Phytochemical Screening and *in vitro* Antioxidant Properties of Methanol and Aqueous Leaf Extracts of *Geophila obvallata*

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*Authors’ contributions*

This work was carried out in collaboration between both authors. Author ILO designed the study, managed the literature searches and wrote the first draft of the manuscript. Author OPN outlined the protocols, supervised the research and performed the statistical analysis. Both authors read and approved the final manuscript.

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**ABSTRACT**

*Aim:* This study investigated the phytochemical constituents and *in vitro* antioxidant properties of methanol and aqueous leaf extracts of *Geophila obvallata* using standard methods.

*Materials and Methods:* The *in vitro* antioxidant assays carried out were 1. 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging ability, Nitric oxide (NO•) radical scavenging activity assay, 2. 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS⁺) radical cation scavenging assay, ferric reducing properties and hydroxyl radical scavenging assays.

*Results:* Phytochemical analysis revealed the presence of alkaloids, flavonoids, phenolic compounds, steroids, saponins, terpenoids and cardiac glycosides in both extracts. Relative to the aqueous extract, the methanol extract contained a higher amount of the secondary metabolites.

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However, both extracts exhibited appreciable and dose-dependent capacities for quenching DPPH, ABTS$^+$ and NO• free radicals, and potent ferric reducing ability to levels comparable to those of ascorbic acid. The crude methanol extract showed significantly increased ($P<0.05$) antioxidant activity than the aqueous extract.

**Conclusion:** It was concluded that the extract possesses strong antioxidant properties due to its content of phytochemicals, and provides scientific basis for its ethno medicinal applications.

**Keywords:** Geophila obvallata; antioxidant activity; phytochemicals; in vitro.

**1. INTRODUCTION**

A free radical is any molecular species possessing unpaired valence electrons. Reactive oxygen species such as superoxide anion ($O_2^-$•), hydroxyl (OH•), hydroperoxyl (OOH•), peroxyl (ROO• alkoxyl) and (RO• peroxide) radicals are oxygen derived free radicals while hydrogen peroxide ($H_2O_2$), hypochlorous acid (HOCl), ozone ($O_3$) and singlet oxygen ($O_2$) are non-free radicals [1,2]. Living organisms when exposed both endogenously (respiration, peroxisomes stimulation of polymorphonuclear leucocytes and macrophages) and exogenously (ionising radiation, tobacco smoke, pollutants, pesticides and organic solvents) to adverse conditions can form these free radicals [3]. These free radicals when produced by the body in excess amounts could destabilise the normal body function leading to oxidative stress, damage to biomolecules and chronic diseases such as diabetes, aging, cancer and other degenerative diseases in humans [4,5]. Any substance that delays or prevents oxidative damage to a target molecule or body cell is referred to as an antioxidant [6]. A unique feature of antioxidants is the ability to scavenge free radicals and terminate the chain reaction before vital molecules are damaged [7].

In recent times, most researchers are involved in searches for medicinal plants with unique antioxidant potentials, achieved by the evaluation of antioxidant phytochemicals such as phenols, flavonoids and tannins which have received more attention for their prospective roles in the prevention of human diseases [8].

The local names of *Geophila obvallata* include “Esetu” in Itshekiri, and “Ebanotu” in Esan tribe of Edo State, Nigeria.

The leaves of this less-studied and greatly under-exploited plant have been extensively reported to possess an array of health benefits translating from its use by the locals in cooking different delicacies [9].

Local traditional herbalists in Edo state, Nigeria, have orally reported that the leaves, stems and roots (Fig. 1) are efficient medicine for the treatment of coughs, facial dermatitis, constipation, hypertension, rheumatism, jaundice, hypertension, stroke and cardiovascular diseases [13,14].

However, there is a dearth of information to validate the ethno medicinal uses of this species of plant. To fill this gap the present study was carried out to investigate the presence of bioactive phytochemicals as well as evaluate the in vitro antioxidant activities of the methanol and aqueous extracts of *Geophila obvallata*.

**2. MATERIALS AND METHODS**

**2.1 Chemicals and Drugs**

All chemicals used in this study were of analytical grade and were purchased from Sigma Chemical Co. Ltd (USA) through a local vendor.

**2.2 Identification and Collection of Plants**

The fresh leaves of *Geophilia obvallata* (GO) were collected (21st of June, 2018) by following leads supplied by a local healer at Ugbowo Quarters, Benin City, Nigeria. They were authenticated by a Taxonomist, Dr. Akinigboso, at the Department of Life Science, University of Benin, Benin City and voucher number UBHa 0312 was deposited at the Herbarium of Department of Plant Biology and Biotechnology, University of Benin for future references.
2.3 Preparation of Geophila obvallata Leaf Extracts

A modified method of Agbai [15] was used. The fresh leaves were washed and air dried for one week. The dried leaves were then ground into fine powder using hammer type milling machine (Meecan, CM/L-1364548, India), weighed and packaged. About (300g) of the fine powder of the leaves were extracted in the soxhlet extractor using both methanol (70%) (1:10 w/v) and distilled water (aqueous) to form two separate extracts respectively [16] followed by homogenisation and continuous agitation for 48 h. The homogenate was then filtered using Whatmans filter paper (No. 1). The filtrate of each was then concentrated to dryness using a rotary evaporator (Laborota 4000, SN 090816862, Germany) with the water bath set at 40°C [17] for 24 hours to obtain about 87.52g of methanol extract and 15.14g of the aqueous extract. They were both dried in a dessicator over anhydrous Copper (II) tetraoxosulphate (VI). The powdered residue were transferred into vials and stored at 4°C in airtight vials before analysis.

2.4 Preliminary Qualitative Phytochemical Analysis

Preliminary qualitative screening was done to identify the secondary bioactive metabolites present in Geophila obvallata methanol and aqueous leaf extracts using standard methods [18,19].

2.5 Quantitative Estimation of Chemical Constituents

2.5.1 Alkaloid content determination

Alkaloid content was estimated using the spectrophotometric reaction of bromocresol green as described by John [20,21].

2.5.2 Total phenolics content determination

The total phenolics content were determined using the Folin-Ciocalteu assay [22,23].

2.5.3 Total flavonoid content determination

The total flavonoid content of the plant extracts were determined by aluminium trichloride (AlCl₃) colorimetric assay using catechin as a reference compound [24].

2.5.4 Total Saponin content determination

The total saponin content of the plant extracts was done using vanillin-sulfuric acid assay [25].

2.5.5 Total condensed tannin contents

The tannin contents were determined by method of Broadhurst [26], using catechin as a reference compound.
2.6 \textit{In vitro} Antioxidant Activity

2.6.1 \textbf{DPPH}\(^+\) (1, 1-diphenyl-2-picryl hydrazyl) radical scavenging assay

The free radical scavenging activities of both extracts were measured in terms of their hydrogen donating or radical scavenging ability using the DPPH radical [27]. For the assay, 0.5 ml of DPPH radical solution in methanol was added to 2 ml of the extract [100–1000 \(\mu\)g/mL] and the reaction mixture was vortexed for 10 s and left in the dark at 37°C for 30 min. The absorbance will be measured at 517 nm using a (UV-VIS 1605 Shimadzu) spectrophotometer. Ascorbic acid and \(\alpha\)-Tocopherol were used as the positive control.

Radical scavenging activity (RSA) will be calculated in percent using the following formulae:

\[
\%\text{RSA} = \frac{(\text{ADPPH} - \text{Asample})}{\text{ADPPH} - \text{Ablank}} \times 100
\]

2.6.2 Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was determined by the method of Leelavinothan and Kalist [28].

Various concentrations of both extracts (250, 500, 750, 1000 \(\mu\)g) were taken and 1 ml of iron EDTA solution, 0.5 ml of EDTA solution, 1 ml of DMSO and 0.5 ml of ascorbic acid were added to it. The mixture was incubated in a boiling water bath at 80°C to 90°C for 15 min. After incubation, 1 ml of ice cold TCA and 3 ml of Nash reagent were added and the reaction mixture was incubated at room temperature for 15 min. The absorbance was read at 412 nm. The % hydroxyl radical scavenging activity is calculated with the formula below, Where, HRSA is the Hydroxyl Radical Scavenging Activity, Abs control is the absorbance of control and Abs sample is the absorbance of the extract.

\[
\text{HRSA} = \frac{([\text{Absctrl}-\text{Abstest}])}{\text{Abscntrl X 100}}
\]

2.6.3 Nitric oxide radical scavenging activity

The modified method as described by Oyedemi [29] was used to determine the nitric oxide radical scavenging activity of both extracts. 2 mL of 10 mM sodium nitroprusside in 0.5 mL of phosphate buffer saline (pH 7.4) was added to 0.5 mL of the plant extracts, ascorbic acid, and BHT at different concentrations (0.2–1.0 mg/mL). The mixture was then incubated for 150 min at a temperature of 25°C. After incubation, 0.5 mL of the mixture was mixed with 0.5 mL of sulfanilic acid reagent and thereafter incubated for 5 min at room temperature. One millilitre of naphthyl ethylenediamine dihydrochloride (0.1% w/v) was finally added to the mixture and again incubated at room temperature for another 30 min, after which the absorbance was measured at 540 nm with a spectrophotometer. The ability of the extract to scavenge nitric oxide radicals was calculated using the given equation below:

\[
\%\text{inhibition} = \frac{([\text{Abscontrol}) - (\text{Abssample})]}{[\text{Abscontrol})] \times 100.
\]

From the equation, Abs control represents the absorbance of nitric oxide radical + acetone; Abs sample represents the absorbance of nitric oxide radical + sample extract/standard.

2.6.4 ABTS\(^+\) 2, 2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) free radical scavenging activity

The total antioxidant activity of the plant extract was measured by [2, 2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)] ABTS\(^+\) radical cation decolourisation assay according to the method of Jamuna [2]. ABTS\(^+\) was produced by reacting 7 mM ABTS\(^+\) aqueous solution with 2.4 mM potassium persulfate in the dark for 12-16 hours at room temperature. The radical was stable in this form for more than two days when stored in the dark at room temperature. Prior to assay, this solution was diluted in ethanol (about 1:89 v/v) and equilibrated at 30°C to give an absorbance of 0.7000±0.02 at 734 nm. Then, 2 ml of diluted ABTS\(^+\) solution was added to the sample concentration at 20 \(\mu\)l (1 mg/ml). After 30 minutes of incubation at room temperature, the absorbance was recorded at 734 nm and percentage of inhibition was calculated and compared with Trolox, Gallic acid and Sodium L-ascorbate. Triplicates were performed.

\[
\%\text{Inhibition} = \frac{([\text{Abscontrol}) - (\text{Abssample})]}{[\text{Abscontrol})] \times 100
\]

2.6.5 Ferric reducing anti-oxidant property (FRAP ASSAY)

The reducing power of both extracts was evaluated according to the method of Amir [30]. The mixture containing 2.5 ml of 0.2M phosphate
buffer (pH 6.6) and 2.5 ml of potassium ferricyanide (K3Fe(CN)6) (1%w/v) was added to 1.0 μg ml of the extract dissolved in 1ml of distilled water. The resulting mixture was incubated at 50°C for 20 min, followed by the addition of 2.5 ml of trichloroacetic acid (TCA) (10%w/v). The mixture was centrifuged at 3000 rpm for 10 min to collect the upper layer of the solution (2.5 ml) and 0.5 ml of (FeCl3) ferric chloride (0.1%w/v). The absorbance was then measured at 700nm against Phosphate buffer (pH 6.6) blank sample. Ascorbic acid was used as a reference standard.

2.7 Statistical Analysis

The statistical comparison among the groups were performed with one way analysis of variance (ANOVA) test using a statistical package program SPSS 21.0 and the significance of the difference between means was determined by Duncan’s Multiple Range Test (DMRT) at (P<0.05) significant level (Stat soft Inc, Tulsa, USA).

3. RESULTS AND DISCUSSION

3.1 Preliminary Qualitative Phytochemical Analysis

The results from qualitative phytochemical analysis revealed the presence of alkaloids, cardiac glycosides, flavonoids, phenols, saponins, steroids, tannins and terpenoids in both extracts (Table 1). However tannins were only detected in the methanol leaf extract while reducing sugars were only present in the aqueous extract. Relative to the aqueous extract, the methanol extract contained a higher amount of the secondary metabolites with a high degree of precipitation (+++). Triterpenoids were determined to be absent (-) in all extracts.

3.2 Quantitative Determination of the Chemical Constituents

The results from quantitative phytochemical screening revealed the presence of alkaloids, saponins, phenolics, tannins and flavonoids in both extracts. The total saponin content was appreciably highest (P<0.05) in the methanol extract while tannin content was the least in both extracts respectively. The crude methanol extract showed the highest quantitative phytochemical content for bioactive chemicals than the aqueous extract excluding the total phenolic content and alkaloid content which were higher in the aqueous extract (Table 2).

3.3 In vitro Antioxidant Activity

3.3.1 DPPH• free radical scavenging assay

The results from the DPPH• free radical scavenging assay indicates that both leaf extracts of Geophila obvallata exhibited a dose dependent free radical quenching ability (P<0.05) from 3.906 μg/mL to 250 μg/mL when compared with the ascorbic acid (standard). However, the methanol extract showed the highest per cent inhibition (Fig. 2).

Table 1. Preliminary qualitative phytochemical analysis of methanol and aqueous leaf extracts of Geophila obvallata

| Parameter          | Methanol extract | Aqueous extract |
|--------------------|------------------|-----------------|
| Alkaloids          | +++              | +               |
| Saponins           | +                | ++              |
| Flavonoids         | +++              | ++              |
| Triterpenoids      | -                | -               |
| Phenols            | +++              | ++              |
| Tannins            | +                | +               |
| Terpenoids         | ++               | +               |
| Steroids           | ++               | +               |
| Cardiac glycosides | +++              | ++              |
| Reducing sugars    | -                | +               |

*+++: Present in high concentration, ++: Moderately present, +: Trace, -: Absent

3.3.2 Hydroxyl radical scavenging (HRS) activity

Fig. 3 shows that the hydroxyl radical scavenging effects of both leaf extracts of Geophila obvallata at different dose levels (3.906μg/mL to 250μg/mL) was concentration dependent. Among them, the methanol extract showed the highest (P<0.05) hydroxyl radical scavenging potential when compared with the standard.

3.3.3 Nitric oxide (NO•) radical scavenging activity

The results from nitric oxide (NO•) radical scavenging assay revealed that the scavenging activity of both extracts were less than the standard (Curcumin). However, the methanol extract showed appreciable scavenging potentials at 15.625(μg/mL) when compared with the control (ascorbic acid) (Table 3).
3.3.4 ABTS** radical scavenging activity

The results from ABTS** radical scavenging assay indicates that the methanol leaf extracts of Geophila obvallata species registered the highest total antioxidant activity, 96.49 μg/mL (Fig. 4.) followed by the aqueous extracts with 94.98 μg/mL when compared with the trolox standard.

3.3.5 Ferric reducing-antioxidant power (FRAP) activity

Table 4 reveals that the ferric reducing-antioxidant power of both extracts were less than the standard (ascorbic acid) in a dose dependent manner. However, the methanol extract showed appreciable reducing power relative to the standard.

3.4 Discussion

The preliminary qualitative and quantitative phytochemical screening of the methanol and aqueous leaf extracts of Geophila obvallata revealed the presence of alkaloids, cardiac glycosides, flavonoids, phenols, saponins, reducing sugars, steroids, tannins and terpenoids (Table 1). However, the saponin content (35.95±0.01%) was significantly higher (P<0.05) in the methanol extract with a high degree of precipitation while the tannin content was the least, 9.95±0.05% in the aqueous extract. This may be due to the high extractive value of the methanol solvent (70% methanol and 30% water) which can liberate a great variety of bioactive constituents relative to the aqueous extract [31].

Table 2. Quantitative phytochemical analysis (%) of methanol and aqueous extracts

| Phytochemicals                        | Methanol       | Aqueous       |
|---------------------------------------|----------------|---------------|
| Saponins (mg DE/100 g extract)        | 35.95±0.01a    | 32.02±0.02a   |
| Tannins (mg GAE/100 g)                | 10.06±0.21b    | 9.95±0.05c    |
| Alkaloids (%)                         | 23.01±0.02c    | 25.83±0.02d   |
| Phenols (mg GAE/100 g)                | 16.36±0.14d    | 12.02±0.15e   |
| Flavonoids (mg RE/100 g extract)      | 14.97±0.13e    | 10.35±0.19f   |

*Values were performed in triplicates and represented as mean ± SD. Mean values followed by different superscript in a column are significantly different (P<0.05)

Table 3. Nitric oxide (NO•) radical scavenging activity of methanol and aqueous leaf extracts of Geophila obvallata

| Sample Conc.(μg/mL) | Methanol (NO• activity) | Aqueous (NO• activity) | Curcumin (NO• activity) |
|---------------------|-------------------------|------------------------|-------------------------|
| 3.906               | 2.06±0.03a              | 3.98±0.00b             | 8.19±0.03a              |
| 7.825               | 7.31±0.10b              | 6.55±0.01c             | 25.31±0.01a             |
| 15.625              | 13.61±0.02c             | 11.10±0.08c            | 35.61±0.02a             |
| 31.250              | 29.51±0.04d             | 23.76±0.03c            | 47.41±0.03a             |
| 62.500              | 47.86±0.01a             | 35.92±0.05c            | 45.38±0.08a             |
| 125.000             | 54.12±0.23b             | 45.71±0.02c            | 60.24±0.04a             |
| 250.000             | 68.25±0.02c             | 62.12±0.01c            | 72.26±0.02a             |

*Values were performed in triplicates and represented as mean ± SD. Mean values followed by different superscripts in a column are significantly different (P<0.05)

Table 4. Ferric reducing-antioxidant power (FRAP) activity of methanol and aqueous leaf extracts of Geophila obvallata

| Sample Conc. (μg/mL) | Methanol (FRAP activity) | Aqueous (FRAP activity) | Ascorbic acid (FRAP activity) |
|----------------------|--------------------------|-------------------------|-----------------------------|
| 3.906                | 0.86±0.02a               | 0.84±0.23c              | 1.89±0.03a                  |
| 7.825                | 2.94±0.01b               | 1.98±0.11c              | 4.29±0.21a                  |
| 15.625               | 6.61±0.05a               | 5.23±0.07c              | 6.13±0.08d                  |
| 31.250               | 13.95±0.07b              | 12.02±0.51c             | 16.43±0.14e                 |
| 62.500               | 16.85±0.04c              | 16.77±0.42c             | 29.48±0.02a                 |
| 125.000              | 18.99±0.23d              | 18.67±0.03c             | 45.11±0.01a                 |
| 250.000              | 26.52±0.08e              | 25.98±0.06f             | 54.22±0.07a                 |

*Values were performed in triplicates and represented as mean ± SD. Mean values followed by different superscript in a column are significantly different (P<0.05)
The presence of saponins which are triterpenoid glycosides in the extracts indicates that the species possesses beneficial (cholesterol lowering) health effects [32], which are useful in cardiovascular therapy [33]. According to Roa, Saponins possess a direct antioxidant activity which may result in reduced risk of cancer and heart diseases [34]. Cardiac glycosides, which have a strong and direct action on the heart in terms of strengthening and support are also appreciably present in the methanol extract [17], [18]. Also, the presence of phenolic compounds like flavonoids in both extracts contributes directly to its antioxidative action thereby inhibiting destructive processes that affect human health via scavenging processes [17]. They mediate health promoting effects such as anticancer, anti-inflammatory, vasoprotective effects. These effects have been linked to an association between flavonoids and the arachidonic acid pathway [18].

The results which revealed a significant presence of phenolic compounds which play an important role in stabilising lipid oxidation in living cells [17] were detected in both extracts due to their ability to bleach the stable DPPH radical, thus registering the presence of antioxidants especially in the methanol extract. This is in agreement with the findings Of Pereira [35] who had similar DPPH+ inhibition for methanol and ethanol extracts of C. citratus and lower scavenging activity for aqueous extracts [36]. Tanaka has also suggested that the consumption of up to 1 gm of fruits and vegetables which contain polyphenolic compounds would drastically reduce mutagenesis and carcinogenesis in man [37].

According to Amir, [30] scavenging the hydroxyl radical is important because of its very high permeability across the cell membranes at specific sites and its potentials for causing cell death. The ability of the extracts of Geophila obvallata to quench hydroxyl radicals seems to be substantial and hence the extracts are good scavengers of active oxygen species, thus terminating the chain reaction of reactive oxygen species.

In addition to reactive oxygen species, nitric oxide concentrations in living cells are risk factors for pathological conditions. An increase in nitric oxide concentration in living cells can alter the structural and functional activities of many cellular components [30]. Although the scavenging activity of both extracts for nitric oxides were less than the standard (Curcumin), the methanol extract showed appreciable potentials in scavenging nitric oxide radicals in sodium nitroprusside relative to the aqueous extract (Table 3).
Fig. 3. Hydroxyl radical scavenging (HRS) assay
Vertical bars are significantly different at * P < 0.05; ** P < 0.001 Mean ± S.E.M = Mean values ± Standard error of means of six experiments

Fig. 4. ABTS•+ radical scavenging assay
Vertical bars are significantly different at * P < 0.05; ** P < 0.001 Mean ± S.E.M = Mean values ± Standard error of means of six experiments

The present study revealed that both extracts possess bioactive constituents with the ability to inhibit potassium persulfate activity while reducing the production of the stable free radical ABTS++. The results of the assay indicate that the methanol leaf extract of the species registered the highest total antioxidant activity, 96.49 μg/mL (Fig. 4.) followed by the aqueous extracts with 94.98 μg/mL which shows that both extracts of the study species have considerable radical scavenging activity.

The ferric reducing-antioxidant power of the methanol and aqueous extracts of Geophila obvallata indicated that the antiradical activity of both extracts were less than the ascorbic acid standard (Table 4). However, the methanol extract was very potent at 15.625 (μg/mL) when
compared with the control (ascorbic acid). This is in agreement with the findings of Senguttuvan who reported similar findings in the leaf and root parts of Hypochaeris radicata.

4. CONCLUSION

The results obtained in the present study indicate that methanol leaf extracts of Geophila obvallata exhibits a significantly higher free radical scavenging ability and reducing power when compared with the aqueous leaf extracts and other standards. The overall antioxidant activity of the methanol leaf extracts might be due to its solubility and high extractive value enabling it to liberate the bioactive constituents restrained by the plant’s cell walls. The findings of the present study suggest that Geophila obvallata possesses strong antioxidant properties due to its content of phytochemicals, and provides scientific basis for its ethno medicinal applications. Hence, it is worthwhile to elucidate the bioactive principles via study using in vivo models.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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