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# Determination of Quantitative Phytochemical Composition and Antioxidants Activities of Methanolic Leaf Extract of *Parinari curatellefolia*.

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**Abstract**

*Parinari curatellifolia* is one of the most valued and cherished medicinal plants. Traditional herbalists extensively use the plant's exceptional components to treat diseases. Methanolic leaf extract of *Parinari curatellifolia* was evaluated for its quantitative phytochemical composition and antioxidant properties in order to set up the relationship between these properties and its acclaimed healing properties in traditional medicine. Plant material was air-dried and methanolic extract was made from the leaf. The free radical scavenging activity of the leaf extract of *Parinari curatell*efolia with the aid of DPPH and H2O2 approach exhibited a concentration-dependent response. Antioxidant activities of the methanolic leaf extract of *Parinari curatellifolia* using 2, 2-diphenyl-1-picryl hydrazyl (DPPH) shows the percentage scavenging property of 41.67%, 72.67%, 77.85% and 80.49% at 20µg/ml, 40µg/ml, 60µg/ml and 80µg/ml respectively. Antioxidant activities of the methanolic leaf extract of *Parinari curatellefolia* using Hydrogen Peroxide Scavenging activity indicates the share scavenging activity of 49.45%, 52.49%, 56.35%, and 60.77% at 20µg/ml, 40µg/ml, 60µg/ml and 80µg/ml respectively. The methanolic leaf extract was partitioned for the quantitative phytochemical composition. The quantitative phytochemical composition of the aqueous partition indicates 9.86% saponin, 0.085% tannin, 4.81% alkaloids, 7.81% flavonoids, 0.44% phenolics, and 3.86% steroids. The quantitative phytochemical composition of the hexane partition indicates 2.23% saponin, 0.014% tannin, 4.10% alkaloids, 7.62% flavonoids, 0.357% phenolics, and 2.56% steroids. The quantitative phytochemical composition of the ethyl acetate partition indicates 5.96% saponin, 0.008% tannin, 5.99% alkaloids, 10.8% flavonoids, 0.127% phenolics, and 0.14% steroids. The presence of a good number of phytochemicals in the methanolic leaf extract of *Parinari curatelliolia* and the antioxidant activities displayed by the methanolic leaf extract of *Parinari curatelliolia* justifies the use of the plant by traditional healers in the management and treatment of disease.

**Keywords: *Parinari curatellifolia,* 2, 2-diphenyl-1-picryl hydrazyl (DPPH), Hydrogen Peroxide ($H_2O_2$), Phytochemical, Antioxidants**.

## INTRODUCTION

The therapeutic uses of herbs are on the basis of plant chemistry. A good information of the chemical composition of plants results in a better understanding of their possible medicinal value. Modern chemistry has described the position of primary plant metabolites in fundamental life functions such as cell division and growth, respiration, storage, and reproduction. They include the components of processes which include

glycolysis, the Krebs or citric acid cycle, photosynthesis, and related pathways. primary metabolites consist of small molecules which includes sugars, amino acids, tricarboxylic acids, Krebs cycle intermediates, proteins, nucleic acids, and polysaccharides. Eventually, the primary metabolites are similar in all living cells (Canarini et al., 2019). Secondary plant metabolites are numerous chemical compounds produced by the plant cell through metabolic pathways derived from the primary metabolic pathways. The idea of secondary metabolite was first defined by Albrecht Kossel, Nobel Prize winner for physiology or medicine in 1910. Thirty years later, Czapek described them as end-products (Hussein and El-Anssary, 2019). In line with him, these products are derived from nitrogen metabolism through what he referred to as 'secondary modifications' such as deamination. In the middle of the 20th century, advances in analytical techniques including chromatography allowed the recovery of increasingly more of these molecules, and this was the idea for the establishment of the field of phytochemistry. Secondary plant metabolites are classified into several classes in line with their chemical structures. The classes of secondary plant metabolites consist of Phenolics, Alkaloids, Saponins, Terpenes, Lipids, and Carbohydrates.

*Parinari curatellifolia* also known as "ganza kuisa' within the Hausa language of West Africa, is broadly utilized in some parts of Northern Nigeria as a recipe in the traditional control of diseases, together with liver-associated illnesses. In traditional medicine, the seed of *Parinari curatellifolia* is used in the healing of wound and skin problems, treatment of malaria, typhoid fever, fracture, and gastrointestinal disorders, whilst the leaf and bark extracts are deployed in the treatment of pneumonia, eye and ear illnesses, and the roots for treatment of cataracts and ear ache and liver disorders (Atawodi *et al.,* 2013). Accumulated research findings support the importance of antioxidants in the prevention and amelioration of various illnesses, inclusive of the healing of wounds and hepatic disorders, *Parinari curatellifolia* was evaluated for its antioxidant and quantitative phytochemical composition to be able to establish the connection between these properties and its acclaimed therapeutic uses in traditional medicine. *Parinari curatellifolia* is a valuable and cherished medicinal plant. Different parts of the plant are widely utilized by traditional herbalists in the treatment of diseases (Atawodi *et al.,* 2017).

## MATERIALS AND METHODS

### Materials:

- 99% methanol
- Powdered leaf of *Parinari curatellifolia*
- 2, 2-diphenyl-1-picryl hydrazyl (DPPH)
- Hydrogen peroxide, $H_2O_2$
- Ascorbic acid
- Analytical balance
- Centrifuge
- Micropipette
- Spectrophotometer
- 10% ethanolic acetic acid.
- filter paper
- Ethyl acetate
- Water
- HCl solution
- Test tube
- Folin-cio Caltean reagent
- Oven
- Desiccator
- Folin-Dennis reagent
- Tanuric acid

**Methods:**

**Collection of plant materials**

The plant material was collected from eastern Nigeria from traditional healers and indigenous herbal merchants. The collected specimen was identified at the Botany Department of the University of Nigeria Nsukka.

**Preparation of plant material**

Plant material was air-dried and methanolic extract was made from the leaf. The methanolic extract of the leaf of the plant was prepared in line with the standard technique (Harborne, 1973; Sofowora, 1982). Briefly, the plant sample collected was air-dried and ground using a milling machine. The powdered material was then transferred into a Soxhlet apparatus and extracted using methanol. The extract was concentrated to dryness and residue was obtained by using a rotary evaporator. stock solutions were prepared by dissolving the dried residues in dimethyl sulphoxide (DMSO). Extract solutions were stored at −20°C till required to be used.

**Free radical scavenging activity of the Leaf Extract of *Parinari Curatellifolia* using DPPH**

The DPPH assay approach described by Burits and Bucar (2000) was used with minor adjustments. The hydrogen atom or electron-donating abilities of the compounds had been measured from the bleaching of the purple-colored methanol solution of 2, 2-diphenyl-1-picryl hydrazyl (DPPH). This spectrophotometric assay makes use of the stable free radical, DPPH as a reagent. 1000 microlitres of diverse concentrations (20-80µg/ml) of the extracts in ethanol were added to 4 ml of 0.004% methanol solution of DPPH. After half-hour of incubation at room temperature, the absorbance was read against a blank at 517nm. The DPPH radical scavenging impact was calculated as inhibition percent (I %) using the following formula:

$$I\% = \frac{\text{A Blank} - \text{A Sample}}{\text{A Blank}} \times 100$$

where A blank is the absorbance of the control (containing all reagents except the test compound) and A sample is the absorbance of the test compound. The values of inhibition were calculated for various concentrations of the extract. Tests were carried out in triplicate.

**Hydrogen Peroxide Scavenging Activity of Leaf Extract of *Parinari curatellifolia***

A solution of $H_2O_2$ (40 mM) was prepared in phosphate buffer (pH 7.4). Extracts (20-80µg/ml) in methanol were added to $H_2O_2$ solution (0.6 ml, 40mM). The absorbance value of the reaction mixture was recorded at 230 nm. The blank solution contained the phosphate buffer without $H_2O_2$. The percentage of $H_2O_2$ scavenging activity was calculated as follows:

$$H_2O_2 \text{ scavenging effect } (\%) = \frac{\text{A Control} - \text{A Sample}}{\text{A Control}} \times 100$$

where:

A control is the absorbance of the control, and A sample is the absorbance in the presence of the sample or standards. (Ruch *et al*., 1989).

**Partitioning of the Methanolic Leaf Extract of *Parinari curatellifolia***

Crude methanolic leaf extract was partitioned using the Kupchan scheme as described by Otsuka, (2006) with moderate modification. Briefly, the extract was dissolved in water and transferred into a separatory funnel. Fifty milliliters (50ml) of hexane were delivered into the same separatory funnel, mixed properly, and set apart for layer separation. The hexane layer was collected into a round bottom flask and the solvent was evaporated to dryness. The remaining water layer was poured into a new round bottom flask. Fifty milliliters (50ml) of ethyl acetate were added to the water layer, mixed properly in a separatory funnel,

and left apart for layer separation. The ethyl acetate layer was collected into a round bottom flask and the solvent was evaporated to dryness. The water layer was additionally evaporated to dryness. The hexane, ethyl acetate, and aqueous partition were added into weight-known vials and labeled $P_H$, $P_E$, and $P_W$ respectively. They were left to dry under the fan.

**Determination Alkaloid**

The determination of the concentration of alkaloids in the leaf of the plant was carried out using the alkaline precipitation gravimetric technique described by Harborne (1973). five grams (5g) of the powdered sample was soaked in 20 ml of 10% ethanolic acetic acid. The mixture was allowed to stand for four (4) hours at room temperature. Thereafter, the mixture was filtered through the Whatman filter paper (No 42). The filtrate was concentrated by evaporation over a steam bath to ¼ of its original volume. To precipitate the alkaloid, concentrated ammonia solution was delivered in drops to the extract until it was in excess. The resulting alkaloid precipitate was recovered through filtration using previously weighed filter paper. After filtration, the precipitate was washed with 9% ammonia solution and dried inside the oven at 60°C for half an hour, cooled in desiccators, and reweighed. The process was repeated two more times and the average was taken. the weight of alkaloids was determined by the differences and expressed as a percentage of the weight of the sample analyzed as shown below.

$$\% \text{ Alkaloid} = \frac{W2-W1}{Weight\ of\ Sample} \times 100$$

Where:
W1 = weight of filter paper
W2 = weight of filter paper + alkaloid precipitate

**Determination Flavonoid**

The flavonoid content of the leaf of the plant was determined by the gravimetric method as described by Harborne (1973). Five grams (5g) of the powdered sample was placed into a conical flask and 50ml of water and 2ml HCl solution was added. The solution was allowed to boil for 30 minutes. The boiled mixture was allowed to cool before it was filtered through the Whatman filter paper (No 42). Ten milliliters (10ml) of ethyl acetate extract which contained flavonoid was recovered, while the aqueous layer was discarded. A pre-weighed Whatman filter paper was used to filter the second (ethyl-acetate layer), the residue was then placed in an oven to dry at 60°C. It was cooled in a desiccator and weighed.

The quantity of flavonoids was determined using the formula.

$$\% \text{ Flavonoid} = \frac{W2-W1}{Weight\ of\ Sample} \times 100$$

Where:

W1= Weight of empty filter paper
W2= Weight of paper + Flavonoid extract

**Determination of phenols**

The concentration of phenols in the leaf extract was determined using the folin-cio Caltean colorimetric method described by Pearson (1976). A powdered sample weighing 0.2g was added into a test tube and 10ml of methanol was added to it and shaken thoroughly. The mixture was left to stand for 15 minutes before being filtered using Whatman (No42) filter paper. One milliliter (1ml) of the extract was placed in a test tube and 1ml folin-cio Caltean reagent in 5ml of distilled water was added. Color was allowed to develop for about 1 to 2 hours at room temperature. The absorbance of the developed color was measured at 760nm wavelength. The process was repeated two times and an average was taken. The percentage phenol content was calculated as follows:

$$\text{Percentage Phenol} = \frac{100}{W} \times \frac{AU}{AS} \times \frac{C}{100} \times \frac{VF}{VA} \times D$$

Where,
W= weight of sample analyzed

AU= Absorbance of the test sample
AS= Absorbance of standard solution
C = concentration of standard in mg/ml
UF= total filtrate volume
VA= Volume of filtrate analyzed
D= Dilution factor

**Determination of saponins**

The saponin content of the samples was determined by the gravimetric double extraction method (Harborne, 1973). A powdered sample weighing 5g was mixed with 50 ml of 20% aqueous ethanol solution in a flask. The mixture was heated with periodic agitation in a water bath for 90 minutes at 55ºC. It was filtered through the Whatman filter paper (No 42). The residue was extracted with 50ml of 20% ethanol and both extracts were mixed together. The combined extracts were reduced to approximately 40 ml at 90°C and transferred to a separatory funnel, to which 40 ml of diethyl ether was added and shaken vigorously. The separation was carried out by discarding the ether layer and retaining the aqueous layer. Re-extraction by partitioning was done repeatedly until the aqueous layer became clear. Saponin was extracted using 60ml of n-butanol. The combined extracts were washed with 5% aqueous sodium chloride (NaCl) and evaporated to dryness in a pre-weighed evaporation dish. This process was repeated twice and the average value was obtained. The percentage of saponins was calculated as follows:

$$\text{Percentage Saponin} = \frac{W2-W1}{Weight\ of\ Sample} \times 100$$

Where
W1 = weight of evaporating dish
W2 =weight of dish + sample

**Determination Steroid**

The steroid content of the extract was determined using the method described by Harborne (1973). Five grams (5g) of the powdered sample was hydrolyzed by boiling in 50ml hydrochloric acid solution for about 30 minutes. It was filtered using Whatman filter paper (No 42), and the filtrate was transferred to a separating funnel. An equal volume of ethyl acetate was added to it, mixed well, and allowed to separate into two layers. The ethyl acetate layer (extract) recovered, while the aqueous layer was discarded. The recovered extract was dried at 100ºC for 5 minutes in a steam bath and heated with amyl alcohol to extract the steroid. The mixture became cloudy and the mixture was properly filtered using re-weighed Whatman filter paper (No42) was used to filter the mixture properly. The dry extract was cooled in a desiccator and reweighed. The process was repeated two times and an average was obtained. The percentage concentration of steroid was calculated as follows:

$$\text{Percentage Steroid} = \frac{W2-W1}{Weight\ of\ Sample} \times 100$$

Where,
W1= weight of filter paper.
W2 = weight of filter paper + steroid

**Determination Tannin**

The tannin content of the extract was determined using the Folin Dennis spectrophotometric method described by Pearson (1976). Two grams (2g) of the powdered sample was mixed with 50 ml of distilled water and shaken for 30 minutes. The mixture was filtered. Five milliliters (5ml) of the filtrate was measured into a 50ml volume flask and diluted with 3 ml of distilled water. Similarly, 5 ml of standard tanuric acid solution and 5 ml of distilled was added separately. One milliliter (1ml) of Folin-Dennis reagent was added to each of the flasks followed by 2.5 ml of saturated sodium carbonate solution. The contents of each flask were filled to the mark. The absorbance of the developed color was measured at 760nm with a reagent blank of zero. The process was repeated two more times to get an average. Tannin content was calculated as follows:

$$\text{Percentage tannin} = \frac{100}{W} \times \frac{AY}{AS} \times \frac{C}{100} \times \frac{VF}{VA} \times \text{D}$$

Where,

W= weight of sample analyzed
AY=Absorbance of the standard solution
C= Concentration of standard in mg /ml.
VA= volume of filtrate analyzed
D= Dilution factor

## RESULTS AND DISCUSSION

**Table 1.** Antioxidant Activities of the Methanolic Leaf Extract of *Parinari curatellifolia using* 2, 2- diphenyl-1-picryl hydrazyl (DPPH) Method.

| Concentration (µg/ml) | Sample (% Scavenging Activity) | Control (Ascorbic Acid) (% Scavenging Activity) |
|---|---|---|
| 20 | 41.67 | 57.37 |
| 40 | 72.67 | 79.29 |
| 60 | 77.85 | 88.11 |
| 80 | 80.49 | 88.31 |

The free radical scavenging activity of the leaf extract of *Parinari curatellefolia* by DPPH by method exhibited a concentration-dependent response. Antioxidant activities of the methanolic leaf extract of *Parinari curatellifolia* using 2, 2-diphenyl-1-picryl hydrazyl (DPPH) shows the percentage scavenging activities of 41.67%, 72.67%, 77.85% and 80.49% at 20µg/ml, 40µg/ml, 60µg/ml and 80µg/ml respectively

**Table 2.** Antioxidant activities of the Methanolic leaf extract of *Parinari curatellifolia using* Hydrogen Peroxide Scavenging Activity

| Concentration (µg/ml) | Sample (% Scavenging Activity) | Control (Ascorbic Acid) (% Scavenging Activity) |
|---|---|---|
| 20 | 49.45 | 49.45 |
| 40 | 52.49 | 54.97 |
| 60 | 56.35 | 70.99 |
| 80 | 60.77 | 84.53 |

The free radical scavenging activity of the leaf extract of *Parinari curatellefolia* by DPPH by method exhibited a concentration-dependent response. Antioxidant activities of the methanolic leaf extract of *Parinari curatellefolia* using Hydrogen Peroxide Scavenging Activity shows the percentage scavenging activity of 49.45%, 52.49%, 56.35%, and 60.77% at 20µg/ml, 40µg/ml, 60µg/ml and 80µg/ml respectively.

**Table 3.** Quantitative Phytochemical Components of the Various Partition of Methanolic Leaf Extract of *Parinari curatellifolia*

| Partition | SAP (%) | TA (%) | ALK (%) | FL (%) | PH (%) | ST (%) |
|---|---|---|---|---|---|---|
| $P_W$ | 9.86 | 0.085 | 4.81 | 7.81 | 0.044 | 3.86 |
| $P_H$ | 2.23 | 0.014 | 4.10 | 7.62 | 0.357 | 2.56 |
| $P_E$ | 5.96 | 0.008 | 5.99 | 10.8 | 0.127 | 0.14 |

Note: **SAP** = Saponin, **TA** = Tannin, **ALK** = Alkaloids, **FL** = Flavonoids, **PH** = Phenolics, **ST** = Steroids, $P_W$ = Aqueous partition, $P_H$ = Hexane partition, $P_E$ = Ethyl acetate partition

The Phytochemical screening of the plant revealed the presence of tannins, alkaloids, flavonoids, phenolics, glycosides, terpenoids, quinines, and steroids (Table 4.1). The result of the quantitative phytochemical composition of the water partition of the leaf extract indicates 9.86% saponin, 0.85mg/ml tannin, 4.81% alkaloids, 7.81% flavonoids, 4.44mg/ml phenolics, and 3.86% steroids. The result of the quantitative phytochemical composition of the hexane partition of the leaf extract indicates 2.23% saponin, 0.135mg/ml tannin, 4.10% alkaloids, 7.62% flavonoids, 3.57mg/ml phenolics and 2.56% steroids. The result of the quantitative phytochemical composition of the ethyl acetate partition of the leaf extract indicates 5.96% saponin, 0.08mg/ml tannin, 5.99% alkaloids, 10.8% flavonoids, 1.27mg/ml phenolics and 0.14% steroids (Table 4.2).

Tannin is a general descriptive name for a group of polymeric phenolic substances capable of tanning leather or precipitating gelatin from solution, a property known as astringency (Oludare and Bamidele, 2015). This group of compounds has received a great deal of attention in recent years since it was suggested that the consumption of tannin-containing beverages, especially green teas and red wines can cure or prevent a variety of ills (Delimont *et al*., 2017). Many human physiological activities, such as stimulation of phagocytic cells, host-mediated tumor activity, and a wide range of anti-infective actions, have been assigned to tannins (Omojate *et al.,* 2014).

Saponins are a class of chemical compounds, one of many secondary metabolites found in natural sources, with saponins found in particular abundance in various plant species. More specifically, they are amphipathic glycosides grouped, in terms of phenomenology, by the soap-like foaming they produce when shaken in aqueous solutions, and, in terms of structure, by their composition of one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative (Velu *et. al*., 2018). Saponins are found to complex with cholesterol to form pores in cell membrane bilayers. This complexation leads to cell lysis (Claereboudt *et. al*., 2018). In addition, the amphipathic nature of saponins make them act as surfactants that can be used to enhance the penetration of macromolecules such as proteins through cell membranes (Zhang *et. al.*, 2020).

Flavonoids are also hydroxylated phenolic substances but occur as a $C_6$-$C_3$ unit linked to an aromatic ring (Karak, 2019). Since they are known to be synthesized by plants in response to microbial infection (Roy *et. al*., 2022), it should not be surprising that the activity observed in this plant may be partly due to its presence in the leaf of this plant. Flavonoids have been found in vitro to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to form complexes with bacterial cell walls as well as extracellular and soluble proteins. More lipophilic flavonoids may also disrupt microbial membranes (Candiracci *et. al.,* 2011).

Steroids are a type of organic compound containing a characteristic arrangement of four cycloalkane rings linked together. Steroids vary by the functional groups attached to the four-ring core and by the oxidation state of the rings (Meher *et. al*., 2013). Sterols are special forms of steroids with a hydroxyl group at position 3 and a skeleton derived from cholestane (Purude, 2011). The activity of this plant extract may be a result of the presence of steroids. Steroids have been reported to have antibacterial properties, and the correlation between membrane lipids sensitivity for steroidal compound indicates the mechanism in which steroids specifically associate with membrane lipids and exerts their action causing leakages from liposomes (Shinde and Mulay, 2015). The presence of all these secondary metabolites with different mechanisms of antimicrobial activities makes this plant a potential source of antibiotics.

Phenolics are a group of highly hydroxylated compounds present in the extractive fraction of several plant materials. Polyphenols in plants include hydroxy coumarins, hydroxycinnamate derivatives, flavanols, flavonols, flavanones, flavones, anthocyanins, and proanthocyanidins often called condensed tannins. The antioxidant activity of polyphenols is mainly due to their redox properties, which may play an important role in the adsorption and neutralization of free radicals, oxygen quenching, or peroxide decomposition. Indeed, the antioxidant activity of some plant extracts is highly correlated with their phenolic content (Tajner-Czopek *et al.*, 2020). Crude

methanolic leaf extract of *P. curatellefolia* showed antioxidant activity which may be relevant in the treatment of oxidative stress.

## CONCLUSION

These Studies have shown that a large number of phytochemicals are present in the methanol extract of *Parinary curatelifolia* leaves and that the methanol extract of *Parinary curateliolia* leaves exhibits antioxidant activities. This justifies the use of plants by traditional healers to treat diseases.

## CONFLICT OF INTEREST

The authors expressed no conflicts of interest.

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