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Antilipoxygenase and Antihelmintic Activity of Ginger (*Zingiber Officinale*) Enriched Cane Jaggery

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Abstract

Zingiber Officinale enriched jaggery (ZOEJ) at 0.05 %, 0.1 % and 0.2 % concentrations from Co 86032, Co419 and Co 62175 sugarcane varieties were evaluated for lipoxygenase inhibitory and antihelmintic activity. In addition total phenol content of the ZOEJ samples were quantified. Total phenol content of ZOEJ indicated significant difference between enriched and control jaggery and showed a dose dependent increase for all the varieties. ZOEJ showed inhibition of lipoxygenase activity with an EC_{50} of 0.34, 0.33 and 0.31 mg/mL at 0.05 % Z. officinale enrichment for Co 86032, Co419 and Co 62175 sugarcane varieties, respectively. A positive correlation (r = 0.961, 0.900 and 0.970) was observed between total phenolics and antilipoxygenase activity of ZOEJ for Co 86032, Co419 and Co 62175 varieties. However, ZOEJexhibited antihelmintic activity at 0.5g/mL concentration in all sugarcane varieties but indicated no significant difference in both paralysis and death time of helmintis upon spice enrichment. Hence, dietary supplementation of Z. officinale enriched jaggery may contribute better health benefits.

Keywords: Jaggery, ginger root, lipoxygenase, total phenols, antihelmintic activity.

Introduction

Functional foods or nutraceutical products include wide range of food and ingredients with variety of bioactive components that are attributed for health benefits and disease prevention[1]. Functional foods can be referred to those foods that are fortified, enriched or enhanced with products that provide health benefits beyond the provision of essential nutrients like vitamins, minerals, bioactive phenolics, etc., when consumed at efficacious levels as part of a varied diet on a regular basis[2]. From times immemorial food habits of human beings, included natural sweeteners and their importance recognized in Indian diets as well. Jaggery, the well-known sweetening agent prepared by concentrating sugarcane juice is added to beverages and foods for increasing palatability. Jaggery being a least processed sugar retains much of phytochemicals that are present in sugarcane juice and exhibits various health benefits[3]. Indian ayurveda medicine considered jaggery as a medicinal sugar and jaggery has been reported to possess protective effect against smoky induced lung damage, arsenic induced chromosomal aberrations, arteriosclerosis and cytoprotection against oxidative damage[4-7]. Spices, food adjuncts are used as natural food additives to enhance sensory properties of foods since antiquity. Spices reported to contain bioactive compounds that impart antioxidant, preservative and antimicrobial properties to the foods. Neutraceutical properties of ginger (*Zingiber Officinale*) compounds have been of great interest in the food processing and pharmaceutical industries. The refreshing pleasant aroma, biting taste and carminative property of ginger make it an indispensable ingredient of food processing throughout the world. The active principle of ginger is known to exhibit antioxidant, anti-inflammatory, antimicrobial, anticancer and cardiovascular protection[8-11]. Phenolic compounds of plant kingdom possess an array ofpotentially beneficial lipoxygenase inhibitory and antioxidant properties and are being used in the treatment of inflammatory diseases[12]. Apart from nutritional role, very less has been studied on the neutraceutical effect of jaggery enriched with ginger (*Z. officinale*) extract. The present investigation aims to evaluate neutraceutical role of *Z. officinale* enriched jaggery (ZOEJ) in terms of lipoxygenase inhibition and antihelmintic activity.

Material and Methods

Preparation of *Z. Officinale* Enriched Jaggery (ZOEJ): ZOEJ was prepared following the method described by Jagannadha Ro a*et al*.,[13]. Briefly, *Z. officinale* powder at different concentrations (0.05 %, 0.1 % and 0.2 %) was added to sugarcane juice extracted from different sugarcane varieties (Co 86032, Co419 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 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 ^{0}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 *Z. officinale* served as control. All the samples were stored at 4 ^{0}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[14]. 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 at 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 ZOEJ: Lipoxygenase Extraction: Lipoygenase enzyme was extracted from soya been seeds following the method described earlier[15]. Ten grams soaked soybean seeds were homogenized with phosphate buffer, pH 6.8 for 20 min at 0-4^{o}C and centrifuged at 10000 rpm for 10 min at 4^{o}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[16]. 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^{o}C with different concentrations (100-500 µg) of ZOEJ 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:

$$\text{Enzyme Inhibition (\%)} = [(A_{Control} - A_{sample})/A_{Control}] \times 100$$

An effective concentration (EC_{50}) for 50% lipoxygenase activity inhibition was also calculated.

Antihelmintic Activity of ZOEJ: Antihelmintic activity of ZOEJ was determined following the method described earlier[17]. The assay was performed *in vitro* using adult Indian earthworm (*Pheretimaposthuma*) owing to its anatomical and physiological resemblance with human intestinal helmintic parasites. Briefly, six worms of equal size were placed in 9 cm diameter petri dish containing 20 ml of ginger 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^{o}C). Piperazine hexahydrate 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 *Z. officinale* enriched jaggery (ZOEJ): Phenolic compounds of plants origin exhibited wide array of physiological and pharmacological activities. Jaggery being a least processed sweetener found to contain polyphenolic compounds that present in sugarcane plant and exhibited antioxidant and cytoprotective activity[7]. ZOEJ indicated a dose dependency increase in total phenol content in all sugarcane varieties as shown in figure-1. Jaggery prepared from Co 86032, Co 419 and Co 62175 exhibited an increase in 11.1, 12.0 and 16.5 % phenol respectively, from its control at 0.05% *Z. officinale* enrichment. However, phenol content in control jaggery samples were found to be 3.16, 3.43 and 3.76 mg/g of jaggery for Co 86032, Co 419 and Co 62175 variety, respectively.

Antilipoxygenase activity of ZOEJ: Lipoxygenases are a family of non-heme iron containing dioxygenases 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 acidmetabolism. Leukotriene function as initiators of inflammation and their inhibition has been considered to be partly responsible for the anti-inflammatory activity[18] and allergic reactions. Fractions anti-lipoxygenase activity was measured as inhibition of linoleic acid's peroxidation to hydroperoxylinoleic acid, a reaction catalyzed by soybean lipoxygenase that constitutes a suitable model for mammalian lipoxygenase[19]. In the present study, the inhibitory effect of ZOEJ of different sugarcane variety on soybean lipoxygenase was examined. ZOEJ of three sugarcane varieties significantly inhibited lipoxygenase activity in a concentration dependent manner and the enzyme inhibition was expressed in terms of EC_{50} values shown in figure-2. The enzyme activity of soybean extract was determined to be 32U.*Z. officinale* enrichment for jaggery has decreased EC_{50} concentration from its respective controls irrespective of sugarcane varieties. At 0.05% *Z. officinale* enrichment, jaggery of Co 86032, Co 419 and Co 62175 had EC_{50} of 0.34, 0.33 and 0.31 mg/mL, respectively. Reference standard indomethacin showed an EC_{50} of 57.2 µg/mL in addition, a good positive correlation (r = 0.961, 0.900 and 0.970) was observed between total phenolics and lipoxygenase inhibitory activity of ZOEJ of Co 86032, Co 419 and Co 62175, respectively.

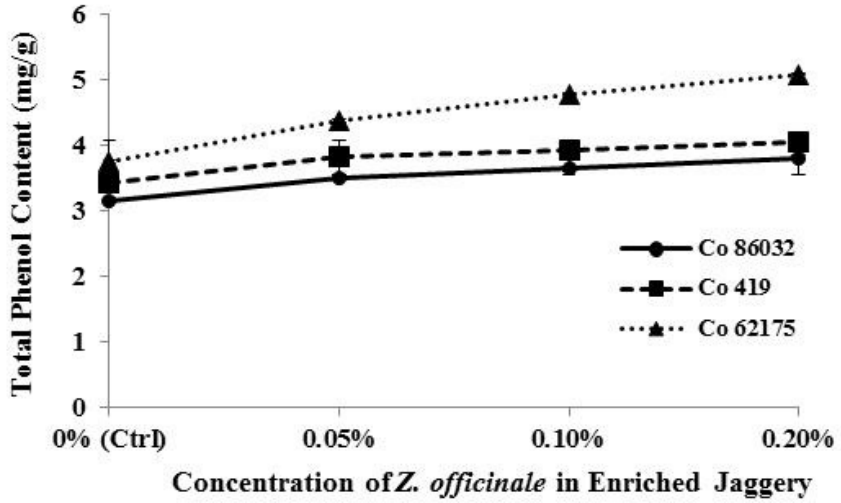

Figure-1
Total Phenol content of ZOEJ of different sugarcane varieties

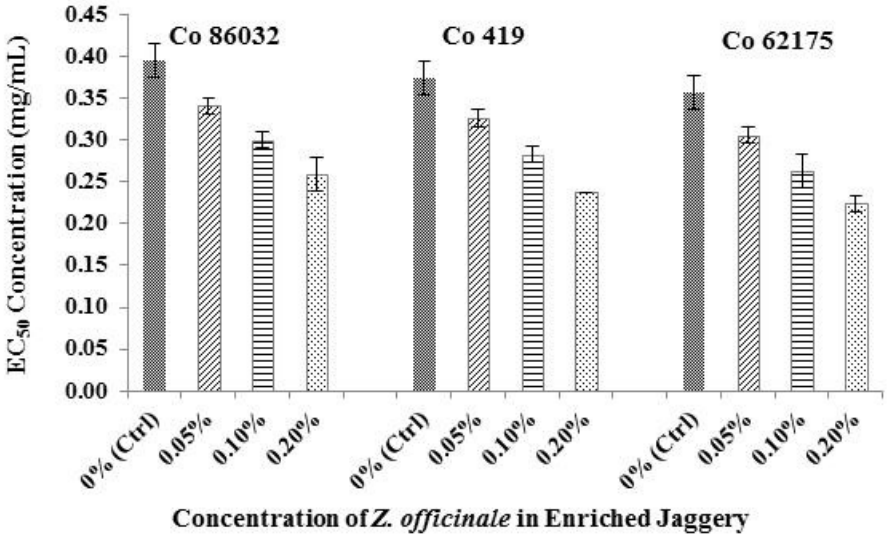

Figure-2
Antilipoxygenase activity of ZOEJ of different sugarcane varieties

Spices reported to contain phenolics and flavonoids that exhibited array of biological activities. Active principle of ginger roots (*Z. officinale*) such as gingerol, gingerdione and 6-shogaol were documented as inhibitors of 5-lipoxygenase activity[20,21]. Hence, active ingredients of *Z. officinale* may have contributed synergetic effect on lipoxygenase inhibitory activity of jaggery upon enrichment in all sugarcane varieties. In addition, antioxidants are known to inhibit plant lipoxygenases[22] and studies implicated oxygen free radicals in the process of inflammation. Phenolic compounds may block arachidonic acid metabolism by inhibiting lipoxygenase activity or may serve as a scavenger of reactive free radicals that are produced during arachidonic acid metabolism[12].

Antihelmintic activity of ZOEJ: Helmintiasis recognized as major problem to livestock production throughout the tropics. The parasitic gastroenteritis infection caused by several species of intestinal helmintis results in weakness, loss of appetite, decreased feed efficiency, reduced weight gain and decreased productivity[23]. *In vitro* anti-helmintic activity of ZOEJ indicated no significant difference in paralytic and death time of helmintis between control and enriched jaggery samples of all sugarcane varieties as tabulated in table-1. Control jaggery and ZOEJ exhibited antihelmintic activity at 0.5g/mL concentration and the death time of worm was found to be at around 20 min. Standard drug piperazine hexahydrate killed earthworm in 26.58 min at 10 mg/mL concentration. However, distilled water (-ve Control) did not shown any effect on helmintis. The predominant effect of piperazine hexahydrate on the worm caused a flaccid paralysis resulting in expulsion of the worm by peristalsis.

Table-1
Antihelmintic activity of ZOEJ of different sugarcane varieties

Sugarcane variety	*Z. officinale* concentration in jaggery*	Paralytic time in min (Mean ± SD)	Death time in min (Mean ± SD)
Co 86032	0% (Ctrl)	16.13 ± 1.2	20.23 ± 2.2
	0.05%	16.25 ± 1.6	21.01 ± 1.7
	0.1%	17.02 ± 1.3	21.46 ± 1.1
	0.2%	17.22 ± 1.9	20.02 ± 1.5
Co 419	0% (Ctrl)	16.45 ± 2.1	19.44 ± 1.3
	0.05%	17.15 ± 1.8	21.09 ± 1.4
	0.1%	17.34 ± 2.5	20.59 ± 1.9
	0.2%	17.04 ± 1.7	20.45 ± 2.1
Co 62175	0% (Ctrl)	15.44 ± 1.4	19.46 ± 1.8
	0.05%	15.55 ± 1.9	19.34 ± 1.1
	0.1%	16.34 ± 2.3	19.20 ± 1.5
	0.2%	16.02 ± 1.5	19.00 ± 2.0
Piperazinehexahydrate (10mg/mL) [+ve control]		23.08 ± 1.8	26.58 ± 1.2
Distilled water [-ve control]		-	-
*Jaggery concentration - 0.5g/mL			

Piperazine hexahydrate 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[24]. However, ZOEJ 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 levels of natural sweeteners may prevent the growth of many helmintis and natural sweeteners have a saturated mixture of saccharides. 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[25]. Some synthetic phenolic compounds like nicolsamide, bithionol and oxyclozanide exhibit antihelmintic activity. They interfere with energy generation in helmintic parasites by uncoupling oxidative phosphorylation[26]. Phenolic compounds in jaggery may also contribute for antihelmintic activity.

Conclusion

Present investigation revealed that enrichment of *Z. officinale*extract during jaggery preparation enhanced phenolic content andinhibitory effect of lipoxygenasein all sugarcane varieties with a potential to act as a potent anti-inflammatory agent.However, no additional antihelmintic activity was observed in jaggery enriched with *Z. officinale*. Hence, ginger addition to jaggery enhances health benefits.

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