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# COMPARATIVE *IN VITRO* ANTIOXIDANT ACTIVITIES OF METHANOL EXTRACTS OF *Phragmanthera incana* LEAVES FROM GUAVA, CASHEW, MANGO AND KOLANUT TREES

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**ABSTRACT:** *The therapeutic effects of several plants used in traditional medicine are usually attributed to their antioxidant potential. Phragmanthera incana belonging to the family Loranthaceae is a species of mistletoe commonly found in South-Western part of Nigeria and used ethno-medicinally in the management of diseases such as diabetes, hypertension and oxidative stress. This study evaluates and compare the antioxidant potential of methanol extracts of P. incana leaves hemi-parasitic on Guava, Cashew, Mango and Kolanut trees using 1,1-diphenyl-2-picrylhydrazyl (DPPH), Ferric Reducing Antioxidant Power (FRAP) and Total Antioxidant Capacity (TAC). P. incana leaves from kolanut exhibited a higher DPPH scavenging activity and reduces ferric ion to ferrous ion in a concentration dependent manner when compared with other test extracts while P. incana leaves from guava demonstrated the highest TAC when compared with other test extracts. The antioxidant profile shown by DPPH scavenging activity, FRAP, and TAC of the methanol extracts of P. incana leaves from the selected host trees in a dose dependent manner indicate that the extracts are rich source of antioxidants and can possibly potentiate the antioxidant potential in vivo and could serve as sources of antioxidants for nutritional and therapeutic purposes.*

**KEYWORDS:** Antioxidant, Medicinal plant, Mistletoes, *Phragmanthera incana*.



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## INTRODUCTION

The medicinal value of plants has assumed a more important dimension owing largely to the discovery that extracts from plants contain not only minerals and primary metabolites, but also a diverse array of secondary metabolites with antioxidant potential (Akinmoladun et al., 2007). These metabolites in plants are capable of preventing and fighting oxidative related diseases (Ayansor et al., 2010). The therapeutic effects of several plants used in traditional medicine are usually attributed to their antioxidant compounds. Antioxidants have been traditionally classified into two main types, based on their mechanism of action: the chain-breaking antioxidants; the primary antioxidants which react with free radicals producing less reactive species or interrupting the radical chain reaction and the secondary antioxidants; the so-called preventive, which act through indirect pathways and delaying the oxidation process (Monreal-Corona et al., 2020).

*Phragmanthera incana* belonging to the family *Loranthecaea* is a species of mistletoe commonly found in South-Western part of Nigeria. They are hemi-parasitic plants which obtain their nutrients and structural support from its host trees. *P. incana* is often referred to as 'Afomo Onishana' because its spicules are like that of a match (Adeyemi et al., 2022). The leaves have been used ethno-medicinally in the management of various diseases, such as hypertension, diabetes, insomnia, among others (Adeyemi et al., 2022; Ogunmefun et al., 2013). The leaves contain different bioactive compounds, such as flavonoids, terpenoids, alkaloids, saponins, tannins and are rich sources of dietary elements essential for biochemical processes and body metabolism (Adeyemi & Osilesi, 2022). They represent a family of plant species with an untapped reservoir of novel compounds for drug discoveries (Adeyemi & Osilesi 2022). The inhibitory effect of methanol extract of *P. incana* leaves harvested from Kolanut and Cocoa on $Fe^{2+}$ induced lipid peroxidation and a higher radical scavenging abilities (Ogunmefun et al., 2015). More host plants have been identified as reported by Adeyemi et al. (2022) and there is no scientific data on their antioxidant activities. Therefore, this study evaluates and compares the antioxidant potential of methanol extracts of *P. incana* leaves hemi-parasitic on Guava (*Psidium guajava*), Cashew (*Anacardium occidentale*), Mango (*Mangifera indica*) and Kolanut (*Kola acuminata*) trees.

## METHODS

### Collection and Identification of Plant Materials

Fresh leaves of *P. incana* from four host trees, Guava, Cashew, Mango and Kolanut trees, were collected from their natural habitat in a forest at Imota, Ikorodu Local Government Area of Lagos State Nigeria. It was identified and authenticated at Forest Herbarium of Forest Research Institute (FRIN) Ibadan.

### Preparation and Extraction of Plant Material

The leaves were washed under running water to remove debris and contaminants and air dried separately under shade for one week. The dried leaves were pulverized using a mechanical grinder and stored in an airtight container until further use. The pulverized samples were extracted for 48h by cold maceration using 70% methanol at 1:6 w/v. The mixture was filtered using Whatman filter paper no. 1, and then concentrated in a rotary evaporator at 40 °C. The


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extracts obtained were stored at below 4 $^{o}$C until further use. Concentrated extracts were reconstituted to make 1 mg/ml stock solution which was used for the antioxidant assays.

**Determination of 1, 1-Diphenyl 2-Picryl Hydrazyl (DPPH) assay**

Free radical scavenging potentials of methanol extracts of *P. incana* leaves, which is based on the capacity of the extracts to reduce 1,1-diphenyl-2-picryl hydrazyl (DPPH), were adopted according to the procedure described by Ayansor et al. (2010). The absorbance of the resulting mixture was measured at 517 nm and converted to percentage antioxidant activity (AA %) using the formula:

$$AA\% = 100 - \frac{(Abs\ sample - Abs\ blank \times 100}{Abs\ control}$$

AA% indicates antioxidant activity of fractions; Abs sample indicates absorbance of sample; Abs blank indicates absorbance of blank (methanol and DPPH) and Abs control indicates absorbance of control. The $IC_{50}$ values which denote the concentration of methanol extract required to scavenge 50% of DPPH radicals were calculated for the samples.

**Determination of Ferric Reducing Antioxidant Power (FRAP) assay**

Ferric Reducing Antioxidant Power (FRAP) measures the reducing ability of antioxidants against oxidative effects of reactive oxygen species. The ferric reducing capacity of the *P. incana* leaves to reduce ferric-cyanide complex to the ferrocyanide complex was determined using a modified method described by Reddy & Grace (2016).

**Total Antioxidant Capacity**

The Total Antioxidant Capacity (TAC) of methanol extracts of *P. incana* was carried out using phosphomolybdenum method according to Priesto et al. (1999).

**Statistical Analysis**

The results were expressed as means ± standard deviation (SD) of three replicates. Where applicable, the data were subjected to one-way analysis of variance (ANOVA) and difference between means was determined by Duncan multiple range test using statistical analysis system (SPSS statistics 21.0). Correlation between extracts antioxidant activity was determined by regression analysis at 95% confidence level. $P < 0.05$ was considered significant.

## RESULTS AND DISCUSSION

**The DPPH antioxidant activity of methanol extracts of *P. incana* leaves**

Data from Figure 1 showed that the standard, Gallic acid ($IC_{50}$ = 178.89 µg/ml) had a significantly high ($p < 0.05$) DPPH scavenging activity compared with the test fractions followed by *P. incana* leaves from *K. acuminata* ($IC_{50}$ = 245.34 µg/mL) < *P. incana* from *A. occidentale* ($IC_{50}$ = 285.39 µg/mL) < *P. incana* from *P. guajava* ($IC_{50}$ = 398.09 µg/mL) < *P. incana* leaf from *M. indica* ($IC_{50}$ = 431.41 µg/mL). However, *P. incana* leaves from *K. acuminata* had a significantly high ($p < 0.05$) scavenging activity when compared with other test extracts (Table 1). The radical scavenging activity indicates that the methanol extracts of


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*P. incana* leaves from the four host trees reduced DPPH to DPPHH in a concentration dependent manner. However, *P. incana* leaves from *K. acuminata* (kolanut) exhibited a higher scavenging activity when compared with other test extracts making it the best among them in this regard.

**Ferric Reducing Antioxidant Power (FRAP) Assay of Methanol Extracts of *P. incana* Leaves**

The antioxidant capacity of *P. incana* leaves extracts to transform $Fe^{3+}$/ferricyanide complex to $Fe^{2+}$/ ferrous form was found to increase slightly in a dose dependent manner. However, the standards Gallic acid and Ascorbic acid had a significantly higher ($p < 0.05$) ferric reducing antioxidant power compared with other test extracts. Methanol extract of *P. incana* leaves from *K. acuminata* (kolanut) exhibited a higher ferric reducing power when compared with other methanol extracts. (Figure 4.2). This finding is similar to the report of Ogunmefun et al., 2015 where *P. incana* from kolanut exhibited a better reducing potential. Methanol extracts of *P. incana* leaves reduced ferric ion to ferrous ion in a concentration dependent manner which might be due to hydrogen donating potentials of Gallic acid as well as the basic structural orientation of the compounds present in the extracts. It has been reported that the ring orientation of compounds determines the ease by which a hydrogen atom from a hydroxyl group can be donated to a free radical as well as the capacity of the antioxidants to support an unpaired electron (Miguel, 2011; Al-Mamary & Moussa (2021).

**Total Antioxidant Capacity (TAC) of Methanol Extracts of *P. incana* Leaves**

The total antioxidant capacity of the different methanol extracts of *P. incana* leaves showed that *P. incana* from *P. guajava* had a significantly higher ($p < 0.05$) total antioxidant capacity (35.01 ± 0.02 mg ascorbic acid equivalent/g) followed by *P. incana* from *K. acuminata* (26.15 ± 0.17) when compared with other extracts (Figure 4.3). The study of the total antioxidant capacity by the various test extracts showed that PIPG demonstrated the best TAC when compared with other test extracts. The observed TAC by the various test extracts may be attributed to the hydrogen and electron donating potentials of phytochemicals present especially by the polyphenolic compounds (Priesto et al., 1999; Unuigbe et al., 2019).

## CONCLUSION

The antioxidant profile shown by DPPH scavenging activity, FRAP, and TAC of the methanol extracts of *P. incana* from the selected host trees in a dose dependent manner indicates that the extracts are rich source of antioxidants and can possibly potentiate the antioxidant potential *in vivo.* This could be a reflection of the bioactive constituents or a further confirmation of the presence of the bioactive compounds present. Methanol extracts of *P. incana* leaves posseses anti-oxidative properties that could serve as sources of antioxidants for nutritional and therapeutic purposes.

**Conflict of Interest**

The authors declares that there is no conflict of interest


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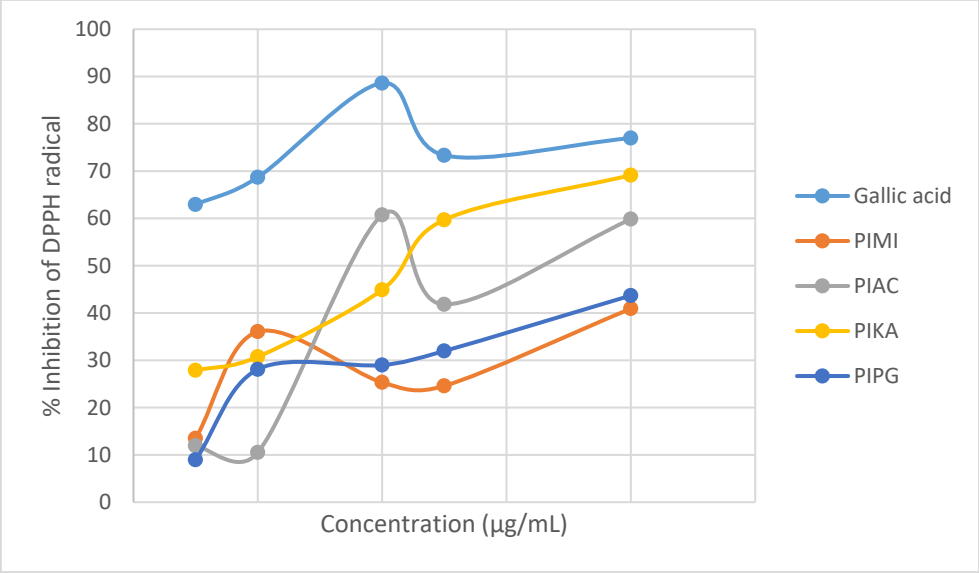


**Figure 1. DPPH radical scavenging activities of methanol extracts of *P. incana* leaves**

PIPG – extract of *P. incana* from *P. guajava* (Guava)

PIKA – extract of *P. incana* from *K. acuminata* (Kolanut)

PIAC – extract of *P. incana* from *A. occidentale* (Cashew)

PIMI – extract of *P. incana* from *M. indica* (Mango)

**Table 1: The DPPH antioxidant activity of methanol extracts of *P. incana* leaves**

| Assay | Extract | $IC_{50}$ (µg/mL) | Concentration (µg/mL) |
|---|---|---|---|
| **DPPH** | Gallic acid | $178.89^{a}$ | 25 – 400 |
| | PIPG | $398.09^{c}$ | |
| | PIKA | $245.34^{b}$ | |
| | PIAC | $285.39^{b}$ | |
| | PIMI | $431.41^{d}$ | |

Values on the same column with different superscript are significantly different from each other at $p < 0.05$

$IC_{50}$ – indicates fifty percent maximal inhibitory concentration

PIPG – extract of *P. incana* from *P. guajava* (Guava)

PIKA – extract of *P. incana* from *K. acuminata* (Kolanut)

PIAC – extract of *P. incana* from *A. occidentale* (Cashew)

PIMI – extract of *P. incana* from *M. indica* (Mango)


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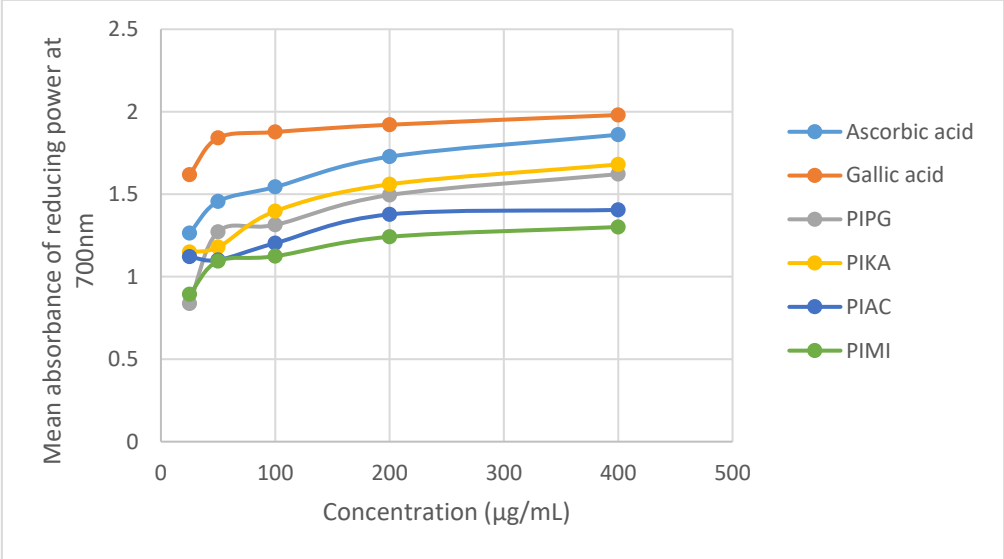


**Figure 2: Ferric reducing antioxidant power methanol extracts of *P. incana* leaves**

PIPG – extract of *P. incana* from *P. guajava* (Guava)
PIKA – extract of *P. incana* from *K. acuminata* (Kolanut)
PIAC – extract of *P. incana* from *A. occidentale* (Cashew)
PIMI – extract of *P. incana* from *M. indica* (Mango)

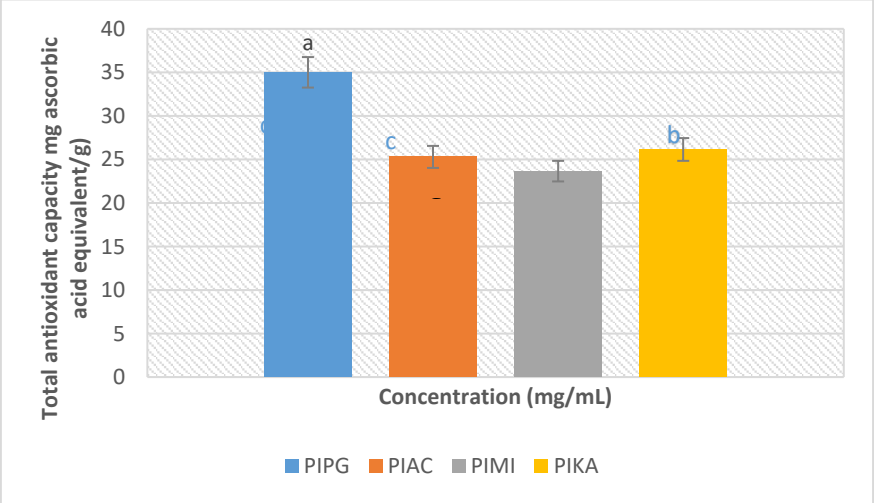


**Figure 3: Total antioxidant capacity of methanol extracts of *P. incana* leaves**

Values with different alphabets are significantly different from each other at $p < 0.05$

PIPG – extract of *P. incana* from *P. guajava* (Guava)
PIKA – extract of *P. incana* from *K. acuminata* (Kolanut)
PIAC – extract of *P. incana* from *A. occidentale* (Cashew)
PIMI – extract of *P. incana* from *M. indica* (Mango)


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