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Research Article

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

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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, acrid, alexiteric, 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 IC_{50} 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 IC_{50} values for anti-inflammatory activity by standard & leaves were 35.04µg/ml and 944.0 µg/ml respectively.

Keywords: antioxidant, anti-inflammatory, elettaria cardamomum, IC_{50} 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-house of the remedies to cure all the ailments of mankind. The knowledge of the drugs has collected over the thousands of years as a result of the man's curious nature so that nowadays it possess many effectual means of ensuring the health-care [2]. The cardamom oil is effectual as an antioxidant and also can increase intensities of glutathione which is a natural antioxidant in our body. The cause is increased by the increasing content of oil from the 100 to 5000 ppm [3]. The plants that acquire therapeutic properties and exert beneficial the pharmacological effects on animal body are by and large designated as the "Medicinal Plants" [4]. Even though there are no noticeable morphological characteristics in medicinal plants producing with them, up till now they acquire some out of the ordinary qualities and virtues that construct them medicinally imperative [5]. The plants which naturally synthesis and also accumulate several secondary metabolites like alkaloids, volatile oils, glycosides, tannins and also contain minerals in addition to vitamins possess the medicinal properties [6]. So from the extremely beginning of civilization, natives have depended on the nature for their essential needs, for the making of the flavors, diet, housing, nourishments, wear and also drugs [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 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 a *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
	0.405	37.32		
Leaves	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
	0.402	2.771		
Leaves	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	Litmus 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]:

$$\% \textbf{ inhibition} = \frac{\textbf{Control} - \textbf{Sample}}{\textbf{Control}}\textbf{X100}$$

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

	Absorbance	Average
	0.468	
Control	0.461	0.465
	0.467	

Table 4: Average absorbance of control

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	

	0.414	64.69			
	0.416	65.42			

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	89.88	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.

	Absorbance	Average
	0.813	
Control	0.817	0.819
	0.829	

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.

Concentration (µg/ml)	Absorbance	% SCV	Average	% SCV ± SEM	IC50 (µg/ml)
62.5	0.451	71.54	71.87	71.87 ± 0.5	
	0.452	71.50			
	0.450	72.57			
125	0.361	81.38	80.80	80.80± 0.37	
	0.369	80.13			
	0.368	80.90			
250	0.297	88.28	88.88	88.88 ± 0.51	106.38
	0.289	89.85			
	0.297	88.53			
500	0.099	96.79	97.28	97.28 ± 0.31	
	0.097	97.40			
	0.096	97.66			
1000	0.077	104.61	104.50	104.50 ± 0.3	
	0.056	104.29			
	0.035	104.60			
2000	0.033	107.66	106.93	106.93 ± 0.7	
	0.030	107.25			
	0.029	105.90			

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

Test Sample	IC_{50}
Leaves	594.47
Standard	106.38

Table 11: Comparative study based on IC_{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.

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 oh this plant. There was also reasonable anti-inflammatory activity in methanolic extract of this plant's leaves. So each part of the *Elettaria cardamomum* has altered constituent and the pharmacological properties of this plant vary in relation to part of the plant evaluated. Here the IC_{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_{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.

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