Exploring the secondary metabolites and antioxidant capacities of methanol extract of *Peperomia pellucida* (L.) Kunth Leaves

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ABSTRACT

Background: Medicinal plants remain vital in the development of natural therapeutic agents due to their rich phytochemical composition. *Peperomia pellucida* (L.) Kunth, a member of the Piperaceae family, is traditionally used to treat inflammation, fever, and oxidative stress-related ailments.

Objective: This study evaluated the phytochemical constituents and in vitro antioxidant activities of the methanol extract of *P. pellucida* leaves collected from Sagamu, Ogun State, Nigeria.

Method: The leaves were air-dried, pulverized, and extracted using 80% methanol. Standard qualitative and quantitative phytochemical analyses were performed to determine the presence of secondary metabolites. Antioxidant activities were assessed using Total Antioxidant Capacity (TAC), Ferric Reducing Antioxidant Power (FRAP), Total Phenolic Content (TPC), and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays.

Results: Phytochemical screening revealed the presence of alkaloids (9.65 ± 0.004 mg/g), terpenoids (1.65 ± 0.001 mg/g), tannins (0.97 ± 0.001 mg/g), saponins (0.70 ± 0.003 mg/g), flavonoids (0.01 ± 0.145 mg/g), and phenols (0.64 ± 0.001 mg/g). The methanol extract demonstrated notable antioxidant activity, with mean TAC (1.40 ± 0.09 mg AAE/g), FRAP (4.08 ± 0.52 mg AAE/g), and TPC (1.62 ± 0.12 mg GAE/g). The DPPH assay showed a concentration-dependent free radical scavenging effect, yielding an IC_{50} value of 205.38 µg/mL compared to 46.5 µg/mL for ascorbic acid.

Conclusion: The findings indicate that *Peperomia pellucida* demonstrate measurable antioxidant potential, possibly due to its content phenolic, flavonoid, and alkaloid. This supports its traditional medicinal use and suggests its potential application as a natural antioxidant source in pharmaceutical and nutraceutical formulations.

Keywords: *Peperomia pellucida*; antioxidant capacity; Secondary Metabolites; phytochemicals

Exploration des métabolites secondaires et des propriétés antioxydantes de l'extrait méthanolique des feuilles de *Peperomia pellucida* (L.) Kunth

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RÉSUMÉ

Contexte: Les plantes médicinales restent essentielles au développement d'agents thérapeutiques naturels en raison de leur riche composition phytochimique. *Peperomia pellucida* (L.) Kunth, membre de la famille des Pipéracées , est traditionnellement utilisée pour traiter l'inflammation, la fièvre et les affections liées au stress oxydatif.

Objectif: Cette étude a évalué les composants phytochimiques et les activités antioxydantes in vitro de l'extrait méthanolique de feuilles de *P. pellucida* récoltées à Sagamu, dans l'État d'Ogun, au Nigéria.

Méthode: Les feuilles ont été séchées à l'air libre, pulvérisées, puis extraites à l'aide de méthanol à 80 %. Des analyses phytochimiques qualitatives et quantitatives standard ont été réalisées afin de déterminer la présence de métabolites secondaires. Les activités antioxydantes ont été évaluées à l'aide des tests suivants : la capacité antioxydante totale (CAT), le pouvoir antioxydant réducteur du fer (FRAP), la teneur totale en composés phénoliques (TCP) et le test de piégeage du radical 2,2-diphényl-1-picrylhydrazyle (DPPH).

Résultats: Le criblage phytochimique a révélé la présence d'alcaloïdes (9.65 ± 0.004 mg/g), de terpénoïdes (1.65 ± 0.001 mg/g), de tanins (0.97 ± 0.001 mg/g), de saponines (0.70 ± 0.003 mg/g), de flavonoïdes (0.01 ± 0.145 mg/g) et de phénols (0.64 ± 0.001 mg/g). L'extrait méthanolique a démontré une activité antioxydante notable, avec une capacité antioxydante totale (CAT) moyenne de 1.40 ± 0.09 mg EAA/g, un FRAP de 4.08 ± 0.52 mg EAA/g et une teneur en composés phénoliques totaux (CPT) de 1.62 ± 0.12 mg EAG/g. Le test DPPH a montré un effet de piégeage des radicaux libres dépendant de la concentration, donnant une valeur IC_{50} de 205.38 µg/mL par rapport à 46.5 µg/mL pour l'acide ascorbique.

Conclusion: Les résultats indiquent que *Peperomia pellucida* possède un potentiel antioxydant mesurable, probablement dû à sa teneur en composés phénoliques, flavonoïdes et alcaloïdes. Ces données soutiennent son usage en médecine traditionnelle et suggèrent son application potentielle en tant que source naturelle d'antioxydants dans les formulations pharmaceutiques et nutraceutiques.

Mots clés: *Peperomia pellucida* ; capacité antioxydante ; métabolites secondaires ; composés phytochimiques

INTRODUCTION

Medicinal plants continue to play a vital role in global healthcare due to their abundance of bioactive compounds with diverse pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer effects.[1] According to Rahman *et al*.,[2] oxidative stress, which arises from an imbalance between reactive oxygen species (ROS) and the body's antioxidant defense mechanisms, has been linked to the etiology of chronic diseases such diabetes, cancer, cardiovascular disease, and neurological disorders. Therefore, identifying natural antioxidants from plant sources represents a promising approach to mitigating oxidative stress-related conditions.

Phytochemicals such as phenolics, flavonoids, alkaloids, tannins, and terpenoids are major contributors to the antioxidant properties of plants.[3,4] According to Knezevic *et al*.,[5] these substances mainly work by inhibiting lipid peroxidation, scavenging free radicals, and chelating metal ions. Among various medicinal species, *Peperomia pellucida* (L.) Kunth belonging to the family Piperaceae has gained scientific attention for its ethnomedicinal and pharmacological properties. It is a small annual herb commonly found in moist, shaded environments of tropical regions. Traditionally, *P. pellucida* is used to treat headaches, inflammation, abscesses, gout, hypertension, and gastrointestinal disorders.[4,6]

Recent studies have shown that *P. pellucida* is rich in alkaloids, flavonoids, saponins, tannins, terpenoids, and phenolic compounds, all known for their potent antioxidant activities.[7] *In vitro* antioxidant assays are frequently used to assess the antioxidant potential of plant extracts, including Total Antioxidant Capacity (TAC), Ferric Reducing Antioxidant Power (FRAP), Total Phenolic Content (TPC), and DPPH radical scavenging.[8,9,10]

Previous findings suggest that methanol extracts of *P. pellucida* exhibit significant antioxidant potential, making it a valuable source of natural antioxidants.[4,11] However, variations in phytochemical composition may occur due to environmental factors, geographic origin, and extraction conditions.[6] Hence, regional evaluation is essential to establish its pharmacological relevance.

This study aimed to investigate the phytochemical composition and *in vitro* antioxidant potential of the methanol extract of *Peperomia pellucida* leaves collected from Sagamu, Nigeria, using standardized assays (TAC, TPC, FRAP, and DPPH).

MATERIALS AND METHODS

Plant collection and preparation

Fresh *Peperomia pellucida* leaves were purchased from the Sagamu local market, Ogun State, Nigeria. The plant was identified and authenticated by Mr. O. A. Adeoti of the Department of Pharmacognosy, Olabisi Onabanjo University (OOU), Sagamu, Ogun State, Nigeria. A voucher specimen (No. PHS/OOU/518) was deposited at the Forest Herbarium, Ibadan (FHI), Nigeria. The leaves were air-dried under shade to prevent photodegradation, ground into coarse powder using a mechanical grinder (Thomas Scientific, USA), and stored in airtight amber bottles until extraction.

Extraction procedure

Two hundred grams of the powdered sample were extracted using a Soxhlet apparatus with 1.0 L of 80 % methanol (methanol: water (80:20 v/v) for 72 hours at approximately 64 ^{o}C. The filtrate was concentrated under reduced pressure using a rotary evaporator and then lyophilized. The dried extract was stored at -20 ^{o}C until further use.

Phytochemical screening

Phytochemical analyses were carried out following standard procedures described by N'goka *et al*.[3] and De Silva *et al*.[12] Tests were performed for tannins, phenols, alkaloids, flavonoids, saponins, and triterpenoids using conventional colorimetric reactions.

Tannins

The presence of tannins was confirmed by the formation of a bluish-black or greenish coloration in a test tube containing 2 mL of the extract and a few drops of ferric chloride solution.[3]

Phenols

A deep bluish or greenish coloration developed when 2 mL of the extract was treated with 1 mL of ferric chloride solution, indicating the presence of phenolic compounds.[3]

Alkaloids

An orange precipitate formed upon adding a few drops of Dragendorff's reagent to 2 mL of the extract, signifying the presence of alkaloids.[3]

Flavonoids
To 2 mL of the extract, an equal mixture of hydrochloric acid and ethanol (50:50, v/v) was added, followed by magnesium shavings. The appearance of a red coloration indicated flavonols, while an orange coloration confirmed flavones.[3]

Saponins
Saponins were identified by the persistence of froth for two to three minutes after vigorous shaking of a test tube containing 2 mL of the extract.[3]

Triterpenes
A red ring showed sterols when 2 mL of chloroform and 2 mL of strong sulfuric acid were carefully added to the extract, while a reddish-brown hue verified triterpenoids.[3]

Antioxidant Activities

Total Antioxidant Capacity (TAC)
In accordance with Phatak and Hendre's[13] methodology, the phosphomolybdenum technique was used to ascertain the methanol extract of Peperomia pellucida's overall antioxidant capability. Through the formation of a green phosphate/Mo(V) combination in an acidic environment, this assay quantifies the extract's reduction of Mo (VI) to Mo(V). The reagent solution was made by combining 1 mL of each of the following: 4 mM ammonium molybdate, 28 mM sodium phosphate, and 0.6 M sulfuric acid. The mixture was then diluted with 50 mL of distilled water. Three milliliters of the reagent solution were added to each sample after the extract was serially diluted (30-200 µg/mL). After 90 minutes of incubation at 95 ^{o}C and cooling to room temperature, absorbance at 695 nm was measured. The amount of antioxidant capacity per gram of dry extract was measured in milligrams of Ascorbic Acid Equivalent (ASAE).

Total Phenolic Content (TPC)
The Folin-Ciocalteu method, as outlined by Ebrahimzadeh *et al*.[14], was used to ascertain the methanol extract's total phenolic content (*Peperomia pellucida*, MEPP). 5 mL of Folin-Ciocalteu reagent (1:10 dilution) was combined with 0.5 mL of each of the extract concentrations (200-1000 µg/mL) that were made. The mixture was incubated for 15 minutes after 5 minutes, then 4 mL of 1 M sodium carbonate was added. Using a UV-Vis spectrophotometer (UV-2600i, Shimadzu, Kyoto, Japan), absorbance was measured at 765 nm. Gallic acid equivalent (GAE) in milligrams per gram of dry extract was used to express the results.

Ferric Reducing Antioxidant Power (FRAP)
The reducing power of the *P. pellucida* methanol extract was ascertained using the FRAP assay, which was detailed by Trease and Evans.[15] The reagent was made up of 300 mM acetate buffer (pH 3.6), 50 mM $FeCl_3 \cdot 6H_2O$, and 10 mM TPTZ in a 1:1:10 ratio. Two milliliters of the FRAP reagent were combined with 75 microliters of the extract (20-100 µg/mL) for the assay. Absorbance was measured against a blank at 593 nm after two minutes.

DPPH radical scavenging activity
The Amarowicz *et al*.[16] approach was used to evaluate the DPPH radical scavenging activity. In methanol, a 0.1 mM DPPH solution was made. The extract, ascorbic acid (standard), and a control (methanol alone) were produced in a range of concentrations (50-250 µg/mL). One milliliter of DPPH solution was added to each sample, and it was then allowed to sit at room temperature for half an hour in the dark. At 517 nm, absorbance was measured. The following was used to determine the % inhibition:

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Sample}) \times 100}{\text{Absorbance of Control}} \quad \ldots\ldots\ldots (1)$$

Linear regression analysis was used to estimate the IC_{50} value, which is the concentration needed to block 50 % of DPPH radicals.[17]

Statistical analysis
Data were expressed as mean ± standard error of the mean (SEM). IC_{50} values were determined by linear regression analysis to estimate the concentration of extract required to inhibit 50 % of DPPH radicals.

RESULTS

Phytochemical Properties of Methanol Extract of Peperomia Pellucida (MEPP) Leaves
Tannins, phenols, alkaloids, saponins, flavonoids, and terpenoids were detected in the methanol extract of Peperomia pellucida leaves according to the phytochemical screening. Among these, alkaloids, terpenoids, and cardiac glycosides were abundantly present, while others were present in moderate to low concentrations. The corresponding quantitative values are shown below in Table 1.

Table 1: Qualitative and Quatitative Phytochemical screening of methanol extract *Peperomia pellucida* leaves

S/N	Qualitative screening	Methanol extract *Peperomia pellucida* leaves	Quantitative Screening
1	Tannin	+	0.97±0.001
2	Phenol	+	0.64±0.001
3	Alkaloid	+	9.65±0.004
4	Saponin	+	0.70±0.003
5	Flavonoid	++	0.01±0.145
6	Terpenoid	++	1.65±0.001

+: Present; ++: Abundantly Present.

Statistical data were indicated as Mean± Standard Error Mean.

Ferric ion reducing antioxidant power assay in methanol extract *Peperomia pellucida* leaves

All tested concentrations of the methanol extract of *Peperomia pellucida* leaves (MEPP) exhibited a clear, concentration-dependent increase in ferric reducing antioxidant power (FRAP) as shown in Table 2. The extract demonstrated progressively higher reducing ability with increasing concentration, reflecting its strong electron-donating capacity and potential to act as a reducing agent in neutralizing free radicals. At 100 µg/mL, MEPP showed the highest reducing power of 5.61 ± 0.52 mg ASAE/g, compared to 4.85 ± 0.52 mg ASAE/g at 80 µg/mL, 3.95 ± 0.52 mg ASAE/g at 60 µg/mL, 3.26 ± 0.52 mg ASAE/g at 40 µg/mL, and 2.74 ± 0.52 mg ASAE/g at 20 µg/mL. The mean FRAP value across all concentrations was 4.08 ± 0.52 mg ASAE/g, indicating that the methanol extract of *Peperomia pellucida* possesses considerable ferric reducing antioxidant potential (Table 2).

Table 2: Ferric ion reducing antioxidant power assay in methanol extract *Peperomia pellucida* (MEPP) leaves

MEPP Concentration (µg/mL)	20	40	60	80	100	Mean ±SEM (FRAP)
(ASAE/g)	2.74	3.26	3.95	4.85	5.61	4.08±0.52

The Mean ±SEM represents the Mean FRAP value in the table.

Total Antioxidant Content (TAC) of *Peperomia pellucida* methanol extract

As presented in Table 3, the total antioxidant content (TAC) of the methanol extract of *Peperomia pellucida* leaves (MEPP) showed a dose-dependent increase in antioxidant activity across the tested concentrations. Antioxidant potential increased progressively with concentration, indicating that higher extract concentrations possess stronger electron-donating and free radical-scavenging abilities. The maximum TAC value was recorded at 200 µg/mL with 1.65 mg of ascorbic acid equivalent per gram (ASAE/g), while the lowest was observed at 30 µg/mL with 1.13 mg ASAE/g. The mean TAC value for MEPP was calculated to be 1.40 ± 0.09 mg ASAE/g, reflecting moderate antioxidant capacity comparable to other medicinal plants with similar phytochemical composition (Table 3).

Table 3: Total Antioxidant Contents (TAC) of methanol extract *Peperomia pellucida* leaves

Concentration of MEPP in µg/mL	30	75	100	150	200	Mean ±SEM (TAC)
(ASAE/g)	1.13	1.33	1.34	1.55	1.65	1.40±0.09

The Mean ±SEM represents the Mean TAC value in the table

DPPH Scavenging Activity (% Inhibition) by Ascorbic Acid (ASA) and Methanol Extract of *Peperomia pellucida* Leaves (MEPP)

Ascorbic Acid (ASA) and Methanol Extract of *Peperomia pellucida* Leaves (MEPP) Inhibit DPPH Scavenging Activity (% Inhibition) DPPH radical scavenging activity findings for varying doses of *Peperomia pellucida* leaf methanol extract (MEPP) and the common antioxidant ascorbic acid (ASA) are shown in Table 4. MEPP demonstrated dose-dependent in vitro DPPH radical scavenging activity at all tested doses. Likewise, ascorbic acid showed an increase in free radical scavenging capacity that was concentration-dependent. 250 µg/mL had the highest scavenging activity for MEPP (54.15 %), while 50 µg/mL had the lowest (31.66 %). With an IC_{50} value of 205.38 µg/mL and a mean DPPH inhibition of 43.19 ± 4.42 %, MEPP demonstrated moderate antioxidant activity. Ascorbic acid, on the other hand, had greater scavenging activity, with an IC_{50} of 46.5 µg/mL and a mean inhibition of 63.31 ± 8.57% (Table 4 and Figure 1).

Table 4: Ascorbic acid and methanol extract-induced DPPH scavenging activity (percent inhibition) Leaves of *Peperomia pellucida* (MEPP)

Concentration of MEPP in µg/mL	50	100	150	200	250	Mean ±SEM	IC_{50}
% Inhibition of DPPH by MEPP	31.66	33.92	46.73	49.50	54.15	43.19±4.42	205.38 (µg/mL)
% Inhibition of DPPH by ASA	53.80	55.13	64.64	70.72	72.24	63.31±8.57	46.5(µg/mL)

The Mean percentage Inhibition of DPPH value in the table is expressed as the Mean ±SEM and half maximal inhibitory concentration (Ic50)

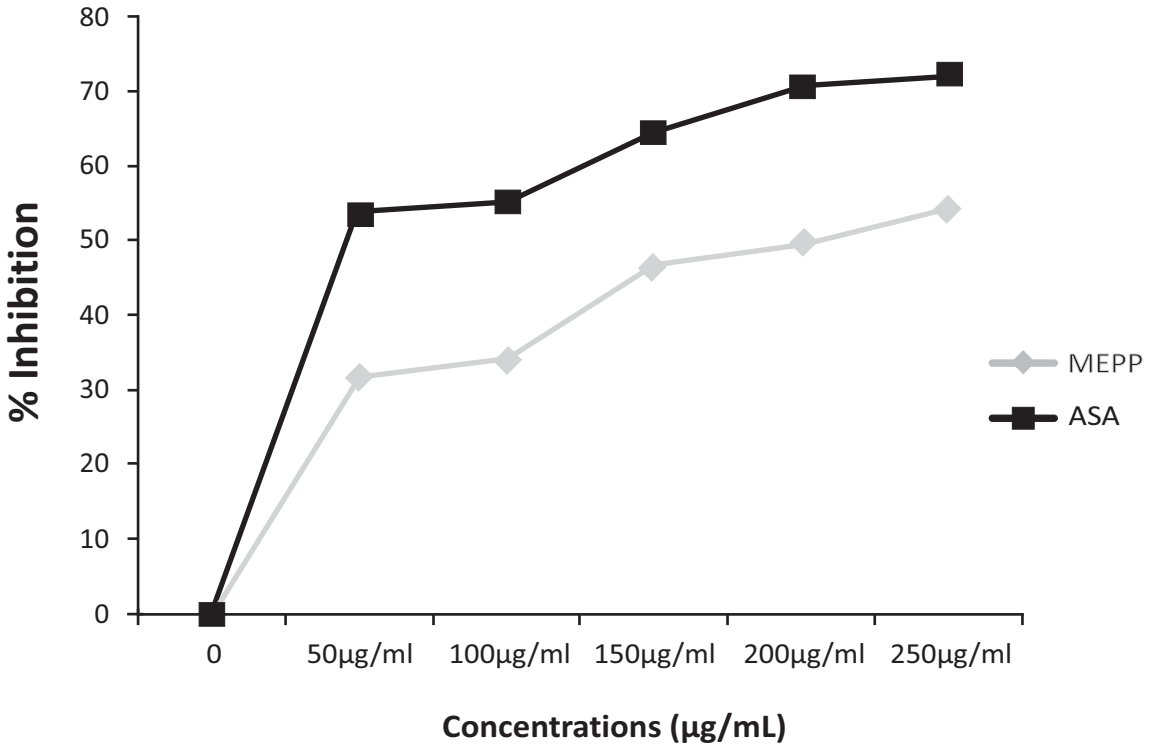

Figure 1: Plot showing the proportion of DPPH radical scavenging between methanol extract and ascorbic acid Leaves of *Peperomia pellucida*

Total Phenolic Contents (TPC) of methanol extract of *Peperomia pellucida* leaves

Gallic acid equivalents (mg GAE) per gram of extract were used to express the total phenolic contents (TPC) of the *Peperomia pellucida* leaf methanol extract (MEPP). Table 5 illustrates how the TPC gradually rose with concentration, suggesting that the extract's phenolic components accumulated in a concentration-dependent manner. 1000 µg/mL, or 1.98 mg GAE/g, had the highest phenolic content, while 200 µg/mL, or 1.28 mg GAE/g, had the lowest. The average TPC of MEPP at all concentrations was determined to be 1.62 ± 0.12 mg GAE/g, indicating that the leaves of *Peperomia pellucida* contain significant amounts of phenolic components that could be responsible for their antioxidant capacity (Table 5).

Table 5: Total Phenolic Contents (TPC) of and methanol extract *Peperomia pellucida* (MEPP) leaves

Concentration of MEPP in µg/mL	200	400	600	800	1000	Mean ±SEM (TPC)
(GAE/g)	1.28	1.50	1.55	1.77	1.98	1.62±0.12

The Mean TPC value in the table is expressed as the Mean ±SEM

DISCUSSION

In this work, the methanol extract of *Peperomia pellucida* (L.) Kunth leaves was analyzed for its phytochemical components and *in vitro* antioxidant properties. The extract contained bioactive substances with antibacterial, anti-inflammatory, and antioxidant qualities, including anthraquinones, phenols, flavonoids, alkaloids, saponins, terpenoids, cardiac glycosides, tannins, and steroids.[3,7]

The substantial presence of alkaloids (9.65 ± 0.004 mg/g) and terpenoids (1.65 ± 0.001 mg/g) suggests a strong antioxidant potential. These compounds are known to modulate redox signaling and enhance endogenous antioxidant defense mechanisms.[1,5] Flavonoids and phenolics, recognized for their hydrogen-donating and metal-chelating abilities, also contribute significantly to the plant's antioxidant profile.[8,10]

The methanol extract exhibited moderate total antioxidant capacity (1.40 ± 0.09 mg ASAE/g) and total phenolic content (1.62 ± 0.12 mg GAE/g), indicating effective redox potential. FRAP results (4.08 ± 0.52 mg ASAE/g) demonstrated concentration-dependent reducing power, aligning with reports by Men and Tuan6 and Ahmad *et al*.[11]

The DPPH assay showed dose-dependent radical inhibition with an IC_{50} of 205.38 µg/mL-lower than that of ascorbic acid (46.5 µg/mL) but still indicative of significant antioxidant capacity.[4,9] Such variations may result from environmental factors affecting metabolite accumulation.[6]

These antioxidant indices are comparable to those of other Nigerian medicinal plants such as Ocimum gratissimum and Vernonia amygdalina.[2] The methanol extract's ferric-reducing and radical-scavenging abilities confirm its role as both a primary and secondary antioxidant.[7] Additionally, saponins may enhance antioxidant defenses by modulating oxidative pathways.[5,8]

In summary, the methanol extract of *P. pellucida* leaves exhibits promising antioxidant potential, validating its ethnomedicinal use against oxidative stress-related disorders. Further chromatographic and spectroscopic analyses are warranted to isolate and characterize the specific phenolic and flavonoid compounds responsible for these activities.

CONCLUSION

This study revealed that the methanolic leaf extract of *Peperomia pellucida* contains key phytochemicals that contribute to its antioxidant activity, as confirmed by DPPH, FRAP, TAC, and TPC assays. The extract showed concentration-dependent free radical scavenging and reducing power, supporting its potential as a natural antioxidant. These findings validate its traditional use and highlight its promise in pharmaceutical and nutraceutical applications. Further studies on active compounds, mechanisms, and in vivo effects are recommended, alongside efforts toward sustainable cultivation.

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