.186 mg QUE/g), while the lowest level was obtained in the root extract (NPH 17) of *C. racemosum* (63.97 ± 0.121 mg QUE/g) of Combretaceae family. The leaf extract of *C. racemosum* (NPH 16) had a content of 247.84 ± 0.368 mg QUE/g. For other plants where many parts were analyzed, different levels were obtained for different parts. *C. albidum* fruit exocarp (NPH 35) had a high level of flavonols of 609.31 ± 0.134 mg QUE/g, while those of the

fruit (NPH 36) and the seed (NPH 38) extracts were obtained to be 312.32 ± 0.052 and 170.09 ± 0.745 mg QUE/g, respectively, with the highest levels among them occurring in the leaf extract (NPH 37) (746.71 ± 0.105 mg QUE/g) (Table 2). *N. oleracea* leave extract (NPH 31) had a content of 145.71 ± 0.076 mg QUE/g, while the root extract (NPH 32) had a content of 175.73 ± 1.034 mg QUE/g. The flavonol contents of fruit exocarp extract (NPH 28) and seed extract (NPH 29) of *P. americana* were determined as 87.31 ± 0.043 and 137.01 ± 0.023

Table 3. Antioxidant activities of the plant extracts.

| Voucher number | Radical scavenging activities of plant extracts and standards at maximum concentration (1 mg/ml) | | | | |
|----------------|--|--------------------------|----------------------------|--------------------------|--|
| | DPPH scavenging activity (%) | IC ₅₀ (mg/ml) | NO scavenging activity (%) | IC ₅₀ (mg/ml) | FRAP (µM FeSO ₄ /mg dry mass) |
| NPH 1 | 75.45 | 0.4459 | 63.51 | 0.6158 | 63.53±0.595 |
| NPH 2 | 46.37 | 0.8986 | 4.56 | 1.5135 | 33.09±0.623 |
| NPH 3 | 16.67 | 1.3508 | 10.94 | 1.3839 | 49.41±0.000 |
| NPH 4 | 51.28 | 0.7810 | 19.77 | 1.3300 | 38.97±0.212 |
| NPH 5 | 23.72 | 1.2176 | 22.86 | 1.2229 | 65.74±0.624 |
| NPH 6 | 21.15 | 1.2323 | 5.05 | 1.5109 | 37.06±0.000 |
| NPH 7 | 17.74 | 1.3430 | 3.80 | 1.5177 | 36.62±1.873 |
| NPH 8 | 59.40 | 0.7215 | 1.83 | 1.5294 | 40.74±0.220 |
| NPH 9 | 73.08 | 0.4662 | 23.15 | 1.2276 | 44.32±0.283 |
| NPH 10 | 31.41 | 1.0810 | 17.14 | 1.3474 | 36.18±0.000 |
| NPH 11 | 39.74 | 1.0254 | 7.76 | 1.3651 | 50.29±0.000 |
| NPH 12 | 17.52 | 1.3446 | 7.77 | 1.4939 | 41.47±0.000 |
| NPH 13 | 73.29 | 0.4644 | 42.52 | 0.9242 | 31.76±1.248 |
| NPH 14 | 98.21 | 0.0377 | 28.49 | 1.1941 | 52.94±0.000 |
| NPH 15 | 15.81 | 1.3544 | 3.27 | 1.5208 | 88.29±0.078 |
| NPH 16 | 80.56 | 0.3330 | 9.24 | 1.4848 | 44.12±1.248 |
| NPH 17 | 90.12 | 0.1838 | 3.79 | 1.5178 | 19.85±1.873 |
| NPH 18 | 63.68 | 0.6141 | 7.47 | 1.4960 | 19.66±0.354 |
| NPH 19 | 7.61 | 1.4939 | 2.46 | 1.5262 | 58.68±0.624 |
| NPH 20 | 5.06 | 1.5108 | 6.90 | 1.4992 | 60.44±1.872 |
| NPH 21 | 24.23 | 1.2147 | 10.91 | 1.3842 | 47.21±6.863 |
| NPH 22 | 38.03 | 1.0382 | 8.92 | 1.4860 | 42.35±0.000 |
| NPH 23 | 73.40 | 0.4636 | 12.02 | 1.3781 | 36.62±0.624 |
| NPH 24 | 24.79 | 1.1268 | 3.39 | 1.5203 | 44.12±0.000 |
| NPH 25 | 43.44 | 0.9196 | 17.22 | 1.3469 | 47.76±0.160 |
| NPH 26 | 39.25 | 1.0399 | 43.31 | 0.9189 | 89.12±0.000 |
| NPH 27 | 11.99 | 1.3783 | 3.49 | 1.5203 | 48.97±1.872 |
| NPH 28 | 6.73 | 1.5108 | 18.23 | 1.3402 | 38.86±0.052 |
| NPH 29 | 13.93 | 1.3665 | 0.58 | 1.5364 | 43.24±1.248 |
| NPH 30 | 43.78 | 0.9157 | 3.62 | 1.5195 | 34.61±0.288 |
| NPH 31 | 9.62 | 1.3999 | 1.36 | 1.5323 | 39.71±0.000 |
| NPH 32 | 85.81 | 0.2794 | 1.77 | 1.5295 | 47.21±0.624 |
| NPH 33 | 18.97 | 1.3347 | 14.02 | 1.3662 | 63.09±3.120 |
| NPH 34 | 7.39 | 1.5055 | 3.41 | 1.5200 | 33.53±0.000 |
| NPH 35 | 55.13 | 0.7636 | 16.88 | 1.3487 | 52.00±0.000 |
| NPH 36 | 23.16 | 1.2299 | 6.75 | 1.4998 | 38.38±1.872 |
| NPH 37 | 98.80 | 0.0299 | 19.68 | 1.3303 | 31.76±0.000 |
| NPH 38 | 21.54 | 1.2400 | 2.38 | 1.5269 | 78.73±0.280 |
| Catechin | 98.93 | 0.0226 | - | - | - |
| Ascorbic acid | 98.53 | 0.0329 | 81.38 | 0.3267 | 94.85±1.872 |

Source: Authors 2022

mg QUE/g, respectively.

Scavenging activity of DPPH radical

The percentage DPPH radical scavenging ability of the

methanol extracts of the 32 plants samples, standard catechin and ascorbic acid at 1 mg/ml, and the corresponding IC₅₀ values in mg/ml are presented in table 3. The leaf extract of *C. albidum* (NPH 37) caused the highest DPPH radical scavenging activity at 98.80%, while the leaf extract of *Lagenaria siceraria* (Molina stanal)

(NPH 20) at 1 mg/ml gave the lowest result of 5.06%. The DPPH antioxidant activity of other parts of *C. albidum*: fruit exocarp (NPH 35), fruit (NPH 36), and seed (NPH 38) as well as catechin and ascorbic acid were 55.13, 23.16, 21.54, 98.93, and 98.53%, respectively. The plant parts preferred by the people of our local communities for the treatment of various ailments appear to have high DPPH radical scavenging activity; this includes root extract of *C. micranthum* (NPH 17) 90.12%, root extract of *N. oleracea* (NPH 32) 85.81%. The IC_{50} in mg/ml was found to decrease with increased DPPH activity. The values vary from 0.0299 mg/ml for NPH 37, 0.0226 mg/ml for catechin, 0.0329 mg/ml for ascorbic acid and 1.5108 mg/ml for NPH 20.

Scavenging activity of NO radical

Biological tissues generate nitric oxide (NO) by specific nitric oxide synthases, which metabolize arginine to citrulline with the formation of NO through a five electron oxidative reaction (Moncada et al., 1989; Marletta, 1989; David, 1999; Virginia et al., 2003; Ghafourifar and Cadenas, 2005; Alam et al., 2013). The NO was generated by sodium nitroprusside and the quantity was determined using the Griess reagent (Perumal et al., 2012).

The maximum percentage of NO scavenging activity at 1 mg/ml exhibited by the methanolic extracts of the 32 plants samples and the standard ascorbic acid, as well as the corresponding IC_{50} values (in mg/ml) are shown in table 3. The results show that the percentage of NO activity is generally low compared to the DPPH activity. The methanol extract of *Acanthus montanus* (Nees) T. Anders root (NPH 1) exhibited the highest NO radical scavenging activity of 63.51% while that of the standard ascorbic acid was 81.38%. The seed extract of *P. americana* (NPH 29) gave the lowest activity of 0.58%. The IC_{50} (mg/ml) which also decreases with increased percentage NO radical scavenging activity of NPH 1, ascorbic acid, and NPH 29 were obtained to be 0.6158, 0.3267, and 1.5364 mg/ml respectively.

Ferric reducing antioxidant power (FRAP) assay

The results of FRAP assay presented in Table 3 is reported as $FeSO_4$ equivalents by reference to the standard curve ($Y = 0.17X + 0.283$; $r^2 = 0.9414$). The methanol extract of unripe fruit and seeds of *Carica papaya* L. (NPH 15) had the highest FRAP assay of $88.29 \pm 0.078 \mu M FeSO_4/mg$, while the leaf extract of *Terminalia catappa* L. (NPH 18) had the lowest assay of $19.66 \pm 0.354 \mu M FeSO_4/mg$. The standard ascorbic acid had the FRAP assay of $94.85 \pm 1.872 \mu M FeSO_4/mg$.

In some plants, more than one part was examined, for example, the leaf extract (NPH 16) and the root extract

(NPH 17) of *C. racemosum* and *C. micranthum* had the FRAP of $44.12 \pm 1.248 \mu M FeSO_4/mg$ and $19.85 \pm 1.873 \mu M FeSO_4/mg$, respectively. The assay for the fruit exocarp of *P. americana* (NPH 28) $38.86 \pm 0.188 \mu M FeSO_4/mg$ was lower than that of its seed extract (NPH 29) counterpart of $43.24 \pm 1.248 \mu M FeSO_4/mg$. Also, *N. oleracea* leaf extract (NPH 31) had a lower FRAP of $39.71 \pm 0.000 \mu M FeSO_4/mg$ than *N. oleracea* root (NPH 32) of $47.21 \pm 0.624 \mu M FeSO_4/mg$. The four different parts of *C. albidum* examined gave the FRAP as follows: *C. albidum* fruit exocarp extract (NPH 35) – $52.00 \pm 0.000 \mu M FeSO_4/mg$, *C. albidum* fruit extract (NPH 36) – $38.38 \pm 1.872 \mu M FeSO_4/mg$, *C. albidum* leaf extract (NPH 37) – $31.76 \pm 0.000 \mu M FeSO_4/mg$, and *C. albidum* seed extract (NPH 38) – $78.73 \pm 0.280 \mu M FeSO_4/mg$ being the highest among the four parts investigated.

DISCUSSION

Phytochemicals which are non-nutritive plants' chemical compounds with protective or disease preventive properties (Padayachee and Bajinath, 2020) consist of an immeasurable wealth of chemical structures which have been and will continue to be a source of new drugs (Ogbole et al., 2018). Some phenolic compounds particularly polyphenols present in different parts of medicinal plants have been found to have antioxidant properties which can assist in the protection of the body from the detrimental effects and oxidative damage of free radicals (Basma et al., 2011; González-Palma et al., 2016; Chaves et al., 2020), as well as pollutants and toxins (Padayachee and Bajinath, 2020). The literature report states that the bioactivity of polyphenols can be related to their ability to chelate metals, the capability of inhibiting or reducing different enzymes such as cyclooxygenase, lipoxygenase, and telomerase, and free radical scavenging (González-Palma et al., 2016).

Isolation of pharmacologically active compounds from these medicinal plants is a long and tedious process. Hence, the unnecessary separation procedures are eliminated during isolation by first carrying out the phytochemical screening of the plant extract. This method not only allows for localization and targeted isolation of new or useful constituents with potential activities but also enables the recognition of known metabolites in extracts or at the earliest stages of separation thereby making the whole process inexpensive (Perumal et al., 2012).

The methanolic extract of the 32 plant samples selected from 22 families of medicinal plants used by people of Southern Nigeria for the management of various diseases shows varying concentrations of total phenolics, flavonoids, and flavonols. Out of five plants from the Acanthaceae family, only three of them have high total phenolic contents in the different parts investigated; viz root of *A. montanus*, whole-plant of

Asystasia gangetica, and leaves of *Hypoestes verticillaries*. The stem bark of *Alstonia boonei* of the Apocynaceae family has high phenolic contents. For the Asteraceae family, two out of four had high total phenolic contents, this includes leaves of *Aspilia africana*, and the whole-plant of *Mikania micrantha*. Other medicinal plants with elevated contents of total phenols were *Cassia alata* leaves of Ceasalpiniaceae family, the whole-plant of *C. ciliata* of Capparidaceae, the leaves and roots of the *C. racemosum*, and the leaves of *T. catappa*, both of the Combretaceae family, whole-plants of *Euphorbia heterophylla* and *Euphorbia hirta* of Euphorbiaceae family. Also, leaves of *Mucuna sloanei* of Fabaceae family, leaves of *I. trichantha* of Icacinaceae family, leaves of *Mistletoe* of Loranthaceae family, the root of *N. oleracea* of Mimosaceae family, and finally, fruit exocarp and leaves of *C. albidum* of Sapotaceae family have high phenolics contents. Most plants with low total phenolic content have high flavonols content.

The emergence of renewed interest in recent years in plant antioxidants may be attributed to some undesirable side effects of some commercial antioxidants and the rising incidence of chronic diseases (Basma et al., 2011; Robbins et al., 2015). Medicinal plants provide an array of bioactive compounds with antioxidant activities which are molecules that are capable of supplying free atoms to the human body thereby inhibiting free radicals that damage cells and cause oxidative stress (Padayachee and Baijnath, 2020). In this study, 32 medicinal plants used by natives of Southern Nigeria to manage various diseases selected from 22 families have been evaluated by various *in vitro* antioxidant assays to obtain new and active antioxidant agents. The bioactive agents will be isolated and characterized in the next phase of the research.

There are two main mechanisms of antioxidants reaction in the human system: a hydrogen atom transfer (HAT) and a single electron transfer (SET) (Craft et al., 2012; Robbins et al., 2015). If the reaction is through the HAT, an antioxidant transfer or donates a hydrogen atom to the radical and in the process quenches the radical thereby forming a more stable one through resonance stabilization. On the other hand, SET is similar to classical redox reaction. If the antioxidant reaction proceeds through SET, an electron is transferred from the antioxidant to quench the radical species.

DPPH radical is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole so that the molecule does not dimerize, as would be the case with most other free radicals. The delocalization of electrons also gives rise to the deep violet colour (Alam et al., 2013). The reduction capability of DPPH radicals induced by antioxidants was determined by the decrease in its absorbance at 517 nm (Basma et al., 2011). The reduced DPPH was pale yellow in colour due to the transfer of a hydrogen atom from the antioxidant substrate. The more the DPPH reduction

occurred, the higher the antioxidant scavenging activity of the extract. The direct relationship of high reduction of DPPH to the high scavenging ability of methanol extract of *Etilingera elatior* has been reported (Lachumy et al., 2010). The percentage DPPH scavenging activity and IC₅₀ in mg/ml which is the amount of antioxidants present in the sample necessary to decrease the initial DPPH concentration by 50% were calculated for the 32 plant samples. The higher the percentage of scavenging activity, the lower the IC₅₀ value, and the higher the antioxidant activity (Basma et al., 2011). The percentage DPPH and NO and the IC₅₀ values of the 38 extracts of the plant samples under the investigation were used to classify the antioxidant activities of the plant extracts into four categories: the first category, classified as low antioxidant activity (LAA) was those with DPPH(%) < 40% and IC₅₀ ≥ 1.0000 mg/ml; the second category, called average antioxidant activity (AAA), were those with DPPH(%) scavenging activity between 40 and 55% and IC₅₀ lying between 0.9000 and 0.7700 mg/ml; the third category, referred to as higher antioxidant activity (HAA), was with DPPH(%) falling within 55.1 and 69.0% and IC₅₀ being within the range 0.7600 to 0.6000 mg/ml; and the fourth category, classified as the best or highest antioxidant activity (BAA) had DPPH(%) ≥ 70% and IC₅₀ value < 0.5000 mg/ml (Table 4). Nine plant extracts in this research were classified under BAA category: root extract of *A. montanus* [DPPH (75.45%); IC₅₀ (0.4459 mg/ml)], leaf extract of *Aspilia africana* [DPPH (73.08%); IC₅₀ (0.4662 mg/ml)], leaf extract of *Cassia alata* L. [DPPH (73.29%); IC₅₀ (0.4644 mg/ml)], whole-plant extract of *Cleome ciliata* [DPPH (98.21%); IC₅₀ (0.0377 mg/ml)], leaf extract of *C. racemosum* [DPPH (80.56%); IC₅₀ (0.3330 mg/ml)], root extract of *C. micranthum* [DPPH (90.12%); IC₅₀ (0.1838 mg/ml)], whole-plant extract of *E. hirta* [DPPH (73.40%); IC₅₀ (0.4636 mg/ml)], root extract of *N. oleracea* Lour [DPPH (85.81%); IC₅₀ (0.2794 mg/ml)] and leaf extract of *C. albidum* [DPPH (98.80%); IC₅₀ (0.0299 mg/ml)] (Table 4). The antioxidant activity of *C. albidum* leaf was slightly greater than that of ascorbic acid [DPPH (98.53%); IC₅₀ (0.0329 mg/ml)] and slightly less than that of catechin [DPPH (98.93%); IC₅₀ (0.0226 mg/ml)]. Basma et al. (2011) reported a high antioxidant activity of the methanol extract of the leaf of *E. hirta* with a percentage DPPH of 72.96 ± 0.78% at 1 mg/ml and low antioxidant activity in the flowers, roots, and stems extract of the plant with scavenging activity of 52.45 ± 0.66, 48.59 ± 0.97, and 44.42 ± 0.94%, respectively. The result of this research corroborates the literature report. The high scavenging activity of the whole plant extract of *E. hirta* in this study could be attributed to the leaf.

The high percentage DPPH and low IC₅₀ antioxidant activity of some of the methanol extracts observed in this research may be attributed to the high value of total phenolics in those plants' extract. Venkatachalam and Muthukrishnan (2012) and Basma et al. (2011) stated that more considerable attention is being given to phenolic

Table 4. Plant groupings based on their antioxidant activity.

| Plant category | Biological name | Plant part used | Voucher number | % DPPH | % NO |
|-------------------------------------|--|--------------------------|----------------|--------|-------|
| LAA | <i>Eramonmastax polysperma</i> (Benth.) | Leaves | NPH 3 | 16.67 | 10.94 |
| | <i>Justicia secunda</i> Vahl | Leaves | NPH 5 | 23.72 | 22.86 |
| | <i>Dracaena arborea</i> (Wild.) | Root | NPH 6 | 21.15 | 5.05 |
| | <i>Cyathula prostrata</i> (L.) Blume | Whole-plant | NPH 7 | 17.74 | 3.80 |
| | <i>Emilia proetermissa</i> | Whole-plant | NPH 10 | 31.41 | 17.14 |
| | <i>Mikania micrantha</i> (L.) Kunth | Whole-plant | NPH 11 | 39.74 | 7.76 |
| | <i>Synedrella modiflora</i> Gaertn | Leaf | NPH 12 | 17.52 | 7.76 |
| | <i>Carica papaya</i> L. | Unripe fruits with seeds | NPH 15 | 15.81 | 3.27 |
| | <i>Vernonia conferta</i> Benth | Leaves | NPH 19 | 7.61 | 2.46 |
| | <i>Lagenaria siceraria</i> (Molina Stanal) | Leaves | NPH 20 | 5.06 | 6.90 |
| | <i>Diocorea villosa</i> L. | Stem tuber | NPH 21 | 24.23 | 10.91 |
| | <i>Euphorbia heterophylla</i> Linn. | Whole-plant | NPH 22 | 38.03 | 8.92 |
| | <i>Cnidoscopus aconitifolius</i> | Leaves | NPH 24 | 24.79 | 3.39 |
| | <i>Selenostemon monostachyus</i> (P. Beauv) | Whole-plant | NPH 27 | 11.99 | 3.49 |
| | <i>Persea americana</i> Mill | Fruit exocarp | NPH 28 | 6.73 | 18.23 |
| | <i>Persea americana</i> Mill | Seed | NPH 29 | 13.93 | 0.58 |
| | <i>Neptunia oleracea</i> Lour. | Leaves | NPH 31 | 9.62 | 1.36 |
| | <i>Fiscus exasperate</i> Vahl | Leaves | NPH 33 | 18.97 | 14.02 |
| | <i>Massularia acuminata</i> (G. Don) | Fruit | NPH 34 | 7.39 | 3.41 |
| | <i>Chrysophyllum albidum</i> G. Don | Fruit | NPH 36 | 23.16 | 6.75 |
| <i>Chrysophyllum albidum</i> G. Don | Seed | NPH 38 | 21.54 | 2.38 | |
| AAA | <i>Asystasia gangetica</i> (Linn.) T. Anders | Whole-plant | NPH 2 | 46.37 | 4.56 |
| | <i>Hypoestes verticillaries</i> (Linn. F) | Leaves | NPH 4 | 51.28 | 19.77 |
| | <i>Mucuna sloanei</i> | Leaves | NPH 25 | 43.44 | 17.22 |
| | <i>Icacina trichantha</i> Oliv. | Leaves | NPH 26 | 39.25 | 43.31 |
| | <i>Mistletoe</i> <i>Viscum album</i> | Leaves | NPH 30 | 43.78 | 3.62 |
| HAA | <i>Alstonia boonei</i> De Wild. | Stem bark | NPH 8 | 59.40 | 1.83 |
| | <i>Terminalia catappa</i> L. | Leaves | NPH 18 | 63.68 | 7.47 |
| | <i>Chrysophyllum albidum</i> G. Don | Fruit exocarp | NPH 35 | 55.13 | 16.88 |
| BAA | <i>Acanthus montanus</i> (Nees) T. Anders | Root | NPH 1 | 75.45 | 63.51 |
| | <i>Aspilia africana</i> (Pers.) C.D. Adams | Leaves | NPH 9 | 73.08 | 23.15 |
| | <i>Casia aleta</i> L. | Leaves | NPH 13 | 73.29 | 42.52 |
| | <i>Cleome ciliate</i> Schum and Thonn | Whole-plant | NPH 14 | 98.21 | 28.49 |
| | <i>Combretum racemosum</i> | Leaves | NPH 16 | 80.56 | 9.24 |
| | <i>Combretum micranthum</i> | Root | NPH 17 | 90.12 | 3.79 |
| | <i>Euphorbia hirta</i> Linn. | Whole-plant | NPH 23 | 73.40 | 12.02 |
| | <i>Neptunia oleracea</i> Lour. | Root | NPH 32 | 85.81 | 1.77 |
| | <i>Chrysophyllum albidum</i> G. Don | Leaves | NPH 37 | 98.80 | 19.68 |

Source: Authors 2022

as well as their strong positive correlation with radical scavenging activity.

The variables, percentage DPPH radical scavenging activity and the total phenolics content were found to be strongly positively correlated, $r(36) = 0.99$, $p < .00001$ ($r^2 = 0.9752$). The result was significant at $p < 0.05$. On the

other hand, total flavonoids and total flavonols were found to be moderately positively correlated, $[r(36) = 0.40$, $p < 0.14$ ($r^2 = 0.1567$)] and $[r(36) = 0.41$, $p < 0.11$ ($r^2 = 0.1683$)], respectively. This result suggests that 97.52% of the plant's antioxidant activity via DPPH results from the activity of phenolic components. Also, it

can be inferred that the antioxidant activity is not restricted to phenolic content only. The activity may come from the presence of other phytochemicals or secondary metabolites such as vitamin C, vitamin E (in particular α -tocopherol), volatile or essential oils, carotenoids, alkaloids, flavonoids, tannins, glycosides, etc., which in this case contribute the remaining 2.48%. This highly positive correlation corroborates the literature reports (Ebrahimzadeh et al., 2010; Yan and Asmah, 2010; Basma et al., 2011; Perumal et al., 2012; Venkatachalam and Muthukrishnan, 2012; Amoussa et al., 2015). Hence, our findings reveal that the antioxidant scavenging activity of the plant extracts, particularly the nine plant extracts classified under the BAA category in this research might be attributable to the phenolic compounds in the plants. Previous studies also reported that the consumption of foods high in phenolic content can reduce the risk of heart diseases by slowing the progression of atherosclerosis since they act as antioxidants (Basma et al., 2011).

In the nitric oxide scavenging activity, the total flavonoid content and percentage NO radical scavenging activity was found to be strongly positively correlated, $r(36) = 1.00$, $\rho < 0.00001$ ($r^2 = 1.000$). The result was significant at $\rho < 0.05$. On the other hand, the total phenolics and total flavonols were found to be moderately positively correlated, [$r(36) = 0.33$, $\rho < 0.04$ ($r^2 = 0.1116$)] and [$r(36) = 0.44$, $\rho < 0.01$ ($r^2 = 0.1954$)], respectively. This result suggests that 99.99% of the plant's antioxidant activity via NO results from the activity of flavonoid content. In this investigation, the methanolic extract of the root of *A. montanus* (Nees) T. Anders with the highest flavonoid content has the highest percentage NO scavenging activity of 63.51% and lowest IC_{50} of 0.6158 mg/ml and can be classified under plant with the best antioxidant activity (BAA) since its percentage DPPH is also as high as 75.45% (table 3). The previous result reported a good correlation of NO with the total flavonoid content (Ebrahimzadeh et al., 2010; Boora et al., 2014).

From the FRAP assay, the presence of antioxidants in the different plant extracts would result in the reduction of Fe^{3+} to Fe^{2+} by the donation of an electron. The amount of iron (II) complex can be monitored by the measurements of Perl's Prussian blue coloured product (ferrous tripyridyltriazine complex) at 593 nm. Increasing absorbance at 593 nm indicates an increase in reductive ability (Basma et al., 2011).

Conclusion

In the present study, the antioxidant activity from 32 medicinal plants used by people in Southern Nigeria to manage various diseases was evaluated spectrophotometrically. The following extracts viz root extract of *A. montanus*, the leaf extract of *A. africana*, the leaf extract of *Cassia alata* L., whole-plant extract of *C. ciliata*, the leaf extract of *C. racemosum*, a root extract of *C.*

micranthum, whole-plant extract of *E. hirta*, a root extract of *N. oleracea* Lour and leaf extract of *C. albidum* exhibited high polyphenols content, significant antioxidant activity by DPPH scavenging activity and ferric reducing power assay. The use of these plants, particularly those whose antioxidant activities are reported here for the first time as a natural antioxidant source could be an alternative to synthetic counterparts. *C. albidum* fruits should be consumed with peels to boost their antioxidant property as the phenolic and flavonol content of the exocarp is higher than that of the fruit. From this research, the fruit exocarp is classified under the category of plants with higher antioxidant activity (HAA), while the fruit is classified under plants with low antioxidant activity (LAA). Further investigation to determine the antioxidant activity of the 9 medicinal plants by *in-vivo* methods, isolation, and characterization of active components should be considered.

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CONFLICT OF INTERESTS

The authors declare that there was no conflict of interests.

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