RESEARCH ARTICLE
ANTILIPOXGENASE AND ANTIHELMINTIC ACTIVITY OF CARDAMOM [ELETTARIA CARDAMOMUM (L.) MATON] ENRICHED CANE JAGGERY

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INTRODUCTION
Nutraceuticals, often referred to as phytochemicals or functional foods, are natural bioactive chemical compounds that provide demonstrated physiological benefits or reduce the risk of chronic diseases, above their basic nutritional function. Although the use of nutraceuticals by people has a long history, only recently scientifically supported nutritional and medical evidence has allowed nutraceuticals to emerge as being potentially effective (Dillard CJ, German JB 2000). Many plants are good sources of antioxidants and anti-inflammatory compounds. In recent years, plant and its products have been the main focus in the search for nutraceuticals to combat oxidative stress induced diseases. Jaggery is an ancient natural sweetener obtained by evaporating sugarcane (Saccharum officinarum) juice which is rich in phytochemicals including polyphenols (Fontaniella B et al., 2003). It is used in folk medicine andayurveda for its attributed health benefits. Sahu AP, Saxena AK(1994) reported the presence of phenolic compounds in the range of 280-320 mg/100g with antioxidant and cytoprotective ability(Harish Nayaka MA et al., 2009). Jaggery is reported to have beneficial effect in treating throat and lung infections. In vivo studies reported that jaggery supplement reduced toxicity of arsenic and also incidence of arteriosclerosis (Nraschant S et al.,2008; Okabe et al., 2009).

Spices, natural food additives used as flavorings and seasonings have long recognized to possess medicinal properties (Nadkarni KM, Nadkarni AK 1976). Literature suggests that “adding spice to your life” has the potential to not only improve the flavor of favorite dishes but also to help fight various chronic diseases. India is the largest producer, consumer and exporter of spices. Cardamom [Elettaria cardamomum (L.) Maton] is a well-known aromatic, expensive spice used worldwide in food preparations for its aroma and flavor. It is used in cuisine for curry, cakes, coffee, bread and flavoring sweet dishes and drinks (El Malti J et al., 2007). The essential oils of cardamom are the main flavorant component with wide array of biological activity like antioxidant, anti-inflammatory, antimicrobial and cardioprotective effects (Huang YB et al., 1999; Crowell PL, Gould MN 1994; Leblebici ME et al., 2013; CaI.,Wu CD 1996). It has been used as medicine in both Indian ayurvedic and Chinese traditional medicines. The present investigation is aimed to determine biological activity namely anti-inflammatory and antihelmintic activity of jaggery enriched with cardamom. The study also included the determination of total phenolic content and its correlation with biological activity. The anti-inflammatory action is determined by a bioassay system that tests the inhibitory effect on soybean lipoxigenase.

MATERIALS AND METHODS
Preparation of E. cardamomum(L.)Maton enriched jaggery (ECEJ)

ECEJ was prepared following the method described by Jagannadha Roa PVK, Madhusweta Das, Das SK (2007). Briefly, E. cardamomum powder at different concentrations (0.05 %, 0.1 % and 0.2 %) was added to sugarcane juice extracted from different sugarcane varieties (Co 86032, Co 419 and Co 62175) and adjusting the pH to 6.6 using milk of lime [Ca(OH)2]. The juice was initially boiled for 10 minutes and the scum formed

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ABSTRACT

Elettaria cardamomum(L.)Maton enriched jaggery (ECEJ) of Co 86032, Co 419 and Co 62175 sugarcane varieties at 0.05 %, 0.1 % and 0.2 % concentrations were evaluated for lipoxigenase inhibitory activity and antihelmintic activity. Total phenol content of ECEJ indicated significant difference between enriched and control jaggery and showed a dose dependent increase for all the varieties. ECEJ(0.2 %) showed an increased inhibitory effect on lipoxigenase activity with an EC_{50} of 0.22, 0.21 and 0.19 mg/mL for Co 86032, Co419 and Co 62175 sugarcane, respectively. A positive correlation (r = 0.991, 0.891 and 0.992) was observed between total phenolics and antilipoxgenase activity of ECEJ for Co 86032, Co 419 and Co 62175 varieties. ECEJ exhibited antihelmintic activity at 0.5g/mL concentration. However, no significant difference in activity was observed between different concentration of spice enrichment and sugarcane varieties. Thus, enrichment of jaggery with E. cardamomum increases its health beneficial potential.
during boiling was completely removed through filtration using muslin cloth. Finally, the juice was heated and concentrated to thick syrup until the temperature reaches 118 °C. The scum formed after subsequent boiling was also completely removed. The syrup was cooled and transferred to moulds. Jaggery prepared without the addition of E. cardamomum served as control. All the samples were stored at 4 °C in a sealed container for further analysis.

**Determination of total phenol content**

The total phenol content of jaggery was determined spectrophotometrically using Folin-Ciocalteu’s method (Singleton VL, Orthofer R, Lamuela-Raventos RM 1999). A sample aliquot of 100 μL (5%) was added to 900 μL of water, 1 mL of Folin-Ciocalteu reagent (1:2, v/v) and 2 mL of 10% sodium carbonate sequentially, mixed thoroughly and incubated for one hour at room temperature. The absorbance was measured to 765 nm in visible spectrophotometer (Systronics India Ltd. Gujarat, India). Gallic acid was used as standard and the total phenolic content expressed as milligrams of gallic acid equivalent (GAE) per gram sample.

**Antilipoxygenase activity of ECEJ**

**Lipoygenase extraction**

Lipoxygenase enzyme was extracted from soyabean seeds following the method described by Vineet Kumar et al., (2006). Ten grams soaked soybean seeds were homogenized with phosphate buffer, pH 6.8 for 20 min at 0-4°C and centrifuged at 10000 rpm for 10 min at 4°C. The supernatant was separated and used as crude extract for assaying lipoxygenase activity.

**Lipoxygenase activity assay**

Lipoxygenase activity in crude extract was analyzed following the method described earlier (Naidu KA 1995). Briefly, reaction mixture (3 mL) in the sample cuvette contained 500 μL of soybean lipoxygenase crude extract in 50 mMTris buffer, pH 7.4 and 1 mL of 50μM Linoleic acid. Increase in absorbance was recorded at 234 nm using spectrophotometer against blank. One unit of enzyme was taken as equivalent to the amount of enzyme that generated an increase in absorbance of 1.0 per min at 234 nm.

**Antilipoxygenase activity**

Soybean lipoxygenase enzyme was pre-incubated for 5 min at 37°C with different concentrations (100-500 μg) of ECEJ as well as control jaggery, prior to initiation of the reaction with substrate, linoleic acid. Later, the assay was performed as described above. Indomethcin was used as reference standard. The percent inhibition was calculated from the following equation:

\[
\text{Enzyme Inhibition} (\%) = \left(1 - \frac{A_{\text{sample}}}{A_{\text{Control}}} \right) \times 100
\]

An effective concentration (EC50) for 50% lipoxygenase activity inhibition was also calculated.

**Antihelmintic activity of ECEJ**

Antihelmintic activity of ECEJ was determined following the method described earlier (Ajaiyeoba EO, Onocha PA, Olarenwaju OT 2001). The assay was performed in vitro using adult Indian earthworm (Phereetina posthuma) owing to its anatomical and physiological resemblance with human intestinal helminthic parasites. Briefly, six worms of equal size were placed in 9 cm diameter petridish containing 20 ml of cardamom enriched jaggery solution (0.5 g/ml). Observations are made for the time taken for paralysis that was noted when no movement of any sort observed except when worms were shaken vigorously. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50°C). Piperazinehexahydrate at 10 mg/ml was used as reference standard. All test solutions and standard drug solution were prepared freshly.

**Statistical analysis**

All the experiments were carried out in triplicates (n = 3) and the results expressed as mean ± standard deviation (SD) using Microsoft Excel software.

**RESULTS AND DISCUSSION**

**Total Phenol Content of ECEJ**

Plant food contains a variety of phytochemicals, which impart health benefits beyond basic nutrition (Sreearamulu D, Raghunath M 2010). Among different phytochemicals, phenolic compounds have attracted the attention of food, pharmaceutical, health and cosmetic industries. E. cardamomum enriched jaggery (ECEJ) indicated a dose dependent increase in total phenol content with respect to cardamom concentration in all sugarcane varieties as shown in Fig 1. Jaggery prepared from Co 86032, Co 419 and Co 62175 had an increased total phenol of 25.9, 22.2 and 26.1 % respectively, compared to its control at 0.2% E. cardamomum enrichment.Total phenol content in control jaggery samples were found to be 3.16, 3.43 and 3.76 mg/g for Co 86032, Co 419 and Co 62175 variety, respectively.

![Fig.1 Total phenol content of ECEJ of different sugarcane varieties](image-url)

**Antilipoxygenase activity of ECEJ**

The mechanism of inflammation involves a series of events in which metabolism of arachidonic acid plays an important role. Since arachidonic acid metabolites are important mediators of inflammation, especially lipoxygenase products, inhibition of lipoxygenase provide good parameter to elucidate drugs for anti-inflammation. Lipoxygenases are dioxygenases which catalyzes the oxygenation of polyunsaturated fatty acids containing a cis-1,4-diene structures to hydroperoxides. It is a key enzyme in leukotriene biosynthesis from arachidonic acid metabolism. Leukotrienes are considered to be involved in the initiation and maintenance of a variety of inflammatory diseases and hence inhibition of leukotriene synthesis may, at least in part, be responsible for the anti-inflammatory action (Ammon HPT et al., 1992) and allergic reactions. Plant lipoxygenase pathway, in many respects is equivalent of the arachidonic acid cascades in animals (Gardner HW 1991) and for this reason, in vitro
inhibition of soybean lipoxygenase constitutes a good model for screening natural anti-inflammatory agents (Abad MJ et al., 1995). In the present study, the inhibitory effect of E. cardamomum enriched jaggery of different sugarcane varieties on soybean lipoxygenase was examined. E. cardamomum enriched jaggery of three sugarcane varieties significantly inhibited lipoxygenase activity in a concentration dependent manner and the enzyme inhibition was expressed in terms of EC\textsubscript{50} values shown in Fig 2. The enzyme activity of soybean extract was determined to be 32 U. E. cardamomum enrichment of jaggery has decreased EC\textsubscript{50} concentration from its respective controls irrespective of sugarcane varieties. At 0.2% E. cardamomum enrichment, jaggery of Co 86032, Co 419 and Co 62175 had EC\textsubscript{50} of 0.22, 0.21 and 0.19 mg/mL, respectively. Reference standard indomethacin showed an EC\textsubscript{50} of 57.2 µg/mL. In addition, correlation coefficient was calculated between lipoxygenase and total phenol content of enriched jaggery. A good positive correlation (r = 0.991, 0.891 and 0.992) was observed between total phenolics and lipoxygenase inhibitory activity of ECEJ of Co 86032, Co 419 and Co 62175, respectively.

**Antihelmintic activity of ECEJ**

Helmintiasis is recognized as a major problem to livestock production throughout the tropics. The parasitic gastroenteritis caused by mixed infection of several species of intestinal helminthi results in weakness, loss of appetite, decreased feed efficiency, reduced weight gain and decreased productivity (Gibbs HC 1986). In vitro anti-helmintic activity of ECEJ indicated no significant difference in paralytic and death time of helminths between control and enriched jaggery samples of all sugarcane varieties as tabulated in Table 1. Control jaggery and ECEJ exhibited antihelmintic activity at 0.5g/mL concentration and the death time of worm was found to be at around 19 min. Standard drug piperazinehexahydrate killed earthworm in 26.58 min at 10 mg/mL concentration. However, distilled water (-ve Control) did not show any effect on helminths. The predominant effect of piperazinehexahydrate on the worm caused a flaccid paralysis resulting in expulsion of the worm by peristalsis. Piperazinehexahydrate by increasing chloride ion conductance of worm muscle membrane produces hyperpolarization and reduced excitability leading to muscle relaxation and flaccid paralysis. There are reports to indicate antihelmintic activity of cane jaggery (Prasad P et al., 2010). However, ECEJ at 0.05, 0.1 and 0.2 % in Co 86032, Co 419 and Co 62175 sugarcane varieties did not indicate any increased antihelmintic activity. Acidic pH level of natural sweeteners may prevent the growth of many helminths and natural sweeteners have a saturated mixture of saccharides.

**Table 1 Antihelmintic activity of ECEJ of different sugarcane varieties**

<table>
<thead>
<tr>
<th>Sugarcane variety</th>
<th>E. cardamomum concentration in jaggery*</th>
<th>Paralytic time in min (Mean ± SD)</th>
<th>Death time in min (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co 86032</td>
<td>0% (Ctrl) 0.05% 0.1% 0.2%</td>
<td>16.13 ± 1.2 16.32 ± 2.0 15.32 ± 1.5 15.29 ± 1.2</td>
<td>20.23 ± 2.2 19.56 ± 1.1 19.52 ± 1.6 18.45 ± 2.1</td>
</tr>
<tr>
<td>Co 419</td>
<td>0% (Ctrl) 0.05% 0.1% 0.2%</td>
<td>16.20 ± 2.1 15.32 ± 1.5 15.29 ± 1.2 15.44 ± 1.7</td>
<td>20.01 ± 1.3 19.44 ± 2.1 19.29 ± 1.4 18.53 ± 1.9</td>
</tr>
<tr>
<td>Co 62175</td>
<td>0% (Ctrl) 0.05% 0.1% 0.2%</td>
<td>15.44 ± 1.4 14.55 ± 1.1 14.34 ± 1.8 14.02 ± 1.3</td>
<td>19.46 ± 1.8 19.29 ± 1.4 19.18 ± 1.2 18.47 ± 2.0</td>
</tr>
<tr>
<td>Piperazinehexahydrate (10mg/mL) [+ve control]</td>
<td></td>
<td>23.08 ± 2.0</td>
<td>26.58 ± 1.2</td>
</tr>
<tr>
<td>Distilled water [-ve control]</td>
<td></td>
<td>-</td>
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</tr>
</tbody>
</table>

*Jaggery concentration-0.5g/mL*

Lipoxygenase are sensitive to antioxidants as antioxidants are involved in inhibition of lipidhydroperoxide formation due to scavenging of lipoxido- or lipidperoxy- radicals which may lead to less availability of lipid hydroperoxide substrate required for its catalysis (Rackova L et al., 2007). Antioxidants inhibit lipoxygenase activity via chelation of its non-heme bound iron (Lin JK, Tsai SH, Lin-Shiau SY 2001) or by reduction of its ferric form (Gutierrez-Lugo MT et al., 2004). E. cardamomum has known for centuries for its flavor, taste and alleged health promoting benefits and has exhibited both antioxidant and anti-inflammatory activity by inhibiting linoleic acid peroxidation in vitro with an EC\textsubscript{50} of 167.29 µg/mL (Souri E et al., 2008). High correlation between total phenolics and inhibitory activity of lipoxygenase indicated the beneficial role of polyphenols in ECEJ. Further, increased phenolic content in ECEJ may have a synergistic effect on lipoxygenase inhibition suggesting the use of cardamom enriched jaggery as a functional food.

Most antioxidants isolated from plants source are polyphenols. Phenolic compounds possess an array of potentially beneficial lipoxygenase inhibitory and antioxidant properties and may be used for the treatment of inflammatory diseases (Sreejayan N, Rao MNA 1996). This mixture has a low water activity and hence water unavailable for worms and makes poor environment for their growth. In addition, polyphenolic compounds show antihelmintic activity (Bate-Smith EC 1962). Some synthetic phenolic compounds like nicolesamide, bithionol and oxyclozamide exhibit antihelmintic effect.
activity. They interfere with energy generation in helminthic parasites by uncoupling oxidative phosphorylation (Martin RJ 1997). Phenolic compounds in jaggery may also contribute for antihelmintic activity.

**CONCLUSION**

Evaluation of ECEJ of different sugarcane varieties for antihelmintic and anti-lipoxygenase activity indicated an enhanced phenolic content and inhibitor effect on lipoxygenase activity with a potential to act as an anti-inflammatory agent. However, no additional antihelmintic activity was observed in jaggery enriched with *E. cardamomum*. Hence, cardamom addition to jaggery enhances health benefits.

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**References**


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