https://chemclassjournal.com/
ChemClass Journal Vol. 9 Issue 2 (2025); 764-771
e-ISSN:3092-8214 p-ISSN:3092-8206
doi.org/10.33003/chemclass-2025-0902/196

Phytochemical Screening and Evaluation of the Anti-Oxidant Activity of *Morinda citrifolia* (Noni) Seeds Extract

Abdullahi Sahal[1*], Fatima Muhammad Balarabe[1], Ahmad Garba[1], Safiya Yusuf Zubairu[1]

[1]*Department of Chemistry and Biochemistry, Nuhu Bamalli polytechnic Zaria, Kaduna.*

(*) Corresponding author: abuhanifa25@gmail.com 07039186436

Abstract

Morinda citrifolia (Noni) seeds are known to possess ethno medicinal values and have been used to treat various diseases. The study was aimed to screen the phytochemicals and evaluate the antioxidant activity of the seeds extract of *Morinda citrifolia*. The extraction was carried out using methanol by maceration method and the extract was screened for the phytochemicals using standard methods. The preliminary and quantitative phytochemical screening revealed some important phytochemicals; saponin (27.85 mg/100 g), alkaloids (1.24 mg/100g), tannin (2.70 mg/100 g) and flavonoid (85.50 mg/100 g). The total phenolic and flavonoids concentrations were found to be 15.65 mgGAE/g and 78.12 mgQE/g respectively. The antioxidant activity of the seeds extract was evaluated using DPPH and H_2O_2 free radical scavenging at concentration of 500 µg/ml to 31.25 µg/ml shows that the seeds possess antioxidant property with percentage radical scavenging inhibition ranging from 38.88% to 10.14%. The 50% scavenging (IC_{50}) of DPPH and H_2O_2 were 13.68 and 18.38 µg/ml respectively. It is therefore recommended that the seeds can be used as source of antioxidant.

Keywords: Antioxidants, *Morinda citrifolia,* Oxidation, Phytochemical

Introduction

Oxidation reactions in human is a biological process that involves electron transfer and when electron transfer becomes uncoupled it generate free radicals [1]. Oxidation may cause some problems when electron flow becomes uncoupled there-by generating free radicals. Free radicals can cause abnormal and uncontrolled oxidation in human systems which lead to damage of cell structure more especially when the antioxidant depend system is weak [2]. Cancer occurs due to excessive free radical which causes damage in the genetic DNA, proteins and lipids which further leads to the mutations and conversion of normal cell into cancer cell [3]. Antioxidants are compounds that protect cells against the damaging effect of reactive oxygen species [4]. Healthy and strong human body system maintains balance redox reaction between pro-oxidant and anti-oxidant [5].

Studies have shown that plants with vast phytochemicals help in inhibiting and eliminating free radicals. Fruits are one of the major sources of antioxidant phytochemicals [6], that play significant role in preventing various degenerative

diseases which is attributed to the natural antioxidant content present and consequently their consumption can protect one from high risk of cancer and heart diseases [7-8]. A significant relationship was established between the intakes of anti-oxidant containing plants and reduced mortality cause by oxidative stress [9]. This necessitated the need for continued screening of plants so as bring out their biological application which can serve as bio-resources in manufacturing drugs.

Morinda citrifolia popularly known as Noni is an evergreen shrub whose ripe fruit has a strong butyric acid smell and flavour. *Morinda citrifolia* is cultivated in the tropical and subtropical region of the world [10-11]. The fruit is traditionally used in treating diabetes, hypertension and cancer. It is widely used as an alternative therapy in treating gastritis, skin diseases, respiratory infections, menstrual disorder, urinary tract infection and venereal diseases [12-13]. *Morinda citrifolia* fruit contains a number of antioxidants such as beta-carotene, ascorbic acid, terpenoids, alkaloids, beta-sitosterol, carotene, polyphenols such as flavonoids, flavone glycosides, rutin etc. [14]. It can also increase immune system activity. Previous study by Sunder [15] showed that the seeds exhibit pronounced inhibitory activity than the various part of the plant. However, not much has been reported on the seeds ability to scavenge free radicals. Therefore this present study focus on screening the phytochemicals and evaluating the anti-oxidant activity of methanol extract of the seeds of *Morinda citrifolia*.

Materials and Methods

Sample Collection and Identification

Fresh Noni fruits were collected at Sabon Gari Zaria Local Government Area of Kaduna State and were identified as *Morinda citrifolia* by a botanist at Ahmadu Bello University Zaria (V/No. ABUH0382).

Sample Preparation

The seeds of *Morinda citrifolia* were separated from the fruits; 150g of the seeds were rinsed thoroughly with water and air-dried at room temperature. The seeds were then pulverized in a mortar with a pestle to fine powder. The powder was packed and stored in an air tight bag prior to analysis.

Preparation of Methanol Extract

Powdered sample (200 g) was weighed in a beaker and added 1000 cm^3 of methanol ensuring that the plant material is completely immersed. The container was tightly sealed and left for a period of 48 hours at room temperature, with intermittent shaking. The extract was then filtered using filter paper. The filtrate was concentrated using a rotary evaporator at a low temperature (40°C) until a semi-solid residue was obtained.

Phytochemical Screening

The methanolic extract was screened for the presence of alkaloids, flavonoids, phenols, tannins, saponins, terpenoids, and glycosides using standard

qualitative test as described by Saddiqui [15] & Harborne [17].

Quantitative Phytochemical Analysis

The quantitative phytochemical screening of the seeds methanol extract was carried out using standard spectrophotometric analytical methods as described by Trease and Evans [18].

Alkaloids: The extract (1 ml) was measured with 20ml of ethanol and 20% H_2SO_4 (1:1 v/v). The filtrate (l ml) was added to 5ml of 60% H_2SO_4. After 5 minutes 5 ml of 0.5 % formaldehyde in 60 % H_2SO_4 was mixed with the mixture and allowed to stand for 3 hrs. the absorbance was read at 565 nm.

Flavonoids: The extract (1 ml) was macerated with 20 ml of ethyl-acetate for 5 mins and filtered. To the filtrate 5 ml of dilute ammonia was added and shaken for 5 mins. The upper layer was collected and the absorbance was read at 490 nm.

Saponins: The extract (5 ml) was macerated with 10 ml of petroleum ether and decanted into a beaker. Another 10 ml of the petroleum ether was added into the beaker and filtrate evaporated into dryness. The residue was dissolved in 6ml of ethanol. The solution (2 ml) was put in a test tube and 2 ml of chromogen solution added into it. It was left to stand for 30min and the absorbance was read at 550 nm.

Tannins: The extract (1 ml) was macerated with 50 ml of methanol and filtered. 0.3 ml of 0.1 M Ferric chloride and 0.3 ml of 0.0008 M Potassium ferricyanide were added to the filtrate and the absorbance was read at 720 nm.

Determination of total phenolic content

The total phenolic content of the extract was determined using the method of Macdonald [19]. Calibration curve was prepared by mixing ethanol solution of Gallic acid (1 ml; 0.025-0.400 mg/ml) with 5 ml Folin-Ciocalteu reagent (diluted tenfold) and sodium carbonate (4 ml, 0.7M). Absorbance values were measured at 765 nm and the standard curve was drawn. One milliliter of the methanol extract was also mixed with the reagents above and after 30 min the absorbance was measured to determine the total phenolic contents. The total phenolic compound in the extract in gallic acid equivalents (GAE) was calculated by the following formula:

$$T = \frac{C.V}{M}$$

Where T = total phenolic contents (milligram per gram plant extract) in GAE, C = the concentration of gallic acid established from the calibration curve (milligram per milliliter), V= the volume of extract (milliliter), M = the weight of methanol plant extract (gram).

Determination of total flavonoids content

The flavonoids content in the plant extracts was estimated according to Chang [20] with quercetin as reference standard. About 0.5 mL of the crude extract was mixed with 0.5 mL of 2% methanolic solution aluminium chloride ($AlCl_3$). The reaction mixture was kept at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm using a UV-VIS Spectrophotometer-1 800, Shimadzu. The value of

optical density was used to calculate the flavonoids content present in the sample and the calibration curve was plotted by using quercetin solutions at concentrations 12.5 to 100 µg/ml in methanol

Antioxidant Activity

a. DPPH Radical Scavenging Activity:

The free radical scavenging ability of the extract was evaluated using DPPH (1,1- diphenyl-2-picryhydrazyl) method. One milliliter of the extract was mixed with 1 mL of the 0.4 mM methanolic solution of the DPPH, and then the mixture was left in the dark for 30 min before measuring the absorbance at 517 nm. The control (ascorbic acid) consisted of methanol instead of the sample and the radical scavenging ability of the sample was calculated as:

$$\text{Scavenging activity (\%)} = \frac{[(A_{control} - A_{sample})]}{A_{control}} \text{ x } 100$$

Afterwards, a graph of scavenging activity against the concentration of the extract was made and determines the IC_{50} value (concentration required to scavenge 50% of DPPH radicals).

b. Hydrogen Peroxide Scavenging Activity

Phosphate buffer solution (50 ml) was added to an equal amount of hydrogen peroxide to generate the free radicals and solution is to be kept aside at room temperature for 5 minutes to complete the reaction. Extracts (1.0 ml) in distilled water was added to 0.6ml hydrogen peroxide solution, and the absorbance was measured at 230 nm in a spectrophotometer (UV-1 800, UV-VIS spectrophotometer, Shimadzu) against a blank solution containing phosphate buffer solution without hydrogen peroxide. Concentrations selected for extract were 500, 250, 125, 62.5 and 31.25 µg/ml. Ascorbic acid was used as the standard. The experiments were repeated in triplicates. The percentage of scavenging of H_2O_2 of the extract was measured using the equation:

$$\text{\% Radical scavenging activity} = \frac{A_C - A_S}{A_C} \text{ x } 100$$

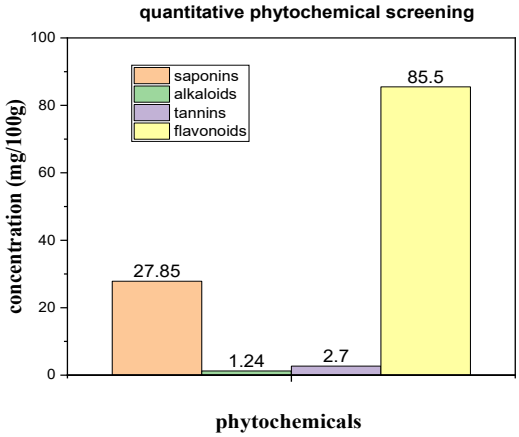
Where As = the Absorbance of the sample and Ac = the Absorbance of the control

Results and Discussion

Table1. Preliminary phytochemical screening of methanol seeds extract of *Morinda citrifolia*

S/N	Phytochemical Constituent	Test	Result
1	Alkaloids	i. Dragendorff's	+
		ii. Mayer's	+
2	Flavonoids	i. Shinoda	+
		ii. lead acetate	+
3	Saponins	i. Froth	+
		ii. Hemolysis	+
4	Tannins	i. Ferric chloride	+
		ii. Gelatin	+
5	Terpenoids	i. Salkowski	+
		ii. Liebermann-Burchard	+
6	Steroids	i. Salkowski	+
		ii. Liebermann-Burchard	+
7	Phenolic compounds	i. Ferric chloride	+
		ii. Lead acetate	+
8	Glycosides	i. Keller-Killiani	+
		ii. Legal's	+
9	Quinones	-	-
10	Anhraquinone	-	-

Key; + = present
- = absent



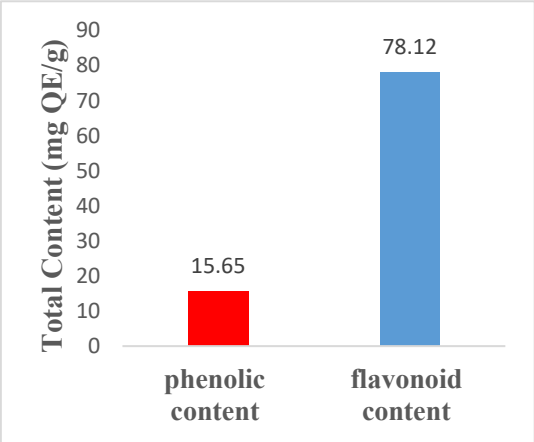


Figure 1: Quantitative phytochemical screening **Figure 2: Total content of phenolic and flavonoids**

Table 2: Free radical scavenging activity of seeds methanol extract of *Morinda citrifolia*

Concentration	NSME DPPH	ASBCA DPPH	NSME H_2O_2	ASBCA H_2O_2
31.25	3.53±1.83	70.90±0.24	6.96±0.87	95.30±0.56
62.5	10.21±1.41	74.70±0.54	12.99±1.11	91.95±0.14
125	17.14±4.37	80.80±0.45	19.50±1.19	90.44±0.36
250	24.45±5.24	82.10±0.34	25.51±2.77	89.01±0.04
500	38.88±0.47	84.52±0.28	30.99±1.1	85.79±0.23
IC_{50}	638.30	954.82	857.95	2534.50

Key
NSME = noni seeds methanol extract
ASBCA = ascorbic acid

In the present study, seeds extract of *Morinda citrifolia* was screened for the qualitative and quantitative Phytochemicals following the standard methods. The results revealed that methanol extract of *Morinda citrifolia* seeds contains broad spectrum of phytochemicals as presented in Table 1.0. The quantity of some of these phytochemicals are in order; flavonoids > saponins > tannins > alkaloids (Figure 1 & 2). These phytochemicals are considered as natural source of antioxidant, antimicrobial and anti-inflammatory agents which have been shown to reduce the risk and progression of many diseases such as cancer and diabetes [21]. A similar result of this phytochemicals was reported by Nnaoma *et al*. [22].

Total phenolic content (TPC) and Total flavonoids (TFC) were estimated from the calibration curve of gallic acid and quercetin standard. The result showed that the methanol seeds extracts of *Morinda citrifolia* contain concentration of phenolic compounds (15.65±1.24 GAEµg/mL) and flavonoid (78.12±1.76 QE µg/mL) respectively. Phenolic and flavonoids content of any plants is directly related to their anti-oxidant properties. They act as reducing agents, hydrogen donors and

are capable of scavenging free radicals [23]. Phenolic compounds have high potent radical terminators by donating hydrogen atom to free radical species thereby inhibiting lipid oxidation. Moreover they play an important role in protection against oxidative stress and related diseases such as cardiovascular diseases, cancer, neurodegenerative diseases, diabetes and allergic diseases [24]. Flavonoids plays the role of antioxidant through terminating free radicals, reducing the oxygen concentration, transforming primary products of oxidation into non-oxidant molecules and acts as metal chelators [25].

The radical scavenging activity of the *Morinda citrifolia* methanol seeds extract was evaluated using the stable free radical assays of DPPH and H_2O_2 (Table 2). The result of the scavenging activity of the seeds extract increase with increasing concentration, 500µg/ml concentration exhibited the highest % radical scavenging effect (38.88±0.47% - 30.99±1.1%). The lowest activity was found at the concentration of 31.25 µg/ml with inhibition of 6.96±0.87% - 3.53±1.83%. The extract was found to have low scavenging activity when compared to standard antioxidants (ascorbic acid) but higher than what Sajani and Maya reported [26]. The IC_{50} values were 13.68 µg/ml and 18.38 µg/ml as the concentration of the extract required to give 50% DPPH and H_2O_2 radical scavenging activity. The present study shows that, there is linear relationship between the phytochemical and antioxidant activity of *Morinda citrifolia* seeds extract.

Conclusion

The present study clearly proved the potential antioxidant activity of *Morinda citrifolia* seeds methanol extract which may be due to the various phytochemicals present in the seeds extracts as revealed by qualitative and quantitative phytochemical investigations.

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