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# ANTIOXIDANT ACTIVITY OF LEAF EXTRACTS OF *GLOBIMETULA BRAUNII* (ENGLER) VAN TIEGH PARASITIZING ON *PILIOSTIGMA THONNINGII* AND *PARKIA BIGLOBOSA*

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## Graphical abstract

## Abstract

The dried leaves of *Globimetula braunii* parasitizing on *Piliostigma thonningii* and *Parkia biglobosa* were successively extracted using *n*-hexane, dichloromethane, ethyl acetate and methanol by cold extraction method. All the extracts were evaluated for antioxidant activities using FRAP, ABTS and DPPH assays. The MeOH extract of *G. braunii* parasitizing on *P. thonningii* displayed the highest DPPH scavenging activity with $SC_{50}$ value 2.82 µg/mL while the EtOAc extract of *G. braunii* parasitizing on *P. biglobosa* showed highest antioxidant scavenging capacity in the ABTS assay with $SC_{50}$ value of 138.9 µg/mL. The MeOH extract of *G. braunii* parasitizing on *P. biglobosa* showed the highest FRAP equivalent value of 9.68 ± 0.14 mM. These results showed that EtOAc and MeOH are suitable solvents for extraction of active antioxidant agents from the leaves of *G. braunii* parasitizing on both hosts.

*Keywords*: *Globimetula braunii*, *Piliostigma thonningii*, *Parkia biglobosa*, FRAP, ABTS, DPPH

**Abstrak**

Daun kering *Globimetula braunii* yang tumbuh menumpang pada *Piliostigma thonningii* dan *Parkia biglobosa* telah diekstrak secara berturutan menggunakan n-heksana, diklorometana, etil asetat dan metanol dengan kaedah pengekstrakan sejuk. Semua ekstrak telah dinilai untuk aktiviti antioksidan menggunakan cerakinan FRAP, ABTS dan DPPH. Ekstrak MeOH *G. braunii* yang tumbuh menumpang pada *P. thonningii* menunjukkan aktiviti memerangkap DPPH tertinggi dengan nilai $SC_{50}$ 2.82 µg/mL manakala ekstrak etil asetat EtOAc *G. braunii* yang tumbuh menumpang pada *P. biglobosa* menunjukkan keupayaan memerangkap antioksidan tertinggi dalam cerakinan ABTS dengan nilai $SC_{50}$138.9 µg/mL. Ekstrak MeOH *G. braunii* yang tumbuh menumpang pada *P. biglobosa* menunjukkan nilai setara FRAP tertinggi iaitu 9.68 ± 0.14 mM. Keputusan ini menunjukkan bahawa EtOAc dan MeOH merupakan pelarut yang sesuai untuk mengekstrak agen aktif antioksidan daripada daun *G. braunii* yang tumbuh menumpang pada kedua-dua perumah.

*Kata kunci: Globimetula braunii, Piliostigma thonningii, Parkia* biglobosa, FRAP, ABTS, DPPH

## 1.0 INTRODUCTION

Antioxidants are active compounds that can protect plant and animal tissue from harm by free radicals. The free radicals, which are derivative of oxygen and nitrogen comprises of atoms, ions, or molecule with free single electrons, unstable and very active often referred to as reactive oxygen species (ROS) or reactive nitrogen species (RNS). These molecules attacked the adjacent molecules such as lipids, amino acids in proteins, nucleic acids DNA and RNA, and sugars [1]. Antioxidants can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. Phenolic compounds are well-known to contain group of compounds with high antioxidant activity due to their redox properties, which can perform a significant function in adsorbing and deactivating free radicals, reducing singlet and triplet oxygen, or disintegrating peroxides [2]. Evidences gathered in recent years indicated that free radicals and other oxidants that are formed naturally in the body are highly reactive chemicals that have the potential to harm healthy cells and may cause impairments, illnesses and several disorders. Cells destruction triggered by free radicals appeared to be a main causative agent to aging and ailments like heart disease, cancer and weakening of immune system and brain function. The ongoing controversy on the safety of synthetic antioxidants such as BHA, BHT and gallates that are reasonably anticipated to be a human carcinogen led to an increased curiosity in secondary metabolites having antioxidant properties [3]. Medicinal plants have been pondered to contain several antioxidants capable of stabilizing or deactivating free radicals before they attack cells. Plant secondary metabolites with antioxidant activity can scavenge reactive chemical species as well reduce the oxidative pressure resulting from unnecessary light energy acquaintances. Several plants metabolites are significant constituents of both human and animal foods and they are safe to be consumed [4]. Polyphenols including flavonoids are secondary metabolites of plants that are generally involved in defense against ultraviolet radiation, aggression by pathogens and in the last decades as antioxidant due to their proton donation activity. Polyphenols and other antioxidants were thought to protect cell constituents against oxidative damage through scavenging of free radicals [3, 5-6]. Therefore, the discovery of new antioxidants from medicinal plants would be a promising approach for treatment of various diseases.

*Globimetula braunii* (Engler) Van Tiegh belongs to family Loranthaceae. It is a hemi-parasitic plant or well known as mistletoe that lives on tree branches and woody stems. The plant leaves are simple, succulent and perennial that attached to their host through timbered suckers adventitious roots. It is a bushy parasitic plant found in variety of host plants from tropical Africa such as Ghana, Cameroun and Nigeria [7]. Scientific evidence has revealed that their compositions or medicinal activities are dependent on the host plant, harvesting period and species [8]. *G. braunii* has been widely used as ethnomedicine for hepatic illness, epilepsy, diabetes, malaria, stomach problems, infertility, constipation and cardiovascular disease [9-11]. So far, several activities have been reported for *G. braunii* parasitizing on different plants [12-15], report on the phytochemicals and antioxidant activity of *G. braunii* in the literature is lacking. So far, several activities have been reported for *G. braunii* parasitizing on different plants [12-15]. Report on the phytochemicals and antioxidant activity of *G. braunii* is still lacking.

This study, therefore is the first attempt aimed at evaluating the *in vitro* antioxidant activity of leaf extracts of *G. braunii* parasitizing on *Piliostigma thonningii* and *Parkia biglobosa* using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and ferric reducing antioxidant power (FRAP) assays to validate its use in ethnomedicine.

## 2.0 METHODOLOGY

### 2.1 Plant Material

Leaves of *Globimetula braunii* parasitizing on *Piliostigma thonningii* and *Parkia biglobosa* were collected in October 2014, from Sheda Science and Technology complex (SHESTCO), Abuja, Nigeria. The plants species were confirmed and validated at the herbarium section of the Biological Sciences Department, Ahmadu Bello University, Zaria, Nigeria. Voucher specimens for *G. braunii*, *P. thonningii* and *P. biglobosa* were labeled as 9016, 7151 and 2846, respectively.

### 2.2 Extraction of the Leaves of *G. braunii*

The powdered leaves of *G. braunii* obtained from *P. thonningii* (3.3 kg) and *P. biglobosa* (3.5 kg) were extracted successively by cold extraction method using *n*-hexane, dichloromethane ($CH_2Cl_2$), ethyl acetate (EtOAc) and methanol (MeOH). The extracts were concentrated using rotary evaporator to obtain black gummy extracts of *n*-hexane, $CH_2Cl_2$, EtOAc and MeOH. Each extract (6 mg) was dissolved in MeOH (6 mL) and these stock samples were used for the antioxidant assays.

### 2.3 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay

The DPPH assay was conducted according to method described by Najihah *et al*. [16] with slight modification. The DPPH reagent (100 µL) were added to the extracts (100 µL) with concentration ranging from 1000-10 µg/mL in MeOH obtained from serial dilution. The DPPH MeOH solution was used as control while butylated hydroxyanisole (BHA) was used as

the reference. The mixtures (DPPH + extract) were incubated for 30 minutes in dark room at room temperature. The absorbance of the resulting solutions was measured at 517 nm. DPPH free radical scavenging activity in percentage (%) antioxidant was calculated using the formula:

$$\% \text{ DPPH scavenging} = \frac{(A_{\text{blank DPPH}} - A_{\text{Sample}})}{A_{\text{blank DPPH}}} \times 100$$

$$*A_{\text{sample}} = (A_{\text{sample}} - A_{\text{blank DPPH}})$$

Where $A_{\text{blank DPPH}}$ is the absorbance of DPPH reagent with MeOH and $A_{\text{sample}}$ is the absorbance of the test sample with DPPH reagent.

### 2.4 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) Assay

ABTS assay was performed using method described by Zou *et al*. [17] with minor modification. ABTS and potassium persulfate were dissolved with distilled water to obtain concentrations of 7 and 4.9 mM respectively. Equal amount of these two solutions were mixed and the mixture was kept for 12 to 16 hours at room temperature in the dark before use. The ABTS solution was dissolved with distilled water until the absorbance reaches 0.7 at 734 nm. The extracts (10 µL) were added to 96-well plates together with ABTS solutions (190 µL) and allowed to react at room temperature in dark for 30 minutes. The absorbance of the mixture was recorded at 734 nm. Inhibition of ABTS radical scavenging was calculated using the following formula.

$$\% \text{ ABTS scavenging} = \frac{A_{\text{blank ABTS}} - A_{\text{Sample}}}{A_{\text{blankABTS}}} \times 100$$

$$*A_{\text{Sample}} = A_{\text{ABTS + Sample}}$$

Where $A_{\text{blank ABTS}}$ is the absorbance of ABTS solution with MeOH and $A_{\text{Sample}}$ is the absorbance of the test sample with ABTS solution.

### 2.5 Ferric Reducing Antioxidant Power (FRAP)

FRAP assay was carried out according to method described by Channarong *et al*. [18] and Shahwar *et al*. [19] with minor modifications. The working FRAP reagent was prepared by mixing acetate buffer (300 mM), $FeCl_3.6H_2O$ (20 mM) and TPTZ (2,4,6-tripyridyl-s-triazine) 10 mM in HCl (40 mM) in the ratio of 10: 1: 1. Test sample (0.5 µL), MeOH (15 µL) and working FRAP reagent (150 µL) were added to the 96-well plates. When a ferric-tripyridyltriazine ($Fe^{3+}$-TPTZ) complex is reduced to the ferrous ($Fe^{2+}$) an intense blue color with absorption maximum will be developed and the absorbance was read at 593 nm after incubation in water bath at 37°C [9]. The known $Fe^{2+}$ concentration calibration curve was obtained from the solutions of $FeSO_4.7H_2O$ ranging from 0.1 to 1.0 mM in MeOH. The antioxidant concentration of ferric-TPTZ reducing capacity corresponding to $FeSO_4.7H_2O$ (0.1 to 1.0 mM) was considered as the parameter equivalent concentration.

### 2.6 Statistical Analysis

Data obtained from biological activities were expressed as mean ± standard deviation. The statistical analyses were carried out by employing independent t-test where data was considered significant at $P<0.05$. A statistical package (SPSS version 16.0) was used for the data analysis. Three replicates of each tested sample were used. Calculation of the $IC_{50}$ measurements were run on Graph prism for windows (version 5.02) software after determining all percentage inhibitions using EXCEL program.

## 3.0 RESULTS AND DISCUSSION

The preliminary phytochemical screening carried out previously on EtOAc extracts of *G. braunii* obtained from *P. thonningii* revealed the presence of numerous secondary metabolites such as flavonoids, saponins, and tannins that are known to be biologically active constituents responsible for different activities such as antioxidant, antimicrobial, and anticancer [12]. These phytochemical constituents have been reported in several studies of medicinal plants to play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decompose formation of peroxides owing to the presence of conjugated rings or carboxylic acid [21-22].

Antioxidant activity can be evaluated by observing its reaction with a stable radical of DPPH in MeOH solution. The sturdy absorption band positioned at about 517 nm made DPPH free radical to produce deep violet colour in solution. The colour turned to pale yellow when the DPPH radicals were scavenged [23]. Table 1 showed the percentage of scavenging at 400 µg/mL and the scavenging concentration (SC) to acquire 50% of the maximum inhibition capacity of DPPH by the tested samples.

**Table 1** DPPH scavenging activity of leaf extracts of *G. braunii*

| Extracts | Percentage inhibition at 400µg/mL | $SC_{50}$ (µg/mL) |
|---|---|---|
| ***G. braunii*** from ***P. thonningii*** | | |
| *n*-Hexane | 43.20±0.33* | 224.0 |
| Dichloromethane | 93.73±3.00* | 18.48 |
| Ethyl Acetate | 98.74±0.15** | 3.93 |
| Methanol | 101.00±1.27* | 2.82 |
| ***G. braunii*** from ***P. biglobosa*** | | |
| *n*-Hexane | 36.42±1.92** | 233.7 |
| Dichloromethane | 40.93±0.48* | 253.7 |
| Ethyl Acetate | 96.98±0.41* | 6.36 |
| Methanol | 96.09±0.64** | 12.07 |
| **Standard** | | |
| BHA | 84.33±0.31* | 11.30 |

$SC_{50}$ (µg/mL) of antioxidant activity < 50 (very strong), 50-100 (strong), 101-150 (moderate), 151-200 (weak), 201-250 (very weak) and >251 (inactive). All values are expressed as mean ± SD for three replicates experiment; * P<0.05, ** = P>0.05 compared with control

The DPPH scavenging capacity of eight different extracts of *n*-hexane, $CH_2Cl_2$, EtOAc and MeOH from dried leaves of *G. braunii* parasitizing on *P. thonningii* and *P. biglobosa* at 100 – 1000 ppm concentration were determined and compared at 517 nm. The highest scavenging capacity was showed by the MeOH extract of *G. braunii* parasitizing on *P. thonningii* with $SC_{50}$ value of 2.82 µg/mL followed by the EtOAc extract (3.93 µg/mL). The EtOAc extract of *G. braunii* parasitizing on *P. biglobosa* also showed very strong scavenging capacity with $SC_{50}$ value of 6.36 µg/mL. The scavenging capacities of these extracts were even stronger than the standard control BHA. The DPPH method played a key role such that the antioxidants react with the stable free radical. This resulted in the free radical reaction by converting DPPH to 2,2-diphenyl-1-picrylhydrazine with colour change. The phytochemical screening on the EtOAc extract showed the extract contain flavonoids and phenolic compounds [12]. These classes of compounds were known to be able to decolourize DPPH solution with their high hydrogen donating capacity and could act as free radicals scavenging serving as primary antioxidant [24].

The ABTS assay is based on the generation of a blue/green ABTS that can be reduced by antioxidants and act as radical scavengers to their hydrogen and electron donating capacity. Table 2 showed the ABTS radical scavenging activities of extracts which were expressed as scavenging concentration at 50% ($SC_{50}$) and in mM Trolox Equivalent Antioxidant Capacity (TEAC). The antioxidant activities of the extracts in this assay were compared with standard reference, BHA and Trolox.

**Table 2** ABTS scavenging activity of leaf extracts of *G. braunii*

| Extracts | Percentage inhibition at 400µg/mL | $SC_{50}$ (µg/mL) | TEAC (mM) |
|---|---|---|---|
| ***G. braunii*** from ***P. thonningii*** | | | |
| *n*-Hexane | 16.57±1.24* | 2444 | 0.61±0.06* |
| Dichloromethane | 20.89±2.05** | 1320 | 0.68±0.01** |
| Ethyl Acetate | 91.09±1.84* | 146.5 | 1.00±0.07* |
| Methanol | 73.39±1.65** | 222.9 | 0.97±0.03** |
| ***G. braunii***f from ***P. biglobosa*** | | | |
| *n*-Hexane | 36.42±1.92** | 1603.0 | 0.65±0.01** |
| Dichloromethane | 40.93±0.48* | 734.9 | 0.82±0.02* |
| Ethyl Acetate | 96.98±0.41* | 138.9 | 1.01±0.02* |
| Methanol | 96.09±0.64** | 219.7 | 0.94±0.02** |
| **Standard** | | | |
| BHA | 52.60±1.90 | 317.7 | - |
| Trolox | 47.36±2.91 | 354.0 | - |

All values are expressed as mean ± SD for three replicates experiment; * P<0.05, ** P>0.05 compared with control;Trolox $R^2$ = 0. 9979.

The EtOAc and MeOH extracts reacted quickly with $ABTS^{\bullet+}$ but the *n*-hexane and $CH_2Cl_2$ extracts of *G. braunii* parasitizing on *P. thonningii* and *P. biglobosa* showed weak scavenging activity. Phenolic compounds and flavonoids have the ability to donate electron in order to scavenge the free radicals. The number and positions of the hydroxyl group on the aromatic ring binding site and the type of the substituents on flavonoids and phenolic compounds plays a significant role on the antioxidant activity [17, 25].

Reducing power of an antioxidant can be quantified by the reductive ability of the antioxidant and it is assessed by the reduction of reduce $Fe^{3+}$ to $Fe^{2+}$. $Fe^{2+}$ can be monitored by the formation of Pearl's Prussian blue and measured at 593 nm. BHA and Trolox were used as standard reference (Table 3). The results for FRAP were calculated from the calibration graph which were linear over the calibration range with $R^2$ value of 0.9843. The MeOH and EtOAc extracts of *G. braunii* showed the highest FRAP equivalent values and also scavenged free radicals such as $ABTS^{\bullet+}$ radical cation quickly. Consequently, it can be conveyed that, the extracts of *G. braunii* may act as free radical scavengers, efficient in converting reactive free radicals species into stable non radical products. The FRAP activity observed may be due to the present of compounds with phenolic groups and double bond in their structures which describe the ability of the extracts to act as reducing agent [19].

**Table 3** Ferric Reducing Antioxidant Power (FRAP) of leaf extracts of *G. braunii*

| Extracts | FRAP (mM equivalent to $FeSO_4.7H_2O$) 0.2 mM | 0.6 mM | 1.0 mM |
|---|---|---|---|
| ***G.braunii*** from ***P.thonningii*** | | | |
| n-hexane | - | - | - |
| Dichloromethane | - | - | - |
| Ethyl Acetate | 1.41±0.35* | 5.66±1.09* | 7.17±0.59*** |
| Methanol | 0.71±0.37* | 3.99±0.11** | 4.70±0.18** |
| ***G.braunii*** from ***P.biglobosa*** | | | |
| n-hexane | - | - | - |
| Dichloromethane | - | - | 3.94±0.49* |
| Ethyl Acetate | 1.17±0.31** | 4.50±0.14** | 5.74±0.77* |
| Methanol | 1.13±0.17* | 3.39±0.63*** | 9.68±0.14* |
| **Standards** | | | |
| BHA | 2.26±0.33 | 3.24±0.04 | 8.69±0.42 |
| Trolox | 0.68±0.04 | 1.89±0.26 | 2.75±0.20 |

All values are expressed as mean ± SD for three replicates experiment; *P<0.05, ** P>0.05, *** P>0.01compared with control, - = inactive.

## 4.0 CONCLUSION

The findings obtained in this study revealed that the EtOAc and MeOH leaf extracts of *G. braunii* possessed substantial antioxidant capacity by comparing with the standard reference BHA and Trolox. This might be due to unequal distribution of

phenolic compounds in the different extracts. Therefore, EtOAc and MeOH are better solvents for extraction of the therapeutic capabilities of *G. braunii* since they possessed stronger antioxidant properties than *n*-hexane and $CH_2Cl_2$. Further research is ongoing to isolate and identify the bioactive compounds responsible for the observed antioxidant activities.

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