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Biotransformation of Gallotannins From Fresh Fruit Juice of *Emblica officinalis* in *In-vitro* System

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ABSTRACT

Gallotannins, esters of carbohydrates or polyols with gallic acid present in fresh fruit juice of *Emblica officinalis* and has been found to be pharmacologically active. The knowledge of dietary intake of gallotannins and their bioaccessibility in gastrointestinal track are key factors in assessing their significance in human health. This study illustrates a biotransformation of gallotannins from fruit juice in gastrointestinal fluids and analysis of bioactive marker by rhodanine assay. Total gallic acid was determined by rhodanine assay from fruit juice of *Emblica officinalis*. Gastric juice and intestinal fluid caused hydrolysis of fruit juice during incubation for 8 h. The hydrolysis product from fruit juice was gallic acid. *In vitro* studies indicated that dissociation of gallic acid from ester complexes was apparent in both the fluid but gallotannins were rapidly hydrolyzed in gastric juice rather than intestinal fluid. Thus, our data suggest that hydrolysis of gallotannin in gastrointestinal fluid produced gallic acid which is possibly responsible for pharmacological effects.

Key words: *Emblica officinalis*, gallotannins, gastrointestinal fluids, hydrolysis, rhodanine assay

INTRODUCTION

Gallotannins are polyphenolic compounds found in legumes, vegetables, fruits and beverages and are the most abundant antioxidants in our diets. Gallotannins were reported to possess multiple biological activities including anticancer, antioxidant, antiallergic, anti-inflammatory, antihyperglycaemic, lipid lowering and antimicrobial activities and their consumption may contribute to prevent stroke, cardiovascular heart disease, neurodegenerative diseases (Manach *et al.*, 2005; Bhattacharya *et al.*, 2002; Mathur *et al.*, 1996; Patel and Goyal, 2011a).

Emblica officinalis Geart. (Family: Euphorbiaceae) commonly known as "Amla" or the Indian gooseberry, have been reported to contain constituents with variable biological activity. Phytochemical investigations of fruits of *Emblica officinalis* show that it is having high amount of polyphenol content like low and high molecular weight gallotannins such as L-malic acid 2-O-gallate, mucic acid 2-O-gallate, mucic acid, 1-O-galloyl- β -D-glucose, mucic acid 6-methyl ester 2-O-gallate, mucic acid 1,4-lactone 2-O-gallate, mucic acid 1-methyl ester 2-O-gallate, mucic acid 2-O-gallate, mucic acid 1,4-lactone 6-methyl ester 2-O-gallate, mucic acid 1,4-lactone 3-O-gallate, mucic acid 1,4-lactone 3,5-di-O-gallate, corilagin (Fig.1) (Zhang *et al.*, 2001; Kumaran and Karunakaran, 2006; Anila and Vijayalakshmi, 2002; Ghosal *et al.*, 1996). Gallotannins present in number of medicinal plants reported to possess various pharmacological activities (Patel and Goyal, 2012;

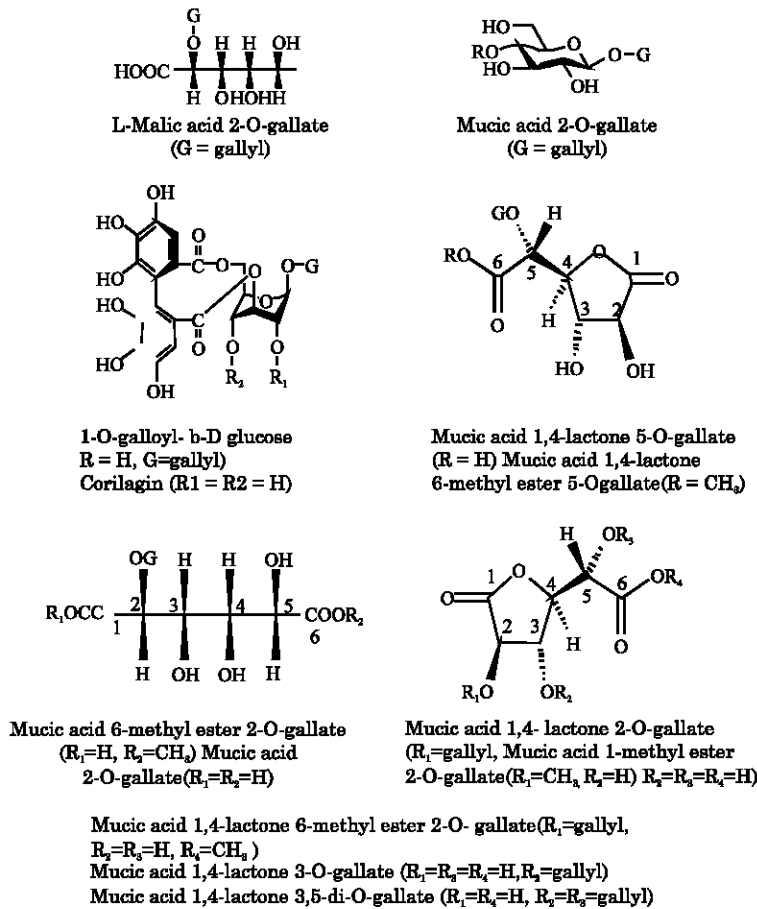


Fig. 1: Structures of gallotannins from fruit juice of *E. officinalis*

Rahman *et al.*, 2009). The gallotannins are hydrolysable tannins which contain a central core of carbohydrates which are esterified by phenolic like gallic acid and, unlike condensed tannins are readily hydrolyzed by acids, bases, or certain enzymes (Salunkhe *et al.*, 1989; White, 1957). On this basis, gallotannins is expected to be subject to degradation within the gut of mammals. Gallotannin which has esterified to gallic acid, is expected that, like other hydrolysable tannins, gallotannin will release those gallic acid residues in free form upon hydrolysis. On this basis, possible hydrolysis of gallotannin, resulting in the release of gallic acid, during incubation with gastrointestinal fluid, the present investigations was carried out in the simulated gastric and intestinal fluid and release of gallic acid residue in free form upon hydrolysis was determined by rhodanine assay. Despite many biological activities of gallotannins no data available for bioavailability of gallotannins from fruit juice in gastrointestinal tract. Thus aim of this present work was to estimate the amount of total gallotannin consumed in a fruit juice of *Embblica officinalis* and their gastrointestinal bioaccessibility.

MATERIALS AND METHODS

The fresh fruits of *Embblica officinalis* Gaertn. were purchased in the month of January of 2008 from Gaziabad, Madhypradesh, India and authenticated by Department of Pharmacognosy, L.M.College of Pharmacy, Gujarat University, Ahmedabad, India and study was carried out for

period of one week. Juice from fruits was freshly prepared in our laboratory before study. Other chemicals used were of analytical reagent grade.

Preparation of simulated gastric juice: Sodium chloride (2.0 g) and purified pepsin (3.2 g), with an activity of 8000 to 2500 units per mg of protein dissolved in 70 mL of 1 M HCl. Sufficient water was added to make 1000 mL and the pH of resulting solution was adjusted to 1.5 with HCl.

Preparation of simulated intestinal juice: Monobasic potassium phosphate was dissolved in 250 mL of water. The solution was mixed with 77 mL of 0.2 N NaOH and 500 mL of water. Pencreatin (10 g) was mixed to the solution. The pH of resulting solution was adjusted 6.8 either with 0.2 N NaOH or 0.2 N HCl and diluted with sufficient water to make 1000 mL.

Incubation of fruit juice of *E. officinalis* with simulated gastric juice and intestinal juice: Equal volumes (10 mL) of simulated gastric or intestinal juice and fruit juice of *E. officinalis* were mixed; the mixtures were incubated at 37°C under anaerobic condition. During incubation, 1 mL of sample was withdrawn at different time interval up to 8 h. The sample was used for gallic acid determination as described below.

Determination of gallic acid by rhodanine assay: The hydrolysate was diluted to appropriate concentration for determination of gallic acid. 200 µL of blank, hydrolysate and standard were mixed with 0.3 mL 0.667% methanolic rhodanine solution. After exactly 5 min, 0.2 mL of 0.5 N aqueous KOH solution was added. After 2.5 min. the mixture was diluted to 5.0 mL with distilled water. Five to 10 min later the absorbance was read at 520 nm. The total gallic acid content was expressed as milligrams of gallic acid/1 mL fruit juice (Inoue and Hagerman, 1988).

RESULTS

The biological properties and bioavailability of gallotannins depend on their chemical structure and it is important to study the effect of various pH conditions in gastrointestinal tract. As shown in Table 1, gallotannins present in fruit juice of *E. officinalis* hydrolyzed and generate free gallic acid in simulated gastric juice (3.5 mg mL⁻¹) and intestinal fluid (2.1 mg mL⁻¹). The hydrolysis of gallotannins by simulated gastric juice was relatively rapid and stable over the course of 8 h. On the other hand, hydrolysis of gallotannins by intestinal fluids was slow as compared to gastric juice. The amount of gallic acid produced reached a plateau after 1.5 h of incubation with simulated gastric juice at 37°C. However, plateau was reached after 4 h of incubation with simulated intestinal fluid. Therefore, it is presumed that the administered gallotannins present in fruit juice of *E. officinalis* hydrolyzed to gallic acid by simulated gastric juice and there after in intestinal fluid by acting on the gallic acid esters of gallotannins. Total contents of gallic acid in the fruit juice were found to be 24.3 mg mL⁻¹. Fruit juice contained no gallic acid before incubation either in gastric juice or intestinal fluid.

Table 1: Amounts of gallic acid released over 24 h after incubation of fruit juice of *E. officinalis*

	Mean values of four findings mg mL ⁻¹	
	Simulated gastric juice	Simulated intestinal fluid
Before hydrolysis	nd	nd
After hydrolysis	3.5	2.1

nd: Not detected

DISCUSSION

Tannins are phenolic compounds that occur widely in the plant kingdom. They are broadly classified into two groups based on chemical structure: hydrolyzable tannins and condensed tannins. Hydrolyzable tannins contain a central core of carbohydrates (glucose and polyhydric alcohol), which are esterified by phenolics (gallic acid, ellagic acid) and, unlike condensed tannins, are readily hydrolyzed by acids, bases, or certain enzymes. Figure 1 shows gallotannins present in fruit juice of *Emblica officinalis* such as L-malic acid 2-O-gallate, mucic acid 2-O-gallate, corilagin chebulagic acid, putrajivain A, elacocarpusin, mucic acid, 1-O-galloyl- β -D-glucose, mucic acid 6-methyl ester 2-O-gallate, mucic acid 1,4-lactone 2-O-gallate, mucic acid 1-methyl ester 2-O-gallate, mucic acid 2-O-gallate, mucic acid 1,4-lactone 6-methyl ester 2-O-gallate, mucic acid 1,4-lactone 3-O-gallate, mucic acid 1,4-lactone 3,5-di-O-gallate, emblicanin A and B, punigluconin, pedunculagin, methyl gallate, corilagin, furosin and geraniin. It has been demonstrated by several workers that pH and action of digestive enzymes are the most important factors affecting the hydrolysis of hydrolyzable tannin like gallotannins (Van Buren and Robinson, 1969; Berenbaum, 1980; Martin *et al.*, 1985; Saura-Calixto *et al.*, 2007). Gallotannins are found in legumes, vegetables, fruits and beverages and reported to possess multiple biological activities (Mishra *et al.*, 2011). Phytochemical investigations of fruits of *E. officinalis* shows that it is having high amount of polyphenol content like low and high molecular weight gallotannins. Therefore to elucidate the significance of gallotannins in human health, it is essential to know the amount of gallotannins in the fruit juice of *E. officinalis* and their bioavailability.

The effects of consumption of fruit juice of *E. officinalis* on health have recently received a great deal of attention. Our laboratory studies have demonstrated antidiabetic and cardioprotective activity of fruit juice in different animal models (Patel and Goyal, 2011a, b). Phytochemical investigation revealed that fruit juice possessed above pharmacological activity may be due to higher concentration of polyphenols like gallotannins. The molecular mechanisms for these actions are under investigation. A major problem in investigating the relationship between fruit juice and antidiabetic effect is the lack of quantitative data. Even in studies with animals, mechanistic understanding of the effect of fruit juice against antidiabetic activity is hampered by a lack of information on the bioavailability of the effective components of it. It was found that the gallotannins present in *E. officinalis* is essential for their potent antioxidative and antidiabetic activities. Gallotannins are hydrolysable tannins which may get hydrolyzed into free form of gallic acid in gastrointestinal tract. Gallic acid is the biological marker compound present in the form of esters in fruit juice of *E. officinalis* (Patel and Goyal, 2011b). It is reasonable to speculate gallotannin present in *E. officinalis* are hydrolyzed to gallic acid which in turn produces various pharmacological activities. In the present study, fruit juice of *E. officinalis* incubated with gastric and intestinal fluids of gastrointestinal tract and caused generation of free gallic acid in simulated gastric juice and intestinal fluid by dissociation of the bond between gallic acid and carbohydrate (Fig. 2a). However, hydrolysis with gastric juice was much faster than intestinal fluid after 8 h of incubation (Fig. 2b). It was shown that after 24 h of incubation concentration of gallic acid found to be 16.6 and 8.5 mg mL⁻¹ in gastric and intestinal fluids respectively (Fig. 2a, b). Our result of the study are in coordination with previous reports where in vivo metabolism of esters of gallic acid like propyl and lauryl gallate was carried out and major metabolite obtained was gallic acid (Booth *et al.*, 1959). The metabolic behavior of orally administered esters of gallic acid was investigated by several scientists on various animals like rat, rabbit chicken as well as on human being (Zong *et al.*, 1999; Watanabe and Oshima, 1965; Potter and Fuller, 1968; Shahrzad *et al.*, 2001; Shahrzad and Bitsch, 1998). They have examined effect of change in pH resembling those

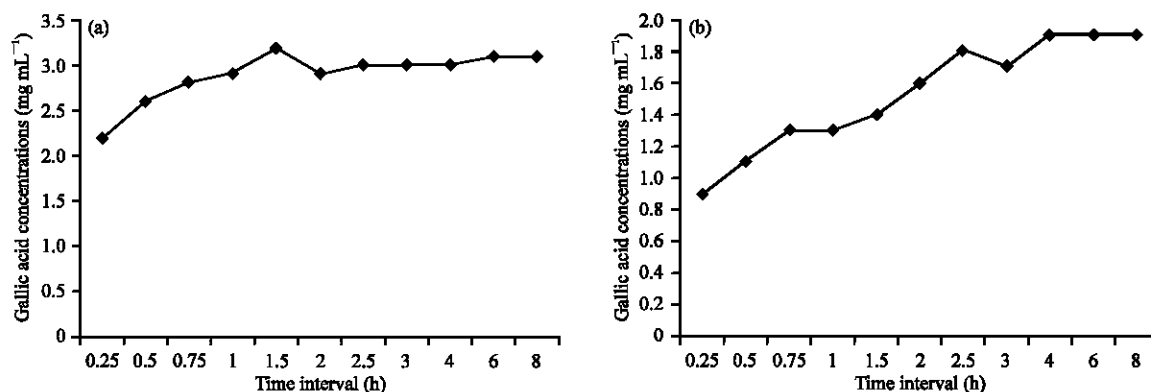


Fig. 2(a-b): Concentration of gallic acid in simulated gastric juice and intestinal fluid at different time intervals

of stomach by measuring the amount of gallic acid released from tannic acid. The results of our study were consistent with their findings. Thus, it can be postulated that, gastric juice facilitated the hydrolysis of gallotannin, as judged by the release of its constituent gallic acid. Thus, it can be postulated that, if fruit juice of *E. officinalis* were consumed, gallic acid would be readily liberated from the ester complexes in the acidic stomach or the alkaline small intestine and thus could be available as sources of nutrients.

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