International Journal of Food Science and Nutrition
ISSN: 2455-4898
Impact Factor: RJIF 5.14
www.foodsciencejournal.com
Volume 2; Issue 6; November 2017; Page No. 239-242

In vitro evaluation of anticoagulant and antibacterial activities of *Capsicum annum* PUSA JWALA

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Abstract
The medicinal value of Indian spices has been known from centuries and they play a vital role in primary health care throughout the world. *Capsicum annuum* has been proposed as a promising candidate for maintaining immune system. The current investigation represents the analysis of anticoagulant and antibacterial activity of aqueous and organic (methanol, chloroform, and acetone) extracts of *Capsicum annuum* (Pusa jwala) by *in vitro* clot lysis assay and disc diffusion method respectively. The methanol chloroform, acetone and aqueous extract have shown 30.1%, 25.1%, 21.2% and 21% clot lysis respectively against control. The antibacterial assay indicated that all tested extracts exert antibacterial activities where as maximum zone of inhibition was showed by methanol extract. The qualitative phytochemical analysis revealed the presence of flavanoids, cardiac glycosides, alkaloids, phenols, saponins, resins and steroids. Thus *C. annuum* is suggested to have efficient potential for pharmaceutical exploitation in the treatment of cardiovascular diseases after further validation.

Keywords: *Capsicum annum*, anticoagulant, antibacterial, streptokinase, disc diffusion assay, *in vitro* clot lysis

1. Introduction
Thrombosis (blood clot) is identified as one of the leading ailment for myocardial infarction, stroke and pulmonary embolism, the leading cause of death. Other than surgical intercession to remove or by pass the blockage, the only treatment available is administration of thrombolytic agents to dissolve blood clots [1]. Thrombolytic agents like, urokinase (UK), tissue plasminogen activator (t-PA), streptokinase (SK) etc. are used worldwide for the treatment of cardiovascular diseases but these anticoagulants have deleterious life-threatening side effects [2]. Limitations of existing anticoagulant and cost effectiveness have propelled scientists and biochemists to go for an alternate novel agent from natural source [3]. Spices such as *Capsicum annum*, *Coriandrum sativum, Curcuma longa, Cinnamomum tamala, Nigella sativa, Eugenia aromaticum* have been the first source of anticoagulant and antithrombotic molecules [4, 5].
Bacterial diseases represent a continuous and increasing threat to human health and welfare [6]. The bacterial organisms including Gram positive and Gram negative like different species of *Bacillus, Staphylococcus, Salmonella* and *Pseudomonas* are the main source to cause severe infections in humans. Because these organisms have the ability to survive in harsh condition due to their multiple environmental habitats [7]. Even though pharmaceutical companies have produced a number of new antibacterial in the last years, resistance to these drugs has increased and now become a global concern [8, 9]. Plants with their wide variety of chemical constituents offer a promising source of new antibacterial agent [10, 11]. Most of the foods borne bacterial pathogens are sensitive to extracts from spices such as capsicum, garlic, mustard, onion and oregano [12].
Spices are dried part of herbs used as flavoring agents in cooking around the globe owing to their taste and aroma [13]. Spices are used as elite, marginalized, and aboriginite masses for ad hoc health care needs because they are readily available in the household [14]. In the traditional Indian system of medicine Ayurveda and Siddha various spices and herbs are described to possess medicinal properties, such as being anti oxidant, antibacterial, antithrombotic, antiatherosclerotic, hypolipidemic, hypoglycemic, antiinflammatory, antiarthritic, etc. [15]. *C. annum* of the family *Solanaceae* is commonly known as 'Red chilli'. Capsaicin, capsaicinoids, volatile oils, and vitamins C and E are some of the active chemical constituents present in *C. annum* [16]. Due to the presence of these active principles, *C. annum* has been found to possess digestive, stimulant, anticough, and analgesic pharmacological properties [17]. The fruit of *C. annum* is a cardiovascular stimulant; capsicum assists in lowering blood pressure and breaking down of cholesterol build-up [18]. It also possesses antimicrobial properties, which suggest its use as a potential natural inhibitor of pathogenic microorganisms in food [19]. Thus, the present study was an attempt to evaluate the anticoagulant and antibacterial effect by *in vitro* analysis.

2. Materials and method
The fruits of *Capsicum annum* (Pusa jwala) were procured from Department of Horticulture, Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur (M.P.). The fruits were washed and dried at room temperature and triturated with blender and stored in hermetically sealed bottles away from light and humidity until use for extract preparation [20].

2.1 Preparation of extract
2.1.1 Aqueous extract
10 gm of fruits powdered sample was dissolved in 100 ml of

D.W. and kept in a boiling water bath for 6 h, then filtered through eight layers of muslin cloth and centrifuged at 10,000 rpm for 15 min. The supernatant was collected and concentrated (by using water bath at temperature not exceeding 40 ˚C) to make final volume i.e. one-fourth of original volume [20].

2.1.2 Solvent extraction

10 Grams of fruits powdered sample was mixed with 100 ml of organic solvent (methanol, acetone and chloroform) and kept in rotary shaker for 3 days. The crude extract was filtered through eight layers of muslin cloth to remove all residual debris and centrifuged at 5000 X g for 15 min. The supernatants were collected and the solvents were evaporated to make the final volume i.e. one fourth of the original volume [20].

The extraction yield of the extract were expressed as-

2.1.3 Extraction yield (%) = (Weight of the dry extract (g)/ weight of the sample used for the extraction (g)) × 100

2.2 Blood clot Lysis assay

2.2.1 Streptokinase

The commercially available lyophilized streptokinase (Sk) of 1,500000 IU per vial was used as positive control. The whole lyophilized powder of the vial was dissolved in 100 ml of water to get 1,500000 IU of Sk solutions which is recommended dose for myocardial patients. We took one tenth of the powder in 10 ml of water for each time to make 1,500000 IU of Sk from where 100 µl was used for *in vitro* clot lysis assay [21].

2.2.2 Clot Lysis

Clot lysis effect of the extracts of *C. annum* was measured according to the method of Prasad *et al*., 2006 [21]. Briefly, venous blood drawn from healthy volunteer who had not taken any medication for last one month was transferred in different pre weighed sterile micro centrifuge tube (500 µl/tube) and incubated at 37 °C for 45 min. After clot formation, serum was completely removed (aspirated out without disturbing the clot formed) and washed several times with distilled water and then each tube having clot was again weighed to determine the clot weight. Each micro centrifuge tube containing clot was properly labelled and 100 µl of each extract was added to the tubes. Tube containing water along with clot serves as a negative and with streptokinase (20 µg) and heparin serves as positive thrombolytic control. All the micro centrifuge tube were then incubated at 37 °C for 6 h and observed for clot lysis. After incubation, fluid obtained after clot lysis was removed and micro centrifuge tube were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis. The test was performed in triplicates.

Clot lysis % = (weight of clot before lysis* -weight of clot after lysis) x 100

*clot weight = weight of clot containing tube -weight of tube alone.

2.3 Antibacterial Assay

The microorganisms *Bacillus subtilis* (BGCC#2386), *Citrobacter freundii* (BGCC#2036), *Staphylococcus aureus* (BGCC#2211), *Shigella dysenteriae* (BGCC#387), *Pseudomonas aeruginosa* (BGCC#2412) used in the study were obtained from BGCC (Bacterial Germplasm Collection Centre) Department of P.G. Studies and Research in Biological Sciences, Rani Durgawati University, Jabalpur, India. A disc diffusion method was employed for the determination of antibacterial activities of the aqueous and organic extract of the spices. The 100 µl of overnight grown bacterial culture was spread over the Muller Hinton agar medium. The sterile filter paper discs impregnated with 10 µl of each extract were placed on the surface of the medium. There were two replicates for each extract and for each bacterium tested. The plates were incubated at 37 ˚C for 24 h. The zone of clearance around each disc after the incubation period confirms the antibacterial activity of each extract [20].

2.4 Phytochemical analysis

Phytochemical analysis to determine the major phytoconstituents of the plant extract was undertaken using standard qualitative methods as described by Harborne (1973) [23], Trease and Evans (1989) [25]. The *C. annum* extract was screened for the presence of various biologically bactive compounds i.e. tannins, flavanoids, terpenoids, resins, carbohydrates, cardiac glycosides, alkaloids, saponins, and steroids.

3. Results and Discussion

Atherothrombotic diseases occur as serious impacts of the thrombus formed in blood vessels. Various thrombolytic agents are used to dissolve the clots that have already formed in the blood vessels; but these drugs have limitations and can lead to serious and sometimes fatal consequences [22]. Therefore, the search for other fibrinolytic enzymes from various sources continues. In our findings, the comparison of aqueous and solvent extracts of *C. annum* in the blood clot lysis assay, with positive control (Streptokinase) and negative control (distilled water) clearly demonstrated that clot dissolution does not occur when water was added to the clot and all the extracts was found to give significant clot lysis activity compared to positive control as presented in graph No. 1. The maximum clot lysis activity was observed in methanol extract (30.1%). Acetone extract are next to methanol extract in clot lysis activity with 25.1% of blood clot lysis. Similar reports on anticoagulant activity of various spices and herbs such as curcumin [23], black pepper [24], pippali [25], coriander [26], garlic [27], ginger [28], and cardamom [29] were also reported. *Capsicum oleoresin* showed reducing effect in serum cholesterol and triglycerides levels in hypercholesterolemic gerbils [30]. Similarly Anwar *et al*. (2013) [31] studied the synergistic thrombolytic potential of Honey and *Capsicum frutescens* and indicated 47.13% clot lysis. Emran *et al*. (2015) [1] noticed very promising effect of *Capsicum annum* in *in vitro* blood clot lysis activity.

The antibacterial efficacy of the aqueous and organic extract of *Capsicum annum* were studied by the disc diffusion method against two Gram positive and Gram negative bacterial strains. All the extracts differed in their activity against all the tested strains. Among them methanolic extract showed broad spectrum of antibacterial activity followed by acetone,

chloroform and aqueous extracts. The diameter of growth of inhibition varies high to low in the range of 23 mm to 3.5 mm. The most significant activity was observed by methanol and acetone extracts against *S.aureus* (23 mm) and *B. subtilis* (19 mm) respectively, whereas the aqueous extracts showed minimum growth of inhibition against all the tested strains. The highest sensitivity of *S. aureus* may be due to its cell wall structure and outer membrane [32]. Our results showed that the growth of selected Gram positive bacteria was more affected by the extracts of *C. annum* than the selected Gram negative bacteria (Table 1). This result is justified as the Gram positive bacteria are expected to be more susceptible due to having only an outer peptidoglycan layer, which is not an effective permeability barrier [33]. Numerous surveys have highlighted the potential importance of extract from *Solanaceae* species as a source of antibacterial agent [34]. Dorantes *et al*. (2000) [35] reported that the *C. annuum* extracts has inhibitory effects on *Salmonella typhimurium, Pseudomonas aeruginosa, Listeria monocytogenes, Staphylococcus aureus* and *Bacillus cereus*, using an agar diffusion method. Hemlatha *et al*. (2013) [36] compared the antimicrobial activity of *C. annum* (Test sample) and *C. frutescens* (Control) and found that test samples showed better results compared to the control capsicum sample. Extraction yield from a plant has a great effect on the overall efficacy and selection for bioprospecting in the calculation of total activity. It was observed that aqueous extraction produced maximum yield of about 9.2% where as other solvent extract (methanol, chloroform and acetone) yielded only 7.3%, 5.2%, 4.3% respectively. The qualitative phytochemical analysis detected the presence of flavanoids, cardiac glycosides, alkaloids, phenols, saponins, resins and steroids.

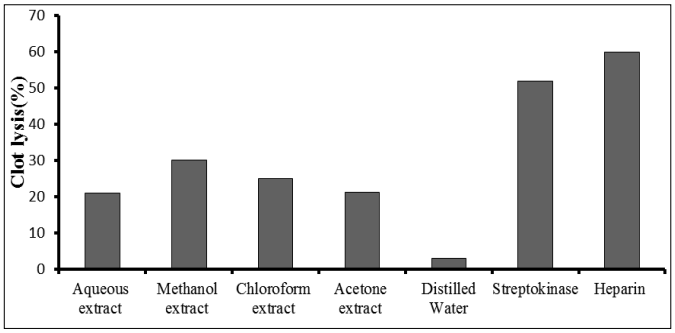

Fig 1: Percentage clot lysis by distilled water, streptokinase and different extracts of *Capsicum annum*

Table 1: Antibacterial activity of *Capsicum annum* by disc diffusion method (mm)

	Solvent extracts			
	Chloroform	**Methanol**	**Acetone**	**Aqueous**
Gram positive bacteria				
B.subtilis	4.5	18	19	2.5
S.aureus	15	23	17.5	3.5
Gram negative bacteria				
C.freundii	8.5	16	18	-
S. typhi	6	15.5	13.5	-
S.dysenteriae	11	14	10.5	-
P.aeruginosa	13	15	14.5	2.1

(-): No activity

4. Conclusion

The present findings corroborate that *C. annum* possess compounds with anticoagulant and antibacterial properties which supports its ethanomedicinal use. The result indicate significant capacity and future scope for the use of the *C. annum* against wide range of pathogenic microbial communities and also show to be a promising agent against anticoagulant drugs. The work can be extended to reveal specific secondary metabolites that attributes to their anticoagulant and antimicrobial activity. The work on preliminary phytochemical screening of *C. annum* extracts certainly encourages future advanced research activities on chromatographic isolation of the active compounds that attributes their anticoagulant and antimicrobial activity.

5. Acknowledgement

Authors are thankful to Head, Department of Post Graduate Studies and Research in Biological Science, Rani Durgavati University, Jabalpur (M.P.), India, for providing Laboratory facilities and Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur (M.P.) for providing spice sample.

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