Demonstration of Phytochemicals and *In-vitro* Antioxidant and Anti-inflammatory activity by methanolic extract of *Elettaria cardamomum*

Tanwy Chowdhury¹ and Md. Shahidul Islam²

¹,² Department of Pharmacy, University of Science and Technology Chittagong, Chattogram, Bangladesh.

*Corresponding Author:* Md. Shahidul Islam, Assistant Professor, Department of Pharmacy, University of Science & Technology Chittagong (USTC), Bangladesh.

**Received date:** November 25, 2020; **Accepted date:** December 01, 2020; **Published date:** December 07, 2020

**Citation:** Chowdhury T. and Md. S. Islam (2020) Demonstration of Phytochemicals and *In-vitro* Antioxidant and Anti-inflammatory activity by methanolic extract of *Elettaria cardamomum*. *J. Pharmaceutics and Pharmacology Research* 3(3); DOI:10.31579/2693-7247/023

**Copyright:** © 2020, Shahidul Islam, This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

**Abstract**

The existing research study attempts to untie novel avenues for development of the medicinal exercises of *Elettaria cardamomum*, fashionable known as the “Queen of Spices” and locally recognized as “elaichi”. Its seeds are utilized as abortifacient, acid, astringent, aromatic, sweet, cardiac tonic, cooling, carminative, digestive, diuretic, expectorant, stimulant and also tonic beneficial in the asthma, haemorrhoids, bronchitis, strangury, renal in addition to vesical calculi, anorexia, halitosis, gastropathy dyspepsia as well as burning sensation. The prime goal of this research work is to evaluate antioxidant as well as anti-inflammatory properties of the traditional Bangladeshi medicinal extracts in addition to examine these activities. The aim in present work was to screen the phytochemical profile as well as pharmacological activities of the methanolic extract of this plant’s leaves. To explore pharmacological actions DPPH scavenging test and the HRBC membrane stabilization techniques were done for the antioxidant and also anti-inflammatory test respectively. The pharmacological works revealed that plant extracts might have noteworthy antioxidant effect which is possibly mediated by the inhibition of DPPH free radical which is accountable for oxidation. The IC₅₀ values by the DPPH scavenging test observed for the standard and the leaves were 106.38µg/ml & 594.47µg/ml respectively. There is also moderate anti-inflammatory activity. The IC₅₀ values for anti-inflammatory activity by standard & leaves were 35.04µg/ml and 944.0 µg/ml respectively.

**Keywords:** antioxidant, anti-inflammatory, elettaria cardamomum, IC₅₀ values

**Introduction**

The three main important necessities of life are food, clothing and also shelter. A host of additional useful manufactured goods are supplied to him by plant kingdom [1]. The nature has offered a complete store shelter. A host of additional useful manufactured goods are supplied to him by plant kingdom [1]. The nature has offered a complete store

[7]. That’s why many medicinal plants have made source of the refined traditional treatment systems that have been in being for the thousands of years and also remain to distribute human with innovative medications [8].

**Materials and Methods:**

**Plant material**

For this research works, the leaves of *Elettaria cardamomum* was accumulated during June, 2019 from the University campus of University of Chittagong, Bangladesh.

**Determination of Total Phenolic Content (TPC)**

As it is known that in the alkaline condition phenols ionize absolutely. WhileFolin-Ciocalteu’s’s reagent is used in this ionized phenolic solution, the reagent will freely oxidize the phenols. Usual color of Folin-Ciocalteu’s reagent is yellow and after the oxidation process the solution converts blue. The strength of the color alteration is restrained in a spectrophotometer at 760 nm. The absorbance value will imitate the total phenolic content of the compound [9].

**Method of sample preparation**

In this research work, the total phenolics of the extracts were evaluated using the Folin and Ciocalteu reagent, following the method designated with slight alterations [12]. The test sample (0.2 mL) was variegated with
0.6mL of water and 0.2mL of Folin-Ciocalteu’s phenol reagent (1: 1). Subsequently, 5min, 1mL of saturated sodium carbonate solution (8% w/v in water) was added to the mixture and the volume was completed up to 3mL with distilled water. Then the reaction was preserved in the dark for 30min and after centrifuging the absorbance of blue color from dissimilar samples was restrained at 760 nm. All determinations were carried out in triplicate [10].

**Method of sample preparation**

In this research work, fifty micro liters (µl) of tannins extract for each sample was occupied in test tube and volume was completed to 1.0 ml with distilled water. Then, 0.5 ml Folin Ciocalteu reagent was added and varied accurately. Then 2.5 ml 20 per cent sodium carbonate solution was added and varied accurately and kept for 40 minutes at room temperature. Moreover, the optical density was reserved at 725 nm in UV spectrophotometer and concentration was assessed [11].

**Results and Discussion**

**Phytochemical screening:**

In Table 3, it is shown that different chemical constituents such as alkaloids, flavonoids, glycosides, phenols, saponins, tannins, terpenoids and triterpenes was present in *Acalyphahispida*. And are clearly accountable for its different therapeutic and pharmacological actions.

| Test sample | Absorbance | TPC (mg of GAE/g) | Average | TPC (mg of GAE/g) ± SEM |
|-------------|------------|------------------|---------|------------------------|
| Leaves      | 0.405      | 37.32            |         |                        |
|             | 0.413      | 36.48            | 36.74   | 36.74±0.5              |
|             | 0.411      | 36.44            |         |                        |

**Table 1:** Total phenolic content (TPC) of Elettaria cardamomum

Total phenolic content (TPC) observed for leaves of *Elettaria cardamomum* was 36.74 ± 0.5 mg of GAE/g.

| Test sample | Absorbance | TTC (mg of TAE/g) | Average | TTC (mg of TAE/g) ± SEM |
|-------------|------------|------------------|---------|------------------------|
| Leaves      | 0.402      | 2.771            |         |                        |
|             | 0.330      | 2.803            | 2.788   | 2.788±0.017            |
|             | 0.398      | 2.787            |         |                        |

**Table 2:** Total tannin content (TTC)

Total tannin content (TTC) observed for leaf of *Elettaria cardamomum* was 2.788 ± 0.017 mg of TAE/g.

| Secondary metabolites | Name of the test | Results |
|-----------------------|------------------|---------|
| Glycosides            | General test     | +++     |
| Flavonoids            | Specific test    | ++ +    |
| Alkaloids             | Wagner test      | ++      |
| Phenols               | Littmus test     | ++ +    |
| Saponins              | Froth test       | +++     |
| Tannins               | Ferric chloride test | ++ |
| Terpenoids            | General test     | +++     |
| Triterpenes           | Salkowski’s test | ++      |

**Table 3:** Different chemical compositions resent in plants

**Anti-inflammatory Activity**

Percent inhibition of protein denaturation was calculated as follows [11]:

\[
\%\text{ inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100
\]

In this research work, the method of HRBC membrane stabilization was selected to estimate anti-inflammatory activities.

| Concentration (µg/ml) | Absorbance | % Inhibition | Average | % Inhibition ± SEM | IC50 (µg/ml) |
|-----------------------|------------|--------------|---------|--------------------|--------------|
| 125                   | 0.539      | 2.43         | 2.41    | 2.41 ± 0.8         |              |
|                       | 0.537      | 1.81         |         |                    |              |
|                       | 0.535      | 2.99         |         |                    |              |
| 250                   | 0.501      | 20.13        | 21.00   | 21.00 ± 0.3        | 944.06       |
|                       | 0.500      | 21.05        |         |                    |              |
|                       | 0.503      | 21.81        |         |                    |              |
| 500                   | 0.452      | 57.05        | 57.47   | 57.47 ± 0.7        |              |
|                       | 0.451      | 58.90        |         |                    |              |
|                       | 0.452      | 56.45        |         |                    |              |
| 1000                  | 0.413      | 63.90        | 64.68   | 64.68± 0.5         |              |
|                       | 0.411      |              |         |                    |              |

**Table 4:** Average absorbance of control
Table 5: Spectroscopic Determination of Anti-inflammatory Activity of leaves.

| Concentration (µg/ml) | Absorbance | % Inhibition | Average | % Inhibition ± SEM | IC50 (µg/ml) |
|-----------------------|------------|--------------|---------|-------------------|--------------|
| 125                   | 0.445      | 57.10        | 57.51   | 91.68 ± 0.5       |              |
|                       | 0.442      | 57.11        |         |                   |              |
|                       | 0.441      | 58.02        |         |                   |              |
| 250                   | 0.340      | 75.68        | 76.46   | 97.58± 0.6        | 35.04        |
|                       | 0.341      | 76.36        |         |                   |              |
|                       | 0.338      | 77.34        |         |                   |              |
| 500                   | 0.226      | 90.88        | 89.96   | 102.69 ± 0.5      |              |
|                       | 0.221      | 90.26        |         |                   |              |
|                       | 0.222      | 89.11        |         |                   |              |
| 1000                  | 0.174      | 99.79        | 100.29  | 106.51 ± 0.5      |              |
|                       | 0.175      | 100.09       |         |                   |              |
|                       | 0.177      | 101.07       |         |                   |              |

Table 6: Spectroscopic Determination of Anti-inflammatory Activity of Standard Compound (Diclofenac - Na)

| Test Sample | IC50       |
|-------------|------------|
| Leaves      | 944.06     |
| Standard    | 35.04      |

Table 7: Comparative study based on IC50

In this research work, it exposed that the plant extracts might have moderate anti-inflammatory effect which is possibly reconciled by HRBC membrane stabilization.

**Antioxidant activity**

Here, the free radical-scavenging action of extracts was assessed with the DPPH assay [11]. In this research work, it exposed that the plant extracts may have important antioxidant effect which is may be reconciled by inhibition of DPPH free radical, which is accountable for oxidation.

Table 8: Average absorbance of control

| Concentration (µg/ml) | Absorbance | % SCV   | Average | % SCV ± SEM | IC50 (µg/ml) |
|-----------------------|------------|---------|---------|-------------|--------------|
| 62.5                  | 0.863      | 21.68   | 21.91   | 9.79 ± 0.6  |              |
|                       | 0.857      | 22.00   |         |             |              |
|                       | 0.852      | 22.07   |         |             |              |
| 125                   | 0.557      | 36.91   | 36.89   | 33.03 ± 0.5 |              |
|                       | 0.551      | 36.69   |         |             |              |
|                       | 0.552      | 37.05   |         |             |              |
| 250                   | 0.415      | 63.98   | 63.99   | 63.99 ± 0.6 | 594.47       |
|                       | 0.410      | 64.87   |         |             |              |
|                       | 0.411      | 63.06   |         |             |              |
| 500                   | 0.214      | 79.61   | 79.08   | 79.08± 0.7  |              |
|                       | 0.210      | 78.20   |         |             |              |
|                       | 0.211      | 79.39   |         |             |              |
| 1000                  | 0.115      | 99.98   | 100.53  | 100.50 ± 07 |              |
|                       | 0.088      | 100.90  |         |             |              |
|                       | 0.088      | 100.67  |         |             |              |
| 2000                  | 0.075      | 108.57  | 108.7   | 108.7 ± 0.6  |              |
|                       | 0.079      | 107.99  |         |             |              |
|                       | 0.077      | 109.17  |         |             |              |

Table 9: Spectroscopic Determination of Antioxidant Activity of Leaves.
Conclusion

By this research study it was concluded that the qualitative estimations indicate substantial existence of saponins, flavonoids, phenols, terpenoids and also triterpenes. In the plant, this was also concluded that glycosides, alkaloids and also tannins are moderately present. The quantitative assessments demonstrate substantial presence of phenols than tannin. There is an outstanding antioxidant activity in methanolic extract of this plant. There was also reasonable antioxidant activity in methanolic extract of this plant’s leaves. So, it is assessed that the IC\textsubscript{50} values by the DPPH scavenging observed for the standard and the leaves were 106.38µg/ml and 594.47 µg/ml separately. Consequently, there is a wonderful antioxidant activity in methanolic extract of this plant. Furthermore, there was also reasonable anti-inflammatory activity in methanolic extract of this plant’s leaves. So, it is assessed that the IC\textsubscript{50} values for the anti-inflammatory action by the standard and the plant leaves were 35.04 µg/ml and 944.06 µg/ml separately.

Table 10: Spectroscopic Determination of Antioxidant Activity of Standard Compound (L- Ascorbic Acid)

| Concentration (µg/ml) | Absorbance | % SCV | Average | % SCV ± SEM | IC50 (µg/ml) |
|-----------------------|------------|-------|---------|-------------|--------------|
| 62.5                  | 0.451      | 71.54 | 71.87   | 71.87 ± 0.5 |              |
| 125                   | 0.361      | 81.38 | 80.80   | 80.80 ± 0.37|              |
| 250                   | 0.297      | 88.28 | 88.88   | 88.88 ± 0.51| 106.38       |
| 500                   | 0.099      | 96.79 | 97.28   | 97.28 ± 0.31|              |
| 1000                  | 0.077      | 104.61| 104.50  | 104.50 ± 0.3|              |
| 2000                  | 0.033      | 107.66| 106.93  | 106.93 ± 0.7|              |

| Test Sample | IC\textsubscript{50} |
|-------------|-----------------------|
| Leaves      | 594.47                |
| Standard    | 106.38                |

Table 11: Comparative study based on IC\textsubscript{50}

In this research work, it exposed that the plant extracts may have important antioxidant effect which is might be reconciled by the inhibition of the DPPH free radical.

References

1. Meyer S. (1982) phytochemical methods a guide to modern techniques of plant analysis. USA: Champian and Hall.
2. Korikanthimath VS, Prasath D, Rao G. (2001) Medicinal properties of cardamom Ellettaria cardamomum. J Med Aroma plant sci. 22/4A & 23/1A, 683-685.
3. AZWANIDA, N. (2015). A review on the extraction methods use in medicinal plants, principle, strength and limitation. Med Aromat Plants. 4, 2167-0412,1000196.
4. Kaushik P, Goyal P, Chauhan A, Chauhan G. (2015) In vitro evaluation of antibacterial potential of dry fruit extract of Ellettaria cardamom Maton (chhoti elaichi). Iranian J Pharma Res. 9 (3), 287-297.
5. PORSOLT, R., BERTIN, A. & JALFRE, M. (1977a). Behavioral despair in mice: a primary screening test for antidepressants. Archives internationales de pharmacodynamie et de therapie, 229, 327-336.
6. Jazila EM, Mountassif D, Amarouch H. (2007) Antimicrobial activity of Elettaria cardamomum: Toxicity, biochemical and histological studies. Food Chemistry 104, 1560-1568.
7. Jamal A, Javed K, Aslam M, Jafri M.A. (2006) Gastroprotective effect of cardamom, Elettaria cardamomum Maton fruits in rats. J Ethnopharmacol 103, 149–153.
8. Verma S.K, Iain V, Katewa S.S. (2009) Blood pressure lowering fibrinolysis enhancing and antioxidant activities of cardamom (Ellatria cardamom). Indian J Biochem biophysics 46, 503-506.
9. Gilani A.H, Jabeen Q, Khan A.U, Shah A.J. (2008) Gut modulatory, Blood pressure lowering, Duretic and Sedative activities of cardamom. J Ethnopharmacol 115, 463–472.
10. WU, W. & SUN, R. (2012). Toxicological studies on plant proteins: a review. Journal of Applied Toxicology, 32, 377-386.
11. Alam MA, Ghani A, Subhan N, Rahman M, Haque MS, Majumder MM, Majumder MEH, Akter RA, Nahar L, Sarkar SD (2008). Antioxidant and membrane stabilizing properties of the flowering tops of Anthocephalus cadamba. Nat. Prod. Commun. 3:65-67.
12. Dubey A, Nayak S, Goupale DC (2011). A review on phytochemical, pharmacological and toxicological studies on Neolamarckia cadamba. Der. Pharm. Let. 3:45-54.