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# *IN VITRO* ESTIMATION OF ANTIOXIDANT ACTIVITY OF *CARYOTA URENS* FRUITS

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**Abstract:**

**Background:** *Complementary and alternative medicine based on plants is the world's oldest form of medicine and recent reports suggest that such therapies still enjoy vast popularity, especially in developing countries where most of the population does not have easy access to modern medicine.* **Aim:** *The objective of this study was to evaluate the antioxidant activity of chloroform fraction (CLF), carbon tetra chloride fraction (CTF) and n-Hexane fraction (NHF) of methanolic extracts of Caryota urens (CU) fruits.* **Method:** *For determination of antioxidant property of the CU fruits extracts, DPPH (2,2-diphenyl-2-picrylhydrazyl) radical scavenging assay was performed.* **Results:** *Among three different fractions CLF showed the highest antioxidant activity (61.58 % scavenging) at 400 µg/ml concentration followed by CTF and NHF. The $IC_{50}$ values for the DPPH radical scavenging test were in the order of CLF (93.45 ± 3.09 µg/ml) > CTF (473.01 ± 12.95) > NHF (613.13 ± 7.64).* **Conclusion:** *Our study suggested that CLF of CU fruits had strong antioxidant effect compared to CTF and NHF.*

***Keywords:*** *Complementary and alternative medicine, Caryota urens, Antioxidant activity, DPPH radical scavenging assay.*

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## INTRODUCTION

Antioxidants are compounds that protect organisms against oxidative damage of lipids, proteins, DNA and other biomolecules [1]. Oxidation of biomolecules is responsible for various human diseases such as cancer, [2] atherosclerosis, [3] heart disease, [4] diabetes, [5] preeclampsia, [6] amyotrophic lateral sclerosis, [7] Alzheimer's disease, [8] Huntington disease, [9] Parkinson disease, [10] Celiac disease [11] etc. Free radicals are atoms or group of atoms with an odd number of electrons **(**reactive oxygen species, ROS and reactive nitrogen species, RNS) that are continuously produced during normal metabolic process and living organisms have developed several defense mechanisms to protect themselves from deleterious effect of free radicals mediated oxidative stress [12]. Proper functioning of the human body depends on the redox homeostasis i.e., the balance between oxidation and anti-oxidation. Redox homeostasis is maintained by antioxidants, detoxifying proteins and other molecules. An imbalance between ROS, RNS and antioxidant factors induce cellular and molecular abnormalities in human body [13].

Antioxidants protect us from above mentioned diseases by fighting against free radicals mediated oxidative damage. The uses of natural antioxidants are increasing day by day, since the currently used synthetic antioxidants such as butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT) etc. were suspected to be carcinogen [14]. Previous studies suggest that the phytocompounds are excellent source of natural antioxidant [15]. Plants are store house of diverse type of phytochemicals like phenolic compounds, flavonoids, tannins, carotenoids and alkaloids contributes to antioxidant effects [16]. Infect plants with dark color fruits are the great source of antioxidants. ACS **(**American Cancer Society**)** stated that yellow, orange, purple and red color fruits and vegetables have anticancer and antioxidant activities [17].

The plant *Caryota urens* (CU) is commonly known as wild coconut and wine palm belongs to the family Arecaceae. CU is naturally found in Myanmar, India and Sri Lanka where it grows on plateau or in rain forest clearings at up to 300 m above sea level [19]. The fruit of this plant matures to a round, red drupe about 1 cm wide and containing a single seed [20]. The plant products have excellent medicinal properties. Traditionally the flower is used to treat gastric ulcer and migraine headaches. The root, bark and the cabbage or terminal bud of this plant is used to treat rheumatic swellings and snake bite. Bark and seed of this plant is used to treat boils and the root is used for tooth ailments [21].

Previous study showed that methanolic extracts of immature fruit and leaf of this plant have strong antioxidant and antibacterial activity [17]. Therefore this study was designed to investigate the antioxidant activity of chloroform fraction (CLF), carbon tetra chloride fraction (CTF) and n-Hexane fractions (NHF) of methanolic extracts of CU fruits.

## MATERIALS AND METHODS

### Chemicals

2,2-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid (AA) and 95 % methanol were purchased from Merck, Germany. Unless otherwise specified, all other chemicals were of analytical grade and purchased from Active Fine Chemicals Ltd., Bangladesh.

### Collection and Identification of Plant Materials

The fresh leaves of CU were collected from Mirpur Beribadh, Dhaka, Bangladesh, in February, 2015 and identified by expert of Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh. Accession number: DACB-54327 for CU.

### Drying and Grinding of Plant Materials

The fresh fruit of the plants were first washed with water to remove adhering dirt. Then fruits were cut into small pieces, sun dried for 5 days and finally dried in an oven at temperature not more than 50 °C for better grinding. After drying, the entire portions were ground into coarse powder by a grinding machine and stored in an airtight container for further use.

### Extraction and Fractionation of Plant Materials

Powdered sample having a weight of 250 g was taken in an amber colored reagent bottle and soaked in 800 ml of 95 % methanol at 25 °C. The bottle with its contents were sealed and kept for a period of about 7 days with occasional shaking and stirring. The whole mixture was then filtered through cotton and then through Whatman No.1 filters paper. Then the filtrate was concentrated with a rotary evaporator under reduced pressure at 50 °C temperature to give crude extracts. The crude methanolic extracts were fractionated, initially with chloroform followed by carbon tetra chloride and finally n-Hexane. Concentrated extracts and different fractions were stored until further use and yield value of these were recorded.

### DPPH Radical Scavenging Assay

According to the method of Molyneux P [22] with some modification the antioxidant activity of the CU fruits was determined by using the DPPH free radical scavenging assay. In 95 % methanol DPPH solution was prepared to attain a concentration of 240 µg/ml. The crude extracts of CU fruits were mixed separately with 95 % methanol to prepare the stock solution to the concentration of 1 mg/ml. The test samples were prepared from stock solution by

dilution with methanol to attain a concentration of 25 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml, and 400 µg/ml. Ascorbic acid (AA) was used as a standard and it was prepared in the same way as described above. For this test freshly prepared 3 ml DPPH solution was added in each of these test tubes containing 100 µl extracts. The mixture was shaken vigorously and left to stand in a dark room for 30 min reaction period at 25 °C. After incubation, the absorbance of the mixture was measured at 517 nm by UV spectrophotometer against methanol as blank. The experiment was repeated for three times. The DPPH solution without sample solution was used as control. Percent scavenging of the DPPH free radical was measured using the following equation:

Scavenging effect (%) = [1- (As/Ac)] × 100

Where, Ac = Absorbance of control,
As = Absorbance of sample/standard solution.

**Statistical Analysis**

Results were expressed as mean ± standard deviation (SD) from three separate observations. Student's t test was used to find the significance of standard and sample for $IC_{50}$ values. Microsoft Excel 2010 (Roselle, IL, USA) was used for the statistical and graphical evaluations. A probability of $p < 0.05$ was considered as significant.

## RESULTS AND DISCUSSION

The percentages of free radical scavenging are given in Figure 1 that shows that the scavenging effects were in the following order: AA > CLF > CTF > NHF. The extracts had dose-dependent activity i.e., as the concentration of the extracts increase, the percentage of scavenged DPPH radical increases. The $IC_{50}$ values of AA, CLF, CTF and NHF were 24.43 ± 0.91, 93.45 ± 3.09, 473.01 ± 12.95 and 613.13 ± 7.64 µg/ml, respectively given in Figure 2. Among these values the $IC_{50}$ value of CLF was statistically significant ($p < 0.001$) with respect to AA. Thus, the results showed that among the three fractions, CLF revealed maximum scavenging activity, 61.58 % compared to CTF scavenging activity, 42.30 % and NHF scavenging activity, 32.62 % at the concentration of 400 µg/ml.

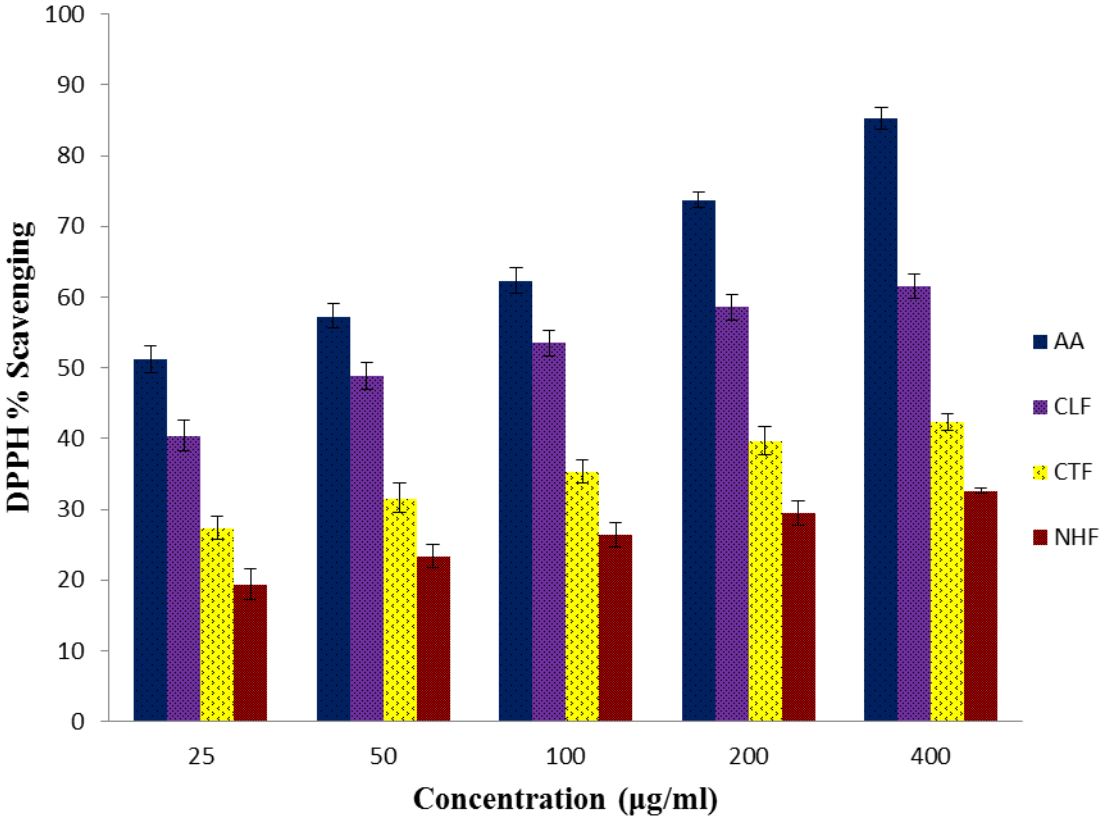


**Fig 1: DPPH radical scavenging activity of standard and CU fruit extracts**. Values are expressed as mean ± SD (n = 3). AA = Ascorbic acid, CLF = Chloroform fraction, CTF = Carbon tetra chloride fraction and NHF = n-Hexane fraction.

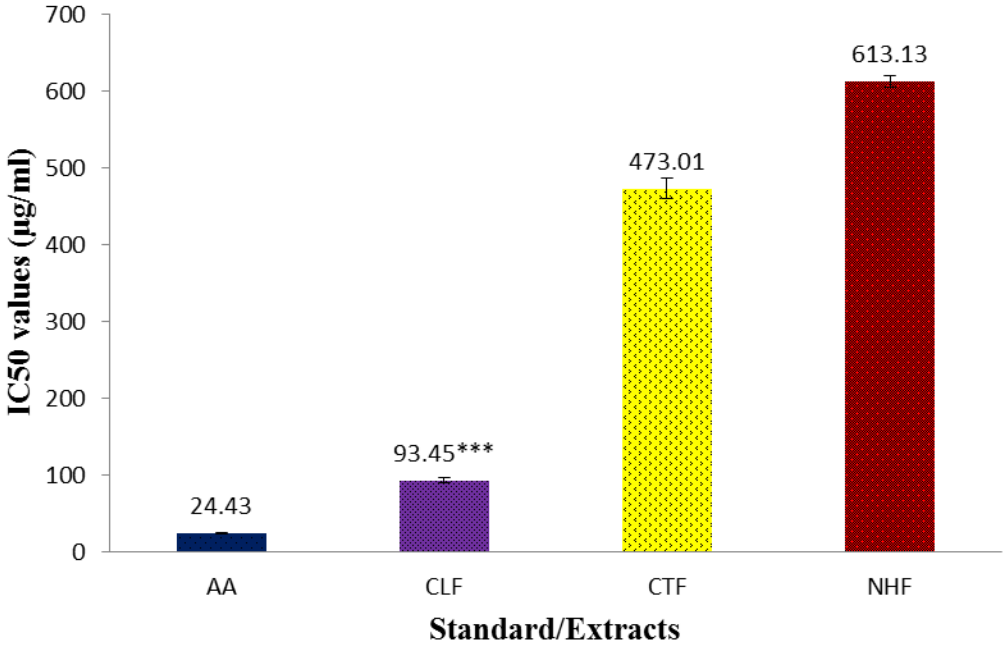


**Fig 2: $IC_{50}$ values of standard and CU fruit extracts.** Values are expressed as mean ± SD (n = 3). AA = Ascorbic acid, CLF = Chloroform fraction, CTF = Carbon tetra chloride fraction and NHF = n-Hexane fraction. *** $p < 0.001$ significance difference from standard

Free radicals are leading causes of pathological manifestations. Antioxidants fight against free radicals and protect us from various diseases. They exert their action either by scavenging the ROS and RNS, preventing free radical chain reactions, protecting the antioxidant defense mechanisms or improving antioxidant status of the body [23]. To evaluate the free radical scavenging effect of plant extracts DPPH radical scavenging assay is a widely used test. The formation of non-radical DPPH-H due to the reduction of DPPH solution in the presence of antioxidant is the basis of this test [24].

In this study, the antioxidant activity of crude methanolic extract and its derived fractions at various concentrations were tested by using DPPH radical scavenging assay. The reduction of the radical is followed by a decrease in the absorbance at 517 nm [25]. In addition to this the degree of discoloration of the DPPH solution from purple to yellow indicates the scavenging potential of the extracts [26].

In this study, among all the extracts tested, the highest capacity to scavenge DPPH radical was found for the CLF with $IC_{50}$ value 93.45 ± 3.09 µg/ml compared to CTF and NHF respectively.

## CONCLUSION

The results of this study showed that CLF of CU fruits had significant antioxidant activity as compared to CTF and NHF and can contribute for prevention of free radicals facilitated oxidative damages of the biomolecules. However, further studies will be necessary to illustrate the promising compounds for possible development of new class of antioxidant drugs.

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## ABBREVIATIONS

*CU: Caryota urens*; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ROS = Reactive oxygen species; RNS = Reactive nitrogen species; BHA = Butylated hydroxy anisole; BHT = Butylated hydroxy toluene; ACS = American Cancer Society; AA = Ascorbic acid; CLF = Chloroform fraction; CTF = Carbon tetra chloride fraction and NHF = n-Hexane fraction; SD = Standard deviation.

## COMPETING INTERESTS

The authors proclaim that there is no competing interests exist about the content of this article.

## AUTHORS' CONTRIBUTIONS

MSU: Designed the study, wrote the protocol,

managed the analyses of the study and prepared the draft of the manuscript. MFH and AAM: Carried out the tests. MSH: Performed statistical and graphical evaluations. MTI: Managed the literature searches. MA: Reviewed the scientific contents of the manuscript. All the authors read and approved the final manuscript.

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