Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

**E-ISSN:** 2278-4136
**P-ISSN:** 2349-8234
JPP 2016; 5(1): 260-263
Received: 17-12-2015
Accepted: 19-01-2016

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# *In vitro* antioxidant and cytotoxic activities of the various extracts of *Coccinia cordifolia* leaves found in Bangladesh

**Md Abu Kawsar Sarker, Samiya Khondaker Rinta, Abu Sayeed Chowdhury, Mahmudul Hasan, Sabbir Ahmed, Md. Abdur Rahman**

**Abstract**
The purpose of the study was to evaluate the antioxidant and cytotoxic activity of different fractions of *Coccinia cordifolia* available in Bangladesh. Antioxidant activity of the extracts was studied using DPPH radical scavenging assay, determination of total phenolic content and total flavonoid content. Cytotoxic activity was evaluated brine shrimp lethality bioassay. Results reflected that the methanol extract had highest antioxidant activity ($IC_{50}$ value for DPPH was 33.25 µg/ml, total phenolic content was 64.38 in mg/g, Ascorbic acid equivalents and total flavanoid content was 212.35 in mg/g, Ascorbic acid equivalents) compared to the carbon tetra chloride and ethyl acetate extract. In brine shrimp lethality bioassay, the $LC_{50}$ values for carbon tetra chloride, ethyl acetate and methanol extracts were 45.51 µg/ml, 28.86 µg/ml and 25.6 µg/ml respectively which revealed mild cytotoxic potential of the extracts.

**Keywords:** *Coccinia cordifolia*, Antioxidant, DPPH, Total Phenolic Content, Total Flavanoid Content, Cytotoxic.

## 1. Introduction
*Coccinia cordifolia* (Telakucha, Ivy gourd) that belongs to Cucurbitaceae family is a perennial herbaceous vine, has been used for centuries in Traditional Medicine for its health promoting properties [1].
It is widely grown in Bangladesh, different parts of India, Pakistan and Srilanka and commonly used for its various medicinal properties and it is also available in other parts of the world [2].
The usual parts of the plant are fruits, seeds, flowers, leaves and roots contain wide variety of bioactive compounds which mainly include tannins, saponins, phenols, flavonoids, terpenoids, steroids, triterpenoids, glycosides, alkaloids, phenols and carbohydrates [3] and also contain proteins, nutrients, especially antioxidant compounds such as total phenol, vitamin C and β-carotene and trace elements which are responsible for its antidiabetic, antiinflammatory, analgesic, antipyretic, anthelmintic, antibacterial, antifungal, hypolipidemic, cytotoxic, antitumor, pesticidal, chemoprotective, cytoprotective, antihepatotoxic, anticancer, antimalarial and anti-ulcer properties.
Additionally it is thought to posses anti anaphylactic as well as anti-histaminic qualities. Several researches have declared this particular vegetable to be constructive at strengthening the defense mechanisms which often serves the entire body in preventing several illnesses. The presence of Vitamin C in this particular vegetable functions at developing the general body structure. It is also well known because of its detoxification capability to cleanse our blood too [4].
A large number of research works on the phytochemistry, pharmacology and several other aspects of *C. cordifolia* have been conducted but there has been no report on *in vitro* bioactivities of different extracts of *C. cordifolia* collected from Bangladesh. So the present investigations were carried out to study *in vitro* antioxidant and cytotoxic activities of the different extracts of *C. cordifolia* available in Bangladesh.

## 2. Materials and methods
**2.1Chemicals and solvents:** DPPH (2,2-Diphenyl-1-piccryhydrazyl) was obtained from Sigma-Aldrich co. USA. Folin-Ciocalteu reagent, ascorbic acid and sodium carbonate were purchased from Merck, Germany. All the other chemicals used, including the solvents were of analytical grade.

**2.2 Plant material and extraction:** Plant sample (Leaves) of *C. cordifolia* was collected from Shreepur, Gazipur on 15th January 2014. About 650 gm of the powdered material was taken in a separate clean, round bottomed flask (5 liters) and soaked in 3.5 liter of methanol. The container with its content was sealed by cotton plug and aluminum foil and kept for a period of 15 days accompanying occasional shaking and stirring. The whole mixture was then filtered through cotton followed by Whatman No.1 filter paper and the filtrate thus obtained was concentrated at 39 °C with a Heidolph rotary evaporation. The concentrated extract was then air dried to solid residue. The weight of the crude methanol extract obtained from the powdered whole plant was 25.18 gm respectively.

**2.3 Preparation of mother Solution:** 5gm of methanol extract was triturated with 90ml of methanol containing 10ml of distilled water. The crude extract was dissolved completely. This is the mother solution.

**2.4 Partition of Mother Solution:** The mother solution was then partitioned off successively on the basis of polarity of solvents by n-Hexane, Carbon tetra chloride, Chloroform and Ethyl acetate respectively.
After partitioning the mother solution carbon tetra chloride, ethyl acetate fraction and the remaining methanol fraction was collected and air dried. These various extracts of *C. cordifolia* leaves were further investigated for different pharmacological properties.

**2.5 Test for antioxidant activity**
**2.5.1 DPPH radical scavenging activity:** The free-radical scavenging activity of *C. cardifolia* of *C. cordifolia* leaves extracts was measured by decrease in the absorbance of methanol solution of DPPH (2, 2-Diphenyl-1-picrylhydrazyl). Scavenging activity was expressed as the percentage inhibition calculated using the following formula:

$$\% \text{ Inhibition} = \frac{(\text{Ao} - \text{A})}{\text{Ao}} \times 100$$

Ao= Absorbance of control and A= Absorbance of sample
Then % inhibitions were plotted against respective concentrations used and from the graph $IC_{50}$ was calculated. Ascorbic acid, a potential antioxidant was used as positive control [5].

**2.5.2 Determination of total phenolic content:** The total phenolic content of the extracts were determined by using Folin-Ciocalteu reagent and Ascorbic acid as standard. 10 fold diluted Folin-Ciocalteu reagent was used to oxidize the extracts which was neutralized with 7.5% sodium carbonate solution. After 60 minutes, absorbances were taken at 765 nm. The total phenolic contents were determined from a standard curve prepared with Ascorbic acid [6-7].

**2.5.3 Determination total Flavonoid Content:** The total flavonoid content of the extract was determined by using Aluminium chloride (AlCl3) colorimetric method. Taking 1.5ml of extract in a test tube, 6ml of distilled water was added. Then 5% of $NaNO_2$was added and incubated for 6 minutes. 10% $AlCl_3$ was added and incubated for 6 minutes. 4% NaOH and 0.6ml distilled water was added respectively. Then it was incubated for 15 minutes. For blank solution 1.5ml methanol was taken and the same procedure was repeated. Then the absorbance of the solution was measured at 510nm using a spectrophotometer against blank [8].

**2.6 Cytotoxic activity**
Brine shrimp lethality bioassay was used following the procedure for evaluating cytotoxic activity using concentrations of 0.78 – 400 µg/ml for each extract. Different concentrations of Tamoxifen were taken as positive control. The percentage of lethality of brine shrimp nauplii was calculated for each concentration of extracts [9-10].

**2.7 Statistical analysis:** Statistical comparisons were performed using Microsoft Excel, 2007.

**3. Results**
**3.1 DPPH radical scavenging activity:** from the analysis of Figure 1, it can be inferred that the scavenging effect of the extracts increases with the concentration. Methanol extract of *C. cordifolia* leaves (MECCL) showed the highest radical scavenging activity whereas carbon tetra chloride extract of *C. cordifolia* leaves (CTCECCL) showed the lowest activity. The $IC_{50}$ values for the standard ascorbic acid and extracts is shown in Table 1.

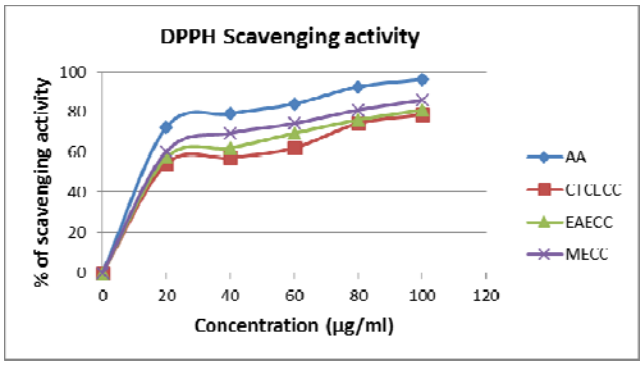


**Fig 1:** DPPH scavenging activity of various extracts of *C. cordifolia* leaves

AA: Ascorbic acid, CTCECCL: carbon tetra chloride extract of *C. cordifolia* leaves, EAECCL: Ethyl acetate extract of *C. cordifolia* leaves, MECCL: Methanol extract of *C. cordifolia* leaves

**Table 1:** $IC_{50}$ value for DPPH radical scavenging activity various extracts of *C. cordifolia* leaves

| Extract/Standard | $IC_{50}$ (µg/ml) |
|---|---|
| AA | 23.36 |
| CTCECCL | 44.10 |
| EAECCL | 38.54 |
| MECCL | 33.25 |

**3.2 Total phenolic content:** Among the three extracts, methanol extract (MECCL) showed the highest amount of phenolic compounds followed by the ethyl acetate (EAECCL) and the carbon tetra chloride extract (CTCECCL) (Table 2).

**Table 2:** Total phenolic content of different extracts of *C. Cordifolia* leaves

| Extracts | Total phenolic content(in mg/g Ascorbic acid equivalents) |
|---|---|
| CTCECCL | 50.15 |
| EAECCL | 58.58 |
| MECCL | 64.38 |

**3.3 Total flavanoid content:** Among the three extracts, methanol extract (MECCL) showed the highest amount of phenolic compounds followed by the ethyl acetate (EAECCL) and the carbon tetra chloride extract (CTCECCL) (Table 3).

**Table 3:** Total flavanoid content of different extracts of *C. cordifolia* leaves

| Extracts | Total flavanoid content(in mg/g Ascorbic acid equivalents) |
|---|---|
| CTCECCL | 180.59 |
| EAECCL | 198.59 |
| MECCL | 212.35 |

**3.4 Cytotoxic activity:** From the analysis of the Figure 2, it can be concluded that the percentage of mortality rate increases with the concentration of extracts. In brine shrimp lethality bioassay, the lowest $LC_{50}$ value showed by the carbon tetra chloride extract (CTCECCL) which is 45.51 µg/ml and the highest $LC_{50}$ value revealed by the methanol extract (MECCL) which is 25.6 µg/ml, whereas, the standard Tamoxifen showed $LC_{50}$ value of 3.72 µg/ml. The extract showed mild cytotoxic activity compared to the standard (Table 4).

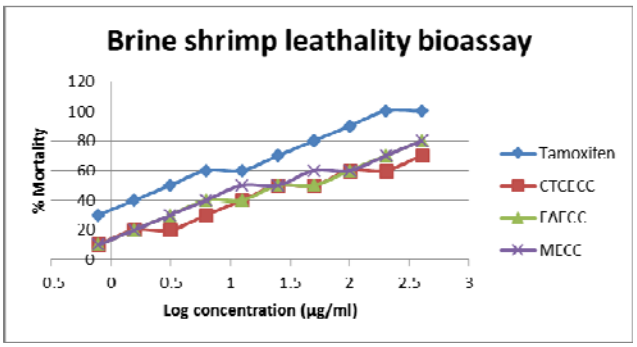


**Fig 2:** Brine shrimp lethality bioassay of tamoxifen various extracts of *C. cordifolia* leaves

CTCECCL: carbon tetra chloride extract of *C. cordifolia* leaves, EAECCL: Ethyl acetate extract of *C. cordifolia* leaves, MECCL: Methanol extract of *C. cordifolia* leaves

**Table 4:** Effect of different extracts of *C. cordifolia* leaves on shrimp nauplii

| Concentration (µg/ml) | LogC | % mortality | | | | $LC_{50}$(µg/ml) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Tamoxifen | CTCECCL | EAECCL | MECCL | Tamoxifen | CTCECCL | EAECCL | MECCL |
| 400 | 2.602 | 100 | 70 | 80 | 80 | | | | |
| 200 | 2.301 | 100 | 60 | 70 | 70 | | | | |
| 100 | 2.00 | 90 | 60 | 60 | 60 | | | | |
| 50 | 1.699 | 80 | 50 | 50 | 60 | | | | |
| 25 | 1.398 | 70 | 50 | 50 | 50 | 3.72 | 45.51 | 28.86 | 25.6 |
| 12.5 | 1.097 | 60 | 40 | 40 | 50 | | | | |
| 6.25 | 0.796 | 60 | 30 | 40 | 40 | | | | |
| 3.125 | 0.495 | 50 | 20 | 30 | 30 | | | | |
| 1.5625 | 0.194 | 40 | 20 | 20 | 20 | | | | |
| 0.78125 | -0.107 | 30 | 10 | 10 | 10 | | | | |

## 4. Discussion

For the health of individuals and communities, medicinal plants were of great importance [11]. Studies suggest that, *C. cordifolia* leaves extract contains alkaloids, flavonoids, phenol and saponin. Presence of these phyto compounds can be correlated to the biological activities of *C. cordifolia* found in this study.

Free radicals are known to play a definite role in a wide variety of pathological manifestations. Antioxidants fight against free radicals and protect us from various diseases. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms [12]. The electron donation ability of natural products can be measured by DPPH radical scavenging activity [13].

In the present study, all the extracts showed strong free radical scavenging activity compared to the standard ascorbic acid and the MECC exhibited highest activity. Phenolic compounds are considered as very important secondary metabolites [14] because their hydroxyl groups confer scavenging ability. Among the extracts, the methanol extract exhibited the highest total phenolic content which can be positively correlated with its DPPH free radical scavenging activity. Flavonoids have been shown to be highly effective scavengers of most oxidizing molecules, including singlet oxygen and various free radicals implicated in several diseases [15]. In this study our findings suggested that phenolic acids and flavonoids may be the major contributors for the antioxidant activity as the $IC_{50}$ values of radical scavenging activity of various extracts of *C. cordifolia* leaves and the contents of phenolics and flavonoids exhibited significant correlation. The methanol extract exhibited significant amount of phenolic and flavonoid content which is correlated to its highest free radical scavenging activity.

Brine shrimp lethality bioassay was performed to compare the cytotoxic activity of different solvent extracts of *C. cordifolia* leaves. From the result of brine shrimp lethality bioassay, it can be concluded that all the extracts of *C. cordifolia* showed moderate activity compared to the standard Tamoxifen.

This study suggestive that *C. cordifolia* leaves can be used as an antioxidant agent in the development of new drugs. Further work is under progress to identify the bioactive principles and elucidate their mechanism of action of specific bioactivities.

## 5. Acknowledgement

The authors are grateful to Dr. Chowdhury Faiz Hossain, Professor and Chairperson, Department of Pharmacy, East West University, Dhaka, Bangladesh for providing proper facilities to conduct the research works.

Our special thanks to Abdullah-al-Faysal, Lecturer, Department of Pharmacy, East West University for giving us his valuable time, constant advising, encouragement and for his kind words during our troubling moments.

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