

Research Article

***In-Vitro* Lipid Peroxidation and Free Radical Scavenging Activity of
Eight Different Polyherbal Formulation Drugs**

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ABSTRACT

The study investigated the in-vitro lipid peroxidation and radical scavenging ability of eight polyherbal drugs namely, Fidson Bitters (FB), Ashetu Bitters Blood Purifying tonic (ABPs), Swedish Bitters (SB), Yoyo Bitters (YB), Ashetu Adams Formula for Diabetes (AD), Pax Herbal Mixture (PHM), Oroki Herbal Mixture (OHM) and Evans Healthy Bitters (EHB). Phytochemical screening, lipid peroxidation and radical scavenging ability of the herbal formulations were measured using standard methods. It was observed that saponin, steroid and cardiac glycoside were present in virtually all the polyherbal products except for FB, SB and PHM, and cardiac glycoside was the most abundant phytochemical while tannin was the least present phytochemical in the eight polyherbals. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical inhibition of the eight polyherbals at 100mg/ml was within 64.41% to 77.15% and all the polyherbals showed high percentage inhibition of DPPH free radicals. AD had the highest DPPH scavenging capacity (77.15%) while OHM showed the lowest inhibition among the polyherbs. All the polyherbal products showed high nitric acid scavenging activity and it was close to that of the standard (garlic). The nitric oxide scavenging activity of the eight polyherbals was within 70.04±0.10% to 82.65±0.19%. ABP showed the highest potential (79.49 %) to inhibit MDA production while EHB showed the least potential (66.38%) at 100mg/ml. FB and PHM had the lowest IC₅₀ value for all the polyherbal for MDA inhibition while EHB had the highest (58.91 mg/ml). This study showed that the polyherbal drugs have good lipid peroxidation and free radical scavenging abilities in-vitro.

Keywords: Polyherbal, Scavenging, Peroxidation, IC₅₀, Bitters.

1.0 Introduction

Medicinal plants are universally known as natural sources of drugs and polyherbal products for

treating diseases (1). This is as a result of medicinal plants being cheap and thought to be

more efficacious than pharmaceutical drugs. Polyherbal formulations have been used from ancient times to treat a wide range of diseases because of their rich phytoconstituents (2). Medicinal plants are the major sources of many polyherbal drugs and have been used for the treatment of diseases like malaria, typhoid, hypertension, fibroids, ulcer, diabetes, asthma, and so forth (3). They deliver therapeutic and preventive benefits to users against various diseases (4) with little adverse effects (2). Africa with its rich plant biodiversity is home to several medicinal plants of economic importance which when properly utilized would reduce expenditure on global drug development and meet the needs of patients. (5).

Polyherbal formulation drugs usage are becoming popular as an alternative therapy in developing countries, and to some extent in developed countries because of their natural origin and less side effects (6). According to the World Health Organization (7), about 80% of the population in South East Asia and sub-Saharan African countries utilize herbal drugs as an alternative therapeutic form of primary health care, due to its affordability and believed efficacy than the conventional drugs. (7). Adisa and Fakeye (8) also reported that there is an increasing use of herbal drugs by trado-medicine practitioners for treatment of diseases in some developing countries. In Nigeria, there has been a rise in the usage of herbal remedies in the last few years, despite the warnings from the regulatory agencies to consumers. In spite of this patronage, little or no scientific and clinical data proving their efficacy, safety and bio-toxicity in humans abound to support manufacturers claims or otherwise. (9-11). In many Nigerian homes, polyherbal mixtures such as Oroki, Ashetu, Pax bitters, Yoyo Bitters, Swedish Bitters, among others have become a common sight as a result of the failure of government regulatory agencies to determine their safety limits and the manufacturers claim (11-13). Free radicals have

been found to be responsible in some pathophysiological diseases, such as diabetes, cancer, heart and neurodegenerative diseases (14). They are unstable atoms or molecules that have unpaired electrons and are produced in cells as a result of metabolic activities. They tend to pair with biomolecules such as lipids, proteins and DNA in living cells to attain stability and as a result, cause damage to proteins and DNA (9). Some polyherbal formulations claim to possess antioxidant abilities, therefore; screening these polyherbal drugs for their free radical scavenging activity can help ascertain the claims or otherwise of the manufacturers (15). It is also important to establish the active components of these herbal extracts (16). Fidson Bitters®, Ashetu Bitters® Blood Purifying tonic (ABPs), Swedish Bitters® (SB), Yoyo Bitters® (YB), Ashetu Adams Formula® for Diabetes (ADs), Pax Herbal Mixture® (PHM), Oroki Herbal Mixture® (OHM) and Evans Healthy Bitters® (EHB) are polyherbal drugs acclaimed for the treatment of ulcer, diabetes, bladder infections, kidney stones, anaemia, indigestion, haemorrhoid etc in Nigeria. Oroki herbal is a mixture of different herbs; *Alstonia congensis*, *Ocimum basilicum*, *Staudtia stipitata*, *Mangifera indica*, *Cassia sieberiana*, *Cyathula prostrata*, *Khaya grandifoliola*, *Securidaca longipedunculata*, *Sorghum bicolor*, *Saccharum officinarum*. Evans healthy bitters comprise of different herbs; *Alhagi camelorum*, *Cassia angustifolis*, *Commiphoramyrrrha*, *Andrographis paniculata*, *Picrorhiza kurroa*, *Tinospora cordifolia*, *Aleo barbadensis*, *Crocus sativus*. Pax Herbal Mixture (PHM) contains lemon grass, green pepper, *Carica papaya* seeds, soya beans leaves, aloe vera, bitter kola, goat weed. Ashetu Adams Formula for Diabetes (ADs) contains the following herbs; aloe vera, *Carica papaya*, *Hoslinda opposita*, raw herbs. Swedish bitter is a combination of different herbs, myrrh, saffron, camphor, rhubarb roots, angelica roots. Fidson healthy bitters is a mixture of the following herbs: aloe vera, *Phyllanthus*

niruri, Ecipta alba, Tephrosia purpurea, Twak cinnamomum, Navang, Kiratikta, Ginseng. Yoyo Bitters (YB) is a combination of different herbs; *Acinos avensis, Chenopodium murale, Citrus aurantifolia, Cinnomum aromaticum.* To the best of our knowledge, there is no work investigating the phytochemical constituents and biological activities of these polyherbal drug and also there is little scientific data on any of these polyherbals to support the therapeutic claims by manufacturers. The popular belief that herbal products are efficacious and without adverse effects prompted this study. Therefore, the objective of this study was to evaluate the antioxidant and free radical scavenging activity as well as the phytochemical composition of these eight popular polyherbal drugs consumed in Nigeria.

2.0 Materials and Methods

2.1 Polyherbal Products

Eight (8) different polyherbal formulation drugs namely; Fidson Bitter, Ashetu Bitters Blood Purifying tonic (ABPs), Swedish Bitter (SB), Yoyo Bitters (YB), Ashetu Adams Formula for Diabetes (ADs), Pax Herbal Mixture (PHM), Oroki Herbal Mixture (OHM) and Evans Healthy Bitter (EHB) used for the treatment of different ailments or as supplements in Nigeria were purchased from three different pharmaceutical stores at No 8 Baale street boundary bus-stop, Ajegunle and No 2 Kunle Osho street, Ikorodu, Lagos, Nigeria. All the polyherbal formulations are approved for use in Nigeria by the National Agency for Food and Drug Administration and Control (NAFDAC). The phytochemical screening and other analysis of the polyherbal drugs were carried out in the Biochemistry Laboratory of the Department of Biochemistry, College of Medicine, University of Lagos, Nigeria.

2.2 Qualitative Phytochemical Screening

Qualitative phytochemical screening were carried out on the polyherbal drugs according to the

method described by Trease and Evans (17); Sahira and Catherine (18) and Komape *et al* (19).

2.3 Quantitative Phytochemical analysis Polyherbal drugs

The flavonoid content was measured according to the method of Bohm and Koupai-Abyazani (20). The concentration of alkaloids was measured by the method of Harborne (21). The saponin content was measured according to the method of Harborne (21), as reported by Obadoni and Ochuka (22). The tannin content was estimated by the method of Van-Burden and Robinson (23), as reported by Belonwu *et al* (24).

2.4 Antioxidant Assay

2.4.1 DPPH Radical Scavenging Activity Assay

The free radical scavenging ability of the polyherbs, based on the scavenging of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was estimated according to the procedure described by (25). 0.5 ml of the polyherb in ethanol (95%) at different concentrations (25, 50, 75, 100mg/ml) was mixed with 2.0ml of reagent solution (0.004g of DPPH in 100ml methanol). The control contained only DPPH solution in place of the sample while methanol was used as the blank. The mixture was vigorously shaken and left to stand at room temperature. After 30 minutes the decrease in absorbance of test mixtures (due to quenching of DPPH free radicals) was read at 517nm using a microplate reader (Epoch, BioTek)

$$\text{Scavenging capacity (\%)} = 100 - \left\{ \frac{(\text{Absorbance of sample} - \text{Absorbance of blank})}{\text{Absorbance of control}} \times 100 \right\}$$

2.4.2 Determination of Malondialdehyde Activity (Lipid Peroxidation)

Malondialdehyde (MDA) an index of lipid peroxidation was determined using the method of Buege and Ausi (26) 1.0ml of the polyherbal at different concentrations (25, 50, 75, 100mg/ml) was added to 2ml of (1:1:1 ratio) TCA-TBA-HCl

reagent (thiobarbituric acid 0.37%, 0.24N HCl and 15% TCA) tricarboxylic acid-thiobarbituric acid-hydrochloric acid reagent boiled at 100°C for 15mins and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm for 10min. The supernatant was removed, and the absorbance read at 532nm against a blank. MDA was calculated using the molar extinction coefficient for MDATBA - complex of $1.56 \times 10^5 \text{ M}^{-1}\text{CM}^{-1}$.

2.4.3 Nitric Oxide Scavenging Activity Assay

Nitric oxide radical scavenging activity of the polyherbs was determined by Griess Ilosvay reaction using sodium nitroprusside (27). 4ml sample of the polyherbs solution at different concentrations (25, 50, 75, 100mg/ml) were taken in different test tubes and 1ml of sodium nitroprusside (5mM in phosphate buffered saline) solution was added into test tubes.

They were incubated for 2hours at 30°C to complete the reaction. A 2ml sample was withdrawn from the mixture and mixed with 1.2ml of Griess reagent (1% Sulphanilamide, 0.1% naphthyethylene diamine dihydrochloride in 2% H_3PO_4). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with naphthyethylene diamine was measured at 550nm (28). Garlic acid was used as standard.

The percentage (%) inhibition activity was calculated using the following equation: $\frac{(A_0 - A_t)}{A_0} \times 100$. Where A_0 is the absorbance of the control and A_t is the absorbance of the extract or standard.

2.5 Statistical Analysis

Data were expressed as mean \pm SEM. Data were analyzed using One-way analysis of variance (ANOVA), followed by Duncan multiple range test (SPSS 20.0 Inc. USA). Significant difference was taken as $p < 0.05$.

3.0 Results

Qualitative and Quantitative Phytochemical Screening

Table 1 shows the qualitative analyses of the phytochemical constituents of the various polyherbal products. The positive or negative sign showed the presence or absence of the phytochemicals in each polyherbal product respectively. It was observed that saponin, steroid and cardiac glycoside were present in virtually all the polyherbal products except for Fidson bitters, Swedish bitters and Pax healthy bitters respectively.

Table 2 shows the quantitative analysis of the phytochemical constituents of the various polyherbal products. Oroki Herbal Mixture gave the highest concentration of alkaloid ($28.41 \pm 0.38 \text{ mg}/100\text{g}$) and cardiac glycoside ($166.38 \pm 1.25 \text{ mg}/100\text{g}$). Evans gave the lowest concentration of alkaloids which was two-folds lower than that of Oroki. The concentration of flavonoids in Yoyo Bitters ($32.88 \pm 0.77 \text{ mg}/100\text{g}$) was significantly different ($P < 0.05$) from Oroki ($11.67 \pm 0.33 \text{ mg}/100\text{g}$), which gave the highest concentration of flavonoids. The Phenol concentration in Swedish Bitter ($32.17 \pm 0.22 \text{ mg}/100\text{g}$) was different from Pax which had the lowest phenol concentration.

Tannin concentration in all the polyherbal were not significantly different from Ashetu Adams Formula for Diabetes (AD) had the highest tannin content ($21.58 \pm 0.33 \text{ mg}/100\text{g}$) while Ashetu Bitters blood Purifying tonic (ABP) had the highest saponin content ($24.77 \pm 0.28 \text{ mg}/100\text{g}$) when compared to other polyherbs. The saponin concentration of all the polyherbals were within $2.97 \pm 0.11 \text{ mg}/100\text{g}$ to $24.77 \pm 0.28 \text{ mg}/100\text{g}$. The concentration of cardiac glycoside in Oroki and ABP was different from Swedish bitters, Fidson, Yoyo bitters and Pax.

Comparatively, cardiac glycoside was the most abundant phytochemical while tannin was the

least phytochemical present in the eight polyherbals.

Table 1: Qualitative phytochemical composition of polyherbal formulation

| PHYTOCHEMICALS | ALKALOID | FLAVONOIDS | PHENOLS | TANNINS | SAPONINS | TERPENOIDS | STEROIDS | CARDIAC GLYCOSIDES | ANTHRAQUINONE | PHLOBATANNIN |
|----------------------|----------|------------|---------|---------|----------|------------|----------|--------------------|---------------|--------------|
| POLYHERBS | | | | | | | | | | |
| OROKI | + | + | - | - | + | + | + | + | + | + |
| SWEDISH | + | - | + | + | + | + | - | + | - | + |
| FID | - | + | - | + | - | + | + | + | - | + |
| YOYO BITTERS. | - | + | - | - | + | + | + | + | + | - |
| EHB | + | + | - | - | + | + | + | + | - | + |
| AD | - | - | + | + | + | - | + | - | + | + |
| ABP | + | + | - | - | + | + | + | + | - | - |
| PAX | + | - | + | + | + | - | + | + | - | + |

+ means present, - means absent, FID: Fidson, ABP: Ashetu Bitters blood Purifying tonic, PAX: Pax Herbal Mixture, OROKI: Oroki Herbal Mixture, AD: Ashetu Adams Formula for Diabetes, EHB: Evans Healthy Bitters, SWEDISH: Swedish bitters.

Table 2: Quantitative phytochemical analysis on various polyherbal formulations.

| PHYTOCHEMICALS | Alkaloid mg/100g | Flavonoid mg/100g | Phenol mg/100g | Tannin mg/100g | Saponin mg/100g | Cardiac glycoside mg/100g |
|--------------------|------------------|-------------------|----------------|----------------|-----------------|---------------------------|
| POLYHERBS | | | | | | |
| OROKI | 28.41±0.38 | 11.67±0.33 | - | - | 5.46±0.28 | 166.38±1.25 |
| SWEDISH | 21.99±0.31 | - | 32.17±0.22 | 6.13±0.24 | 8.28±0.37 | 61.02±0.31 |
| FID | - | 15.76±0.19 | - | 13.32±0.21 | - | 71.38±0.81 |
| YOYO BITTER | - | 32.88±0.77 | - | - | 5.57±0.06 | 78.98±0.31 |
| EHB | 12.92±0.38 | 20.17±0.39 | - | - | 2.97±0.11 | 104.87±1.87 |
| AD | - | - | 22.17±0.33 | 21.58±0.33 | 10.59±0.11 | - |
| ABP | 14.61±0.38 | 22.85±0.32 | - | - | 24.77±0.28 | 131.82±0.19 |
| PAX | 13.46±0.12 | - | 8.90±0.23 | 19.62±0.44 | 9.34±0.19 | 47.22±0.19 |

All values are expressed as Mean±SD, FID: Fidson, SWEDISH: Swedish bitters, ABP: Ashetu Bitters blood Purifying tonic, PAX: Pax Herbal Mixture, OROKI: Oroki Herbal Mixture, AD: Ashetu Adams Formula for Diabetes, EHB: Evans Healthy Bitters.

Antioxidant Assay

Table 3 shows the DPPH scavenging activities of the polyherbs and their IC₅₀ values. From the results, the DPPH free radical inhibition of the eight polyherbals at 100 mg/ml was within 64.41% to 77.15%. All the polyherbals showed good percentage inhibition of DPPH free radicals. Their DPPH scavenging ability was in a concentration dependent manner and Ashetu Adams Formula for Diabetes (AD) which had the highest tannin content, had the highest DPPH scavenging capacity (77.15%) while Oroki showed the lowest inhibition among the polyherbs. The percentage (%) inhibition of DPPH free radicals of all the polyherbals at all the concentrations was slightly lower when compared to the standard (Garlic acid) except for ABP at 25 mg/ml (48.51%). Yoyo bitters had the least percentage (%) inhibition at all concentrations. Yoyo bitters had the highest IC₅₀ (67.34 mg/ml) for DPPH while ABP had the lowest (31.83 mg/ml). The IC₅₀ of ABP and AD was not significantly different from the standard (Garlic acid).

Table 3: DPPH free radicals scavenging activities of the polyherbal formulation at different concentrations and their IC₅₀ values.

| CONCENTRATIONS | 25mg/ml | 50mg/ml | 75mg/ml | 100mg/ml | IC ₅₀ mg/ml |
|-------------------|------------|------------|------------|------------|------------------------|
| POLYHERBS | % | % | % | % | |
| FID | 41.04±0.62 | 48.59±0.62 | 59.40±0.50 | 70.65±0.50 | 53.68 |
| ABP | 48.51±0.25 | 54.40±0.38 | 64.06±0.13 | 76.28±0.25 | 31.83 |
| SWEDISH BITTERS | 45.08±0.37 | 53.43±0.50 | 62.92±0.25 | 71.79±0.62 | 39.98 |
| YOYO BITTERS | 39.55±0.74 | 44.29±0.25 | 53.08±1.00 | 64.41±0.37 | 67.34 |
| AD | 46.49±0.38 | 57.91±0.38 | 66.08±0.50 | 77.15±0.50 | 32.46 |
| PAX | 42.89±0.25 | 48.07±0.38 | 56.15±0.37 | 68.81±0.38 | 57.23 |
| OROKI | 41.39±0.38 | 45.52±0.50 | 57.30±0.74 | 64.50±0.25 | 60.29 |
| EHB | 42.27±0.62 | 49.12±0.37 | 55.27±0.62 | 70.39±0.12 | 53.89 |
| GARLIC ACID (STD) | 46.71 | 75.29 | 83.76 | 89.43 | 27.78 |

All values are expressed as Mean±SD, FID: Fidson, ABP: Ashetu Bitters blood Purifying tonic, PAX: Pax Herbal Mixture, OROKI: Oroki Herbal Mixture, AD: Ashetu Adams Formula for Diabetes, EHB: Evans Healthy Bitters, %: Percentage inhibition, IC₅₀: Half maximal inhibitory concentration.

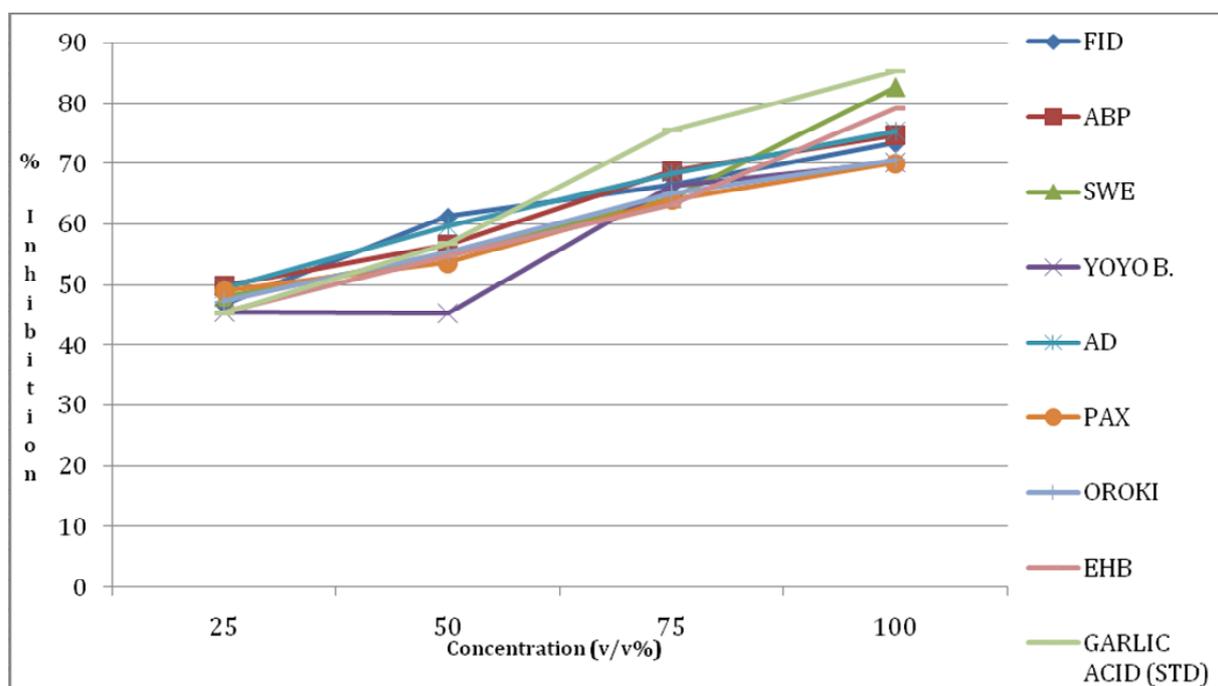


Figure 1: Percentage (%) inhibition of DPPH Free radicals by the polyherbs at different concentrations.

Table 4 shows the Nitric oxide (NO) scavenging activity of the polyherbal products and their IC₅₀ values. All the polyherbal products showed good nitric acid scavenging activity in a concentration dependent manner as their percentage inhibition was close to that of the standard (garlic). The nitric oxide scavenging activity of the eight polyherbals was within 70.04±0.10% to 82.65±0.19%. At 25 mg/ml, all the polyherbals exhibited higher nitric oxide inhibition when compared to the standard (Garlic acid) (45.33%). At 50 mg/ml, Fidson and AD with good tannin content showed higher nitric oxide inhibition compared to the other six polyherbals and the standard. Swedish Bitter had the highest percentage inhibition of NO (82.65 %) while Pax Herbal Mixture (PHM) has the least percentage inhibition (70.04

%) at 100mg/ml. The IC₅₀ of all the polyherbals for Nitric Oxide (NO) was lower than that of the standard (35.97 mg/ml), except for Yoyo bitters (56.99 mg/ml) and Evans bitters (37.06 mg/ml).

Table 4: Nitric Oxide (NO) Scavenging Activities of polyherbs at different concentrations and their IC₅₀ values

| CONCENTRATION | 25mg/ml | 50mg/ml | 75mg/ml | 100mg/ml | IC ₅₀ mg/ml |
|-------------------|------------|------------|------------|------------|------------------------|
| POLYHERBS | % | % | % | % | |
| FID | 46.70±0.19 | 61.16±0.19 | 66.35±0.29 | 73.35±0.29 | 30.66 |
| ABP | 49.86±0.30 | 56.61±0.20 | 68.80±0.10 | 74.73±0.29 | 25.53 |
| SWE | 47.94±0.19 | 54.96±0.39 | 64.19±0.20 | 82.65±0.19 | 32.76 |
| YOYO B. | 45.56±0.10 | 45.32±0.19 | 66.19±0.29 | 70.11±0.40 | 56.99 |
| AD | 49.42±0.19 | 59.71±0.30 | 68.32±0.40 | 75.21±0.19 | 26.42 |
| PAX | 49.04±0.19 | 53.65±0.48 | 63.98±0.30 | 70.04±0.10 | 31.08 |
| OROKI | 47.25±0.39 | 55.24±0.19 | 65.02±0.39 | 70.53±0.19 | 33.99 |
| EHB | 45.46±0.39 | 54.76±0.29 | 63.20±0.39 | 79.13±0.10 | 37.06 |
| GARLIC ACID (STD) | 45.33 | 56.88 | 75.55 | 85.40 | 35.97 |

All values are expressed as Mean±SD, FID: Fidson, ABP: Ashetu Bitters blood Purifying tonic, PAX: Pax Herbal Mixture, OROKI: Oroki Herbal Mixture, AD: Ashetu Adams Formula for Diabetes, EHB: Evans Healthy Bitters, SWE: Swedish bitters, YOYO B.: Yoyo bitters, %: Percentage inhibition, IC₅₀: Half maximal inhibitory concentration.

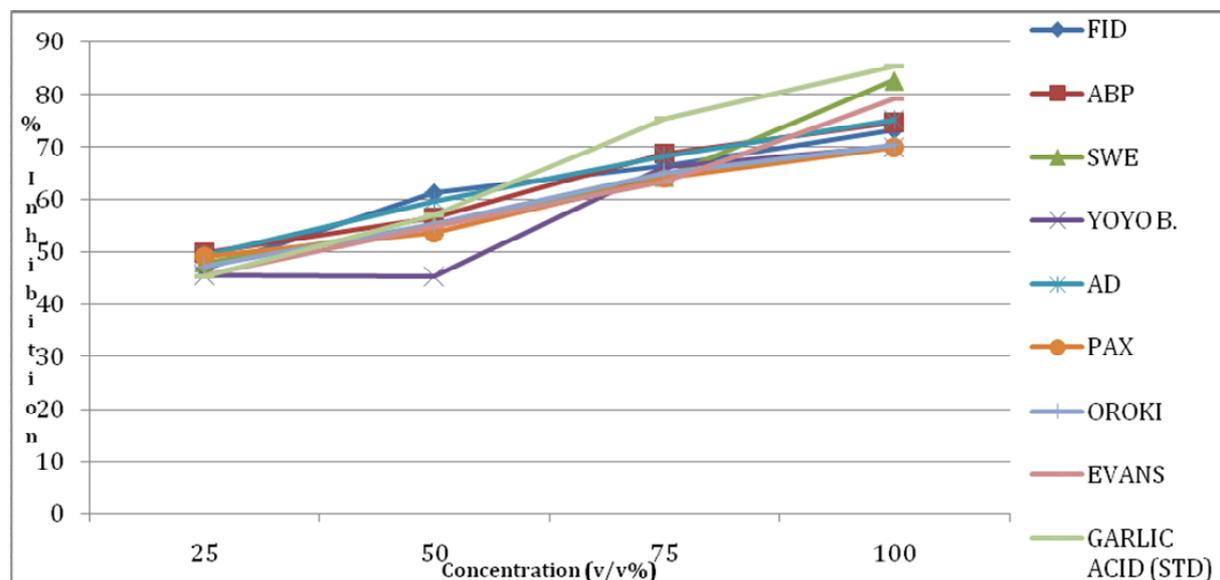


Figure 2: Percentage (%) inhibition of Nitric Oxide (NO) Free radicals by the polyherbs at different concentrations.

Table 5 shows the Lipid peroxidation activity of polyherbal products. All the polyherbal products have high potentials and abilities to inhibit or prevent lipid peroxidation in a concentration dependent manner using the percentage inhibition of MDA production as an index. Ashetu Bitters blood Purifying tonic (ABP) (high saponin content) showed the highest potential (79.49 %) to inhibit MDA production (i.e. prevent lipid peroxidation) while Evans Healthy Bitter (EHB) showed the least potential (66.38%) at 100

mg/ml. Fidson and Pax had the lowest IC₅₀ value for all the polyherbal for MDA inhibition while Evans had the highest (58.91 mg/ml).

TABLE 5: Lipid peroxidation (using mda as index) of polyherbs at different concentrations and their IC₅₀ values.

| CONCENTRATION | 25mg/ml | 50mg/ml | 75mg/ml | 100mg/ml | IC ₅₀ mg/ml |
|----------------|------------|------------|-------------|------------|------------------------|
| POLYHERBS | % | % | % | % | |
| FID | 46.31±6.18 | 64.08±3.78 | 68.45±2.75 | 75.12±1.54 | 25.37 |
| ABP | 46.48±0.17 | 47.89±0.52 | 68.08±1.20 | 79.49±0.86 | 40.59 |
| SWE | 49.91±0.35 | 50.61±1.89 | 55.10±0.69 | 71.97±7.73 | 38.19 |
| YOYO B. | 41.75±2.40 | 55.10±8.92 | 61.53±12.87 | 71.72±8.75 | 43.01 |
| AD | 40.05±0.69 | 52.67±1.03 | 63.35±0.69 | 76.10±0.86 | 45.62 |
| PAX | 46.68±3.60 | 62.26±2.23 | 66.02±2.74 | 71.24±0.86 | 25.27 |
| OROKI | 32.65±1.89 | 63.11±1.72 | 62.27±0.69 | 79.25±1.20 | 45.80 |
| Ehb | 39.69±0.86 | 45.76±0.86 | 53.28±1.89 | 66.38±1.20 | 58.91 |

All values are expressed as Mean±SD, FID: Fidson, ABP: Ashetu Bitters blood Purifying tonic, PAX: Pax Herbal Mixture, OROKI: Oroki Herbal Mixture, AD: Ashetu Adams Formula for Diabetes, EHB: Evans Healthy Bitters, SWE: Swedish bitters, YOYO B.: Yoyo bitters, %: Percentage inhibition, IC₅₀: Half maximal inhibitory concentration.

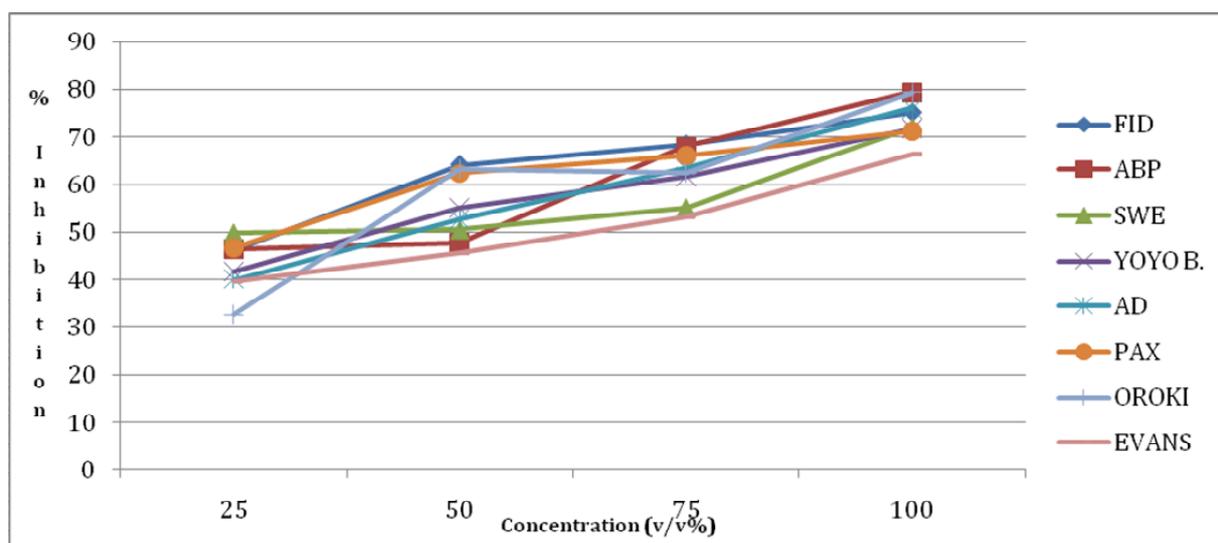


Figure 3: Lipid peroxidation (using MDA as index) of polyherbs at different concentrations.

4.0 Discussion

The investigation reported here shows the presence of phytochemicals such as tannins, alkaloids, phenols, flavonoids, terpenoids, steroids, cardiac glycosides, saponins, anthraquinone and phlobatannins in some of the eight polyherbal drugs and the free radical scavenging abilities of the drugs. The polyherbal

drugs used in this study showed some high level of antioxidant phytochemicals. The high antioxidant ability of some of the polyherbal drugs may relate to their therapeutic effects. This result is against an earlier study on Oroki herbal mixture which reported low free radical scavenging potential and low polyphenolic content (29). However, this investigation showed

high free radical scavenging potential. Sala *et al* (30) showed that alkaloids possess free radical scavenging ability which is linked to their anticancer, antibacterial and anti-inflammatory activities. Alkaloid-rich medicinal plants have been used for the treatment of malaria, cancer, heart disease, and dementia because of the strong antioxidant ability of alkaloids (31-32). The absence of Tannins and Phenols in Yoyo bitters, Ashetu Bitters Blood Purifying tonic (ABPs), Oroki Herbal Mixture (OHM) and Evans Healthy Bitter (EHB) may indicate little or no input by these phytoconstituents to the medicinal benefits of the polyherbals and this may undermine their so-called health benefits (33). Studies have linked several therapeutic benefits to the polyphenols in medicinal plants (34-35). Tannins and phenols have been reported to contribute to antioxidant activities (36-39).

The effect of antioxidants on DPPH is believed to be as a result of their ability to donate hydrogen (40). Free radical scavenging activities are vital to prevent the harmful role of free radicals in some diseases, including cancer. DPPH free radical scavenging is a known mechanism for screening the antioxidant activity of plant extracts. The relative short time required for analysis has made this method extensively useful to predict antioxidant activities. The DPPH assay showed good scavenging potential for the herbal mixtures when compared to garlic acid when the eight different polyherbal drugs were assessed for their free radicals scavenging activity.

AD (with high phenol and tannin content) and ABP (with high flavonoid and saponin content) demonstrated better DPPH free radical scavenging ability among the polyherbals but was slightly lower than the standard (Garlic acid).

This could be as a result of its high phenol and tannin content as noticed in AD. This could be as a result of its high phenol and tannin content. Previous studies have shown that tannins exhibited free radical scavenging abilities in

some medicinal plants (36, 37, 41). The comparable high DPPH scavenging ability of ABP could also be as a result of its high saponin content (42-43). This could explain the therapeutic claims of ABP as a blood purifier. Saponins have been shown to have curative effect on several pathogens (44). It has the ability to bind membrane sterols and reduce cholesterol levels (45).

They form foams in solutions and inhibit microbial growths (46). The ability of the different herbal drugs to significantly scavenge the free radicals implies good antioxidant strength. Polyphenolic compounds have been reported to possess good antioxidant potential against DPPH (47). Polyphenol contents scavenge the DPPH radicals by their hydrogen donating ability (40,48). The results from this study suggest that all the polyherbals showed good radical scavenging activity either by their proton donating or electron transfer ability.

Free radicals interfere with the membrane integrity of the cell by peroxidizing polyunsaturated lipid moieties, in a reaction cascade known as lipid peroxidation (40). The initial reaction step generates a second radical, which reacts with a second macromolecule, leading to a chain reaction and causing cellular abnormalities and membrane impairment. Lipid peroxidation inhibition is considered the most important indicator of antioxidant activity.

In this study, the polyherbals showed more than 60% lipid peroxidation inhibition activity. These results indicated that the polyherbals can prevent cellular abnormalities caused by free radicals by breaking down the chain reactions responsible for lipid peroxidation. Thus, some of the polyherbals are a good source of natural antioxidants and may be useful in treating diseases arising as a result of free radicals. The level of MDA was appreciably raised by Oroki herbal mixture when compared to the control. This may suggest oxidative stress. A previous study by Adeyemi *et al.* (11), reported the potential of herbal mixtures to elevate MDA

and reduce GSH levels in a way that reflects oxidative stress.

NO is involved in the regulation of various physiological processes and is generated by endothelial cells, neurons, macrophages, as an important chemical mediator. Several diseases are associated with the over-production and release of NO in tissues. This compound is responsible for the alteration in the structural and functional behavior of many cellular components (49). Swedish bitters (82.65%), followed by Evans bitters (79.13%) at 100 µg/mL, demonstrated the best inhibitory activity on NO synthesis in-vitro. All the polyherbals had NO inhibitory activity above 70% but was slight lower than the standard (85.40%) at 100 µg/mL. The IC₅₀ of all the polyherbals for Nitric Oxide (NO) inhibition was lower than that of the standard (35.97mg/ml), except for Yoyo bitters (56.99 mg/ml) and Evans bitters (37.06 mg/ml). These results are the antioxidant activities of the some of the polyherbals as claimed by the manufacturers like AD which is a formula for diabetes, ABP as blood purifier and so on. Phytochemical analysis of the polyherbals showed the presence of terpenoids and phenols in some of them. Phenolic compounds and terpenoids have been shown to be associated with antioxidative effect in biological systems, acting as scavengers of singlet oxygen and free radicals (50-51). The NO scavenging activity of phenolic compounds and terpenoids has been reported previously (52-53), and this could explain the NO inhibitory activity of the polyherbal.

5.0 Conclusion

The findings of this study indicate that the eight polyherbals have varying degree of free radical scavenging ability and could prevent cellular abnormalities caused by free radicals which could explain some of their therapeutic uses. Thus, the selected polyherbals are a good source of natural antioxidants and may be used to treat several diseases caused by free radicals.

Competing Interest

Authors have declared that no competing interests exist

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