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Effects on Gastrointestinal Microbes and Blood Clot of *Capsicum annuum* Phytochemicals Obtained by Modified Chloroform Extraction Process

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Abstract*—The scientific name of capsicum is a Capsicum annuum and popularly used in different food preparation as well as freshly consumed as salad. The fruit of capsicum has many nutritional properties and traditionally have taken part of health management. It is occasionally observed that sometimes consumption of capsicum relates the discomfort of stomach. Present study showed that capsicum has distinct antimicrobial effects on GI microorganisms. Excessive damage of GI microbial environment may be responsible for the discomfort of the stomach. The present study also observed the specific effects on blood clot lysis which may be useful for the management of several cardiovascular diseases.*

Keywords*—Capsicum annuum, Shimla mirch, Clot lysis, thrombolytic, Lactobacillus acidophilus, Lactobacillus bulgaricus, Bifidobacterium bifidum.*

I. INTRODUCTION

Capsicum annuum (family: Solanaceae) is locally known as capsicum is a popular vegetable or fruit nowadays widely cultivated in Bangladesh [1]. This is recognised in America as 'Bell peppers' whereas in India it is known as 'Shimla mirch' and only 'Peppers' in the United Kingdom [2]. The vegetable is available in different attractive colors mostly green, red, yellow and orange which make this vegetable indispensable for decoration of salad and foods such as pizza, sandwich, burger, pasta etc. even to the local foods fusca, borhani, meat fired items etc. [3]. The vegetable is not only delicious but also very healthy as it contains huge amount of vitamin C and other nutritional phytochemicals such as capsaicin, capsaicinoids, niacin (vit-B3), pyridoxine (vit-B6), vit-A, vit-K, vit-E as well as contain several inorganic elements including calcium, magnesium, phosphorus, potassium, sodium etc. [3-5]. Capsaicin is an alkaloid [6] which has several potential analogues such as capsiate, dihydrocapsaicin etc. has a multifunctional effect on human body including analgesic and wound healing, alteration of dyslipidemia, provide hypoglycaemic effect, even it can alter the gene expression in various stages of cancer cell survival, angiogenesis and metastasis [7-9]. Whereas capsaicinoids are secondary metabolites of capsaicin and have a distinct effect of boosting metabolism [10-12]. Capsicum being popularized of knowing its several beneficial effects on human health for example, antioxidant, antifungal, anti-inflammatory, antidiabetic, anticancer, analgesic, neuroprotective, antibacterial, immunosuppressive, immunostimulant action [13-15]. A review of randomized controlled trials describes that capsicum supplementation may reduce the risk of metabolic syndrome connected to heart disease as well as type 2 diabetes [16]. However, excessive consumption of capsicum may induce allergic reactions in sensitive individuals [17], sometimes may be responsible for stomach pain and gastric irritation and also for sweating and runny nose [18]. Capsicum bioactive compounds such as peptides, phenolics, capsaicinoids etc. have proven antimicrobial properties, which may create some shorts of unpleasant effects on gut microbes [13].

The present study was designed to assess the effect of capsicum on the microbial environment of the stomach as well as on the anticoagulation effect on human blood. Capsicum generally consumes orally so it is necessary to assess the beneficent or destructive effect on the microbes present in the GI tract. At the same time cardiac patients will be confident to intake capsicum after knowing it's real effects on blood clots.

II. MATERIALS AND METHODS

A. Source and extraction process

In the present study aqueous decoction method (a conventional extraction method) was selected for extracting out the phytochemicals from the fresh fruits of red-bell pepper or red-capsicum (Fig.1) collected from the authentic source of vegetable market of Mohammadpur, Dhaka. The fruits were identified by the botanist served in the school of life Sciences, University of Development Alternative, Dhaka, Bangladesh as Capsicum annuum or red-capsicum and picture and video of the fruits are preserved in the archive for further reference. The fruit was bell shaped, medium in size and healthy in

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appearance. The skin was shiny and thick and the fleshy texture observed inside the fruit.

B. Extraction process

Fresh fruits of *C. annuum* were first washed properly by running tap water followed by the distilled water for removing external debris. After shade drying of the surface water, fruits were chopped and 100 gm was placed in a conventional juice blender machine with addition of distilled water q.s. to 500 ml. The fine juice was passed through a 20-mesh size net for getting fine particles and transferred to a 1000 ml conical flask. The capsicum juice was boiled 10 minutes before filtration by three layers of polyester cloth. The filtrate was equally divided into Part-A and Part-B. Part-A was placed in a water bath for drying at 55^0C temperature and the dried crude extracts were collected in the glass vial, taken weight and preserved in the refrigerator for further study. Part-B was placed in a separating funnel, added 50 ml of chloroform, shaken vigorously and allowed them for phase separation. The chloroform phase was separated. The process was repeated three times and all separated chloroform mixed together and placed under a fume hood for evaporation. Finally, the dried chloroform crude extracts were collected in a glass vial, taken weight and preserved in the refrigerator for further study. The whole extraction process was repeated three times for getting average extract weight. Extraction yield and the phytochemical study of both crude and chloroform extracts were done as per Sadat et al., [19-22].

Fig. 1. Capsicum (red bell pepper)

C. Activity pattern against GI tract microbes

Disc diffusion method [19-22] was used for this study on the commercial probiotic microbes. In the GI tract there are 500-1000 different species of friendly microbes colonized to provide different health benefits. Food has a distinct effect on the health of the microbes present in the GI tract. Probiotic foods contain many of the similar types of microbes generally present in the GI tract. Many commercial probiotic dosage forms similar to the GI tract microbes are also available in the market for management of patients suffering GI discomfort. In the present study commercial probiotic bacteria were used for testing the activity pattern of capsicum extract on the beneficent microbes present in the GI tract. Brand name of the commercial dosage form 'Probio capsule' containing 2 billion *Lactobacillus acidophilus* (Gram +ve), 1 billion *Lactobacillus bulgaricus* (Gram +ve) and 1 billion *Bifidobacterium bifidum* (Gram +ve) produced by Square Pharmaceuticals Ltd., Bangladesh was used for this study. The probio capsule was first dissolved aseptically in a sterilized 40 ml distilled water and mixed properly by vortex mixture. Water was sterilized by a micropore syringe filter. The ultimate concentration was prepared 50,000,00 microbes per ml through a serial dilation method. Finally, 20 µl was inoculated on the nutrient agar media containing approximately 100,000 microbes in each Petridis. 6 mm diameter sterilized filter paper disk was used for application of experimented dosages i.e., 500 µg equivalent of crude and chloroform fraction to the inoculated Petri dish. Zone of inhibition more than 8 mm was considered the effective antimicrobial activity in this study.

D. Study of Clot Lysis properties

Clot lysis properties of capsicum extract were analyzed by slight modification of the method stated by Prasad et al, 2006 [23-24]. Blood samples were collected from 4 healthy (2 male and 2 females) volunteers in the age range 20-30 years with no known evidence of blood related disease and no history of taking any shorts of drugs within the last one month. Approximately 2.5 ml of venous blood was drawn from each volunteer and distributed in 4 pre-labelled and pre-weighed sterile microcentrifuge tubes (0.5 ml/tube) and incubated at 37°C for 45 minutes. Bloods were allowed for clot formation and serum was completely removed without disturbing the clot and clot weight was determined (clot weight = weight of clot containing tube – weight of tube alone). 100 µl containing 100 µg equivalent of crude and chloroform fraction of capsicum extracts were added to the pre-weighed clot and incubated at 37°C for 90 minutes. For positive control, commercially available Streptokinase, 1.5-million-unit vial marketed by the Incepta Pharmaceuticals Ltd. Bangladesh was collected and mixed in 3 ml distilled water for preparation of stoke solution. The stoke solution was further diluted in such a manner that each 100 µl suspension contained 1000 unit of Streptokinase which was finally added to the microcentrifuge tube containing the clot. For negative control, only 100 µl distilled water was added. Both positive and negative control tubes were also incubated at 37°C for 90 minutes and clot lysis was monitored. After incubation, the released liquid was removed and the tubes were reweighed to determine the difference in weight after collapse. The difference in mass taken before and after clot dissolution was expressed as a percentage of clot dissolution (Equion-1) [25]. The experiment was repeated 3 times with the blood samples of 4 volunteers and statistical average value was considered for interpretation.

% of clot lysis= (wt. of released clot /clot wt.) ×100 .. Eq.1

III. Result and Discussion

Fresh fruit of *C. annuum* extract was obtained by the conventional aqueous decoction method, from which chloroform fraction was separated by conventional fraction separation techniques and the ultimate yields were found

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8.27% and 0.58% respectively (Table I). The result indicated that approximately 7.09% organic phytochemicals present in the capsicum fruits may be separated by the chloroform fractional techniques after aqueous decoction. Phytochemical screening tests indicate that chloroform fraction successfully separates organic components such as flavonoid, terpenoids, glycosides, alkaloids etc. from the aqueous extracts (Table-II). A similar composition of phytochemicals in aqueous crude extracts was also observed in other published works [25]. Through the present study, it may be established that chloroform fractional extraction after aqueous decoction method stated in the present study is effective for obtaining a good quantity of organic components from the crude extracts. Both the aqueous and the chloroform fractionated extracts of *C. annuum* were applied on the probiotic microorganisms for testing sensitivity (Table III). It was observed that chloroform fractionated extract has more sensitivity than the aqueous crudes. The result was justified as the chloroform extract contained high percentages of active phytochemicals. From a literature survey it was observed that *C. annuum* fruit has specific antimicrobial and antifungal activities. In the present study antimicrobial sensitivity was observed specifically on the microbes present in the GI tract. Antimicrobial activities of both aqueous and chloroform fraction indicated hostile sensitivity of *C. annuum* to the probiotics (Table III). On the basis of the present study, it might be assumed that excessive oral ingestion of raw *C. annuum* fruits or its herbal dosage form may have a chance of creating some shorts of stomach discomfort. So, measures should be taken before assuming excessive oral ingestion of capsicum fruits, especially in the case of herbal dosage form. There is no chance of overlooking the benefits of capsicum on healthy humans as well as it has recognized benefits for the management of some devastating diseases such as cardiovascular diseases. In the present study, both the aqueous and the chloroform fraction of *C. annuum* fruits were observed promising clot lysis properties compared to streptokinase (Table IV). Fig-2 represents the promising clot lysis properties of capsicum fruits. So, the ingestion of capsicum fruit may be inspirable specially for cardiovascular diseases related patients.

TABLE I. Yield of the extract obtained from crude aqueous and purified chloroform fraction

Extraction Method	Quantity of material used for extraction (gm)	Crude extract (gm)	Yield		Presence of organic compounds in the chloroform extract
			%	Mean ± SD	
Aqueous Extracts	50	3.7	7.4	8.27±0.9	7.09%
	50	4.6	9.2		
	50	4.1	8.2		
Chloroform Fraction	50	0.28	0.56	0.58±0.1	
	50	0.35	0.7		
	50	0.25	0.5		

TABLE II. Phytochemicals present in the crude aqueous and purified chloroform fraction

Sample Extract	A	B	C	D	E	F	G	H	I
Aqueous	+	+	+	-	+	+	+	-	+
Chloroform	+	-	+	-	+	+	+	-	-

Here, A=Tanin, B=Saponin, C=Flavonoid, D=Steroid, E=Terpenoid, F=Clycoside, G=Alkaloid, H=Anthraquinones, I= Vitamin-C

TABLE III. Antimicrobial activity of capsicum on probiotic microorganism

Extract	Quantity	Zone of inhibition
Aqueous	500 µg/disc	9.44 ± 0.41
Chloroform	500 µg/disc	16.89 ± 0.84

TABLE IV. Clot lysis properties of capsicum on healthy human blood sample

Experimented clot lysis solution	Weight (gm)				% of clot lysis
	0.5 ml blood sample	Dry clot	Dry clot after test	clot lysis	
Distilled water (100 µl)	0.60 ± 0.11	0.43 ± 0.07	0.42 ± 0.08	0.013 ± 0.005	3.08 ± 1.68
Streptokinase (1000 IU/100 µl)	0.59 ± 0.09	0.38 ± 0.04	0.19 ± 0.06	0.188 ± 0.057	49.02 ± 13.91
Aqueous extract (100 µg/100 µl)	0.68 ± 0.12	0.47 ± 0.09	0.42 ± 0.07	0.048 ± 0.022	9.78 ± 2.69
Chloroform extract (100 µg/100 µl)	0.54 ± 0.06	0.37 ± 0.03	0.32 ± 0.05	0.055 ± 0.031	15.17 ± 9.72

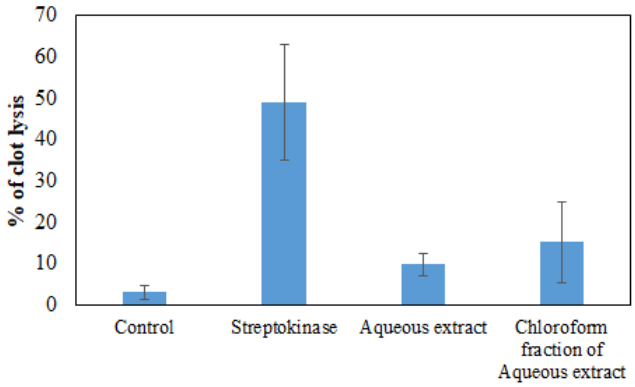

Fig. 2. Comparison of the clot lysis properties of aqueous and chloroform fraction of aqueous extract

IV. Conclusion

Dietary application of C. annuum is popularized day by day due to its availability in the market and application of multi-dimensional food ingredients. The ultimate objective of the present study was to justify the benefits of such popularities. It was observed that people can take these fruits as their normal diet chart for improvement of taste as well as for the management of some common diseases including heart related crises. In the present study it was observed that the fruit of capsicum is rich in several phytochemicals connected to the management of several health issues. The fruit has an identical thrombolytic (clot lysis) property which may be applied for the treatment of dissolving dangerous clots in blood vessels, improve blood flow, and prevent damage to tissues and organs. Commercial thrombolysis management is done by the injection of clot-busting drugs through an intravenous (IV) line or through a long catheter that delivers drugs directly to the site of the blockage. The commercial management of thrombolysis is an expensive procedure and special care is required. On the other hand, habitual ingestion of capsicum or ingestion of herbal dosage form especially in the middle age and obese individuals may prolong the occurrence of such cardiovascular disease related

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emergencies. Moreover, this may be beneficial for preventing heart attacks and ischemic strokes. Untreated deep vein thrombosis (DVT) or clots in the legs, pelvic area and upper extremities increases the risk of broken pieces of the clot which further travel to an artery in the lungs and are responsible for acute pulmonary embolism. The results of the present study suggested that daily intake of capsicum and its derivatives may be beneficial for the management of cardiovascular prone individuals. However, quantification is also important as it has some shorts of sensitivity on the GI tract microbes. The study recommends further study for developing the suitable dosage form and appropriate dose for the daily use of raw capsicum as well as its purified derivatives.

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