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IN-VITRO ANTI-INFLAMMATORY, ANTI-ARTHRITIC AND ANTI-OXIDANT ACTIVITY OF BROMELAIN SUPPLEMENT

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ABSTRACT

<u>Bromelain</u> is a complex mixture of proteinases derived from pineapple stems and fruit. The objective of the present work is to study the *in-vitro* anti-inflammatory, anti-arthritic activity and antioxidant activity of commercially available *bromelain* supplements. *Bromelain* was studied for *in-vitro* anti-inflammatory activity by human red blood cell (HRBC) membrane stabilization method and *in-vitro* anti-arthritic activity by bovine serum protein denaturation method and egg albumin denaturation method. The activity of *bromelain* was compared with standard anti-inflammatory drug Diclofenac. Antioxidant activity was determined by the method of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) with vitamin C as a standard. It was found that *bromelain* showed protection of HRBC in hypotonic solution at IC₅₀ 43.71µg/ml, whereas, standard diclofenac showed, IC₅₀ 0.592µg/ml. In egg albumin denaturation method, that *bromelain* inhibition of egg albumin denaturation found IC₅₀ 23.36µg/ml; whereas, standard diclofenac showed inhibition of egg albumin denaturation at IC₅₀ 25.31µg/ml). *Bromelain* product showed good antioxidant activity (IC₅₀ 28.37µg/ml), as compared with the standard (IC₅₀10.56µg/ml). It was found that *bromelain* was more potent in inhibition of egg albumin denaturation compared with standard. Finally, from results it can be concluded that *bromelain* possesses good *in-vitro* antiinflammatory, anti-arthritic and anti-oxidant activities.

Keywords: Bromelain, Anti-inflammatory, Anti-arthritic, Anti-oxidant, Pineapple enzyme.

1. INTRODUCTION

Inflammation is a normal protective response to tissue injury caused by heat, redness, pain, swelling microbial agents. Inflammation is a protective response of the body to tissue injury/repair [1]. Prolonged inflammation leads to the auto-immune diseases like rheumatoid arthritis, atherosclerosis, etc., [2]. The inflammation of RA can also occur in tissues around the joints, ligaments and muscles [3]. The commonly used drug for the management of inflammatory conditions was non-steroidal anti-inflammatory drugs (NSAIDs), which have several adverse effects especially gastric irritation leading to the formation of gastric ulcers [4,5]. For these reasons, there is a need for ant-inflammatory drugs having less severe side effects to use for chronic inflammatory disease as well. Therefore, in recent times, more interest is shown in alternative and natural drugs for the treatment of various diseases, but there is a lack of proper scientific evidence. Bromelain is a protein found in the pineapple (Ananascomosus), belonging to the group of protease enzymes. Bromelain is

a protease that can hydrolyze proteins [6]. The presence of proteolytic activity in bromelain is beneficial in the therapeutic field [7]. Bromelain obtained commercially from the fruit or stem of pineapple. Different methods are used to prepare fruit bromelain and stem bromelain and they contain different enzymatic compositions. Usually bromelain refers to the stem bromelain. Bromelain is not a single enzyme. It is a mixture of different compounds like thiol endopeptidases, glucosidase, peroxidase, carbohydrates, cellulose and several protease inhibitors. In vitro and in vivo studies demonstrate that bromelain exhibits various biological activities like anti-inflammatory, anti-cancer activities. Bromelain is easily absorbed by the body without any major side effects [8]. Currently bromelain is administered for numerous clinical applications due to its therapeutic effects in the treatment of inflammation and soft tissue injuries [9]. Also bromelain is used as a nutritional supplement [9]. Bromelain is considered as a food supplement and is freely available in health food stores and pharmacies in India. The objective of the present

work is to study the *in-vitro* anti-inflammatory, antiarthritic activity and antioxidant activity of Pineapple *bromelain* commercially available in the health food store.

2. MATERIAL AND METHODS

2.1. Study area

The study was carried out at Chemistry Department, SRK University, Bhopal.

2.2. Chemicals and Instruments

Pineapple *bromelain* capsules were purchased from Healthy Hey Foods LLP, Andheri (E), Mumbai. Capsules containing Pineapple *bromelain* from pineapple, providing 2400 Gelatin Digestive Unit (GDU)/g. Other ingredients: Microcrystalline cellulose (INS 460(i)) and Silicon dioxide (INS 661). Instrument: UV Spectro-photometer (SHIMADZU 1800).

2.3. In-vitro Anti-inflammatory Activity 2.3.1. Human red blood cell (HRBC) membrane stabilization test

Fresh human blood (10 ml) was collected in heparinised centrifuge tubes and centrifuged at 3000 rpm for 10 min and washed thrice with an equal volume of normal saline solution. The volume of the blood was measured and reconstituted as a 10%v/v suspension with normal saline [10]. The reaction mixture (2ml) consisted of 1 ml bromelain and 1 ml of 10% red blood cell suspension. For the control, saline was added instead of bromelain. Diclofenac sodium was used as a standard drug (positive control). The samples were incubated at 56°C for 30 min, centrifuged at 2500 rpm for 5 min and the absorbance of the supernatant measured at 560 nm. The experiment was performed in triplicate. Percent membrane stabilization activity was calculated by the formula given in Inhibition of protein denaturation method section [11], while the percentage of protection was calculated using the following formula:

% inhibition = $100 - A_s / A_c \times 100$

where A_c and A_s are the absorbance (at 560 nm) of the control and sample, respectively.

2.3.2. In-vitro Anti-arthritic Activity

The in-vitro anti-arthritic activity was studied using Egg albumin denaturation method [12]. A mixture of 0.2 ml of egg albumin, 2.8 ml of PBS (pH 6.4) and 2 ml of varying concentrations (10, 20, 30, 40 and 50 μ g/ml) of Bromelain was used as the test sample. The same

mixture replacing the *bromelain* with diclofenac sodium (20, 40, 60, 80 and 100 μ g/ml) was used as the reference drug. A similar volume of double distilled water served the control (represents 100% denaturation). The above mixtures were incubated at $37\pm2^{\circ}$ C, for 15 min and then heated at 70°C for 5min. The test procedure was repeated 6 times. After cooling, the absorbance was measured at 660 nm using a UV spectrophotometer (Shimadzu 1800). The percentage inhibition of denaturation, which is an index of anti-inflammatory activity, was calculated using the following formula [12].

% Inhibition = $100 \times (V_t/V_c - 1)$

Where, V_t = absorbance of the test sample, V_c = absorbance of control. Each experiment was done in triplicate and the average was taken. The extract concentration for 50% inhibition (IC₅₀) was determined by the dose-response curve.

2.4. DPPH scavenging activity

DPPH scavenging activity was determined by the method of Jain and Agarwal [13]. Various concentrations of *bromelain* (10 μ l, 20 μ l, 30 μ l, 40 μ l & 50 μ l) of the test solution and 50 μ l of DPPH (0.659 mM) solution were incubated at 25°C for 20 min and the absorbance was read at 510 nm using Shimadzu UV 1800 spectrophotometer, the same procedure used for control without sample. Ascorbic acid served as a positive control. The % inhibition was calculated according to the following equation [13]:

% Inhibition = $(A_0 - A_t) / A_0 \ge 100$.

Where A_0 was the absorbance of the control (blank, without sample), and A_t was the absorbance in the presence of the extract.

2.5. Statistical Analysis

Microsoft office excel was used to calculate IC₅₀ value.

3. RESULTS

3.1. In-vitro Anti-Inflammatory Activity by HRBC Method

Bromelain at concentration of 10, 20, 30, 40 and 50 μ g/ml showed 29.62, 38.37, 45.34, 58.13 and 61.44% protection of HRBC in hypotonic solution, respectively (IC₅₀ 34.10) (table 1); whereas, standard diclofenac at concentration of 20, 40, 60, 80 and 100 μ g/ml showed 56.28, 60.14, 67.49, 72.78 and 78.69% (IC₅₀ 0.592) (table 2).

3.2. *In-vitro* Anti-arthritic Activity by Egg Albumin Denaturation Method

In *in-vitro* anti-arthritic activity by Egg Albumin denaturation method, at concentration of 10, 20, 30, 40 and 50μ g/ml showed 32.29, 38.54, 46.87, 52.08 and 63.54% inhibition of egg albumin denaturation (IC₅₀ 34.40) (table 3); whereas, standard diclofenac at concentration of 20, 40, 60, 80 and 100 μ g/ml showed 47.76, 57.71, 63.89, 75.87 and 84.81% inhibition of egg albumin denaturation (IC₅₀ 25.31). It was found that *bromelain* was more potent in inhibition of egg albumin denaturation (Table 4).

3.3. In-vitro antioxidant activity (DPPH scavenging activity)

The inhibition concentration at 50% inhibition (IC₅₀) was the parameter used to compare the radical scavenging activity. DPPH radical scavenging was in a concentration-dependent manner (table 5) and the IC₅₀ of *bromelain* was found to be 28.37 μ g/ml. However, the activity was less when compared with the standard, ascorbic acid (IC₅₀ value10.56 μ g/ml). A lower IC₅₀ meant better radical scavenging activity. *Bromelain* showed a good scavenging activity on DPPH radicals.

Table 1: Effect	t of Bromelain on	Human RBC membran	ne stabilization Activity
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Sample	Concentration (µg/ml)	% of Inhibition on Membrane Stabilization	IC ₅₀ value
	10	29.62	
	20	38.37	-
Bromelain	30	45.34	34.10
	40	58.13	_
	50	61.44	-

Table 2: Effect of Diclofenac sodium (Standard) on Human RBCs Membrane Stabilization Activity

oncentration (µg/ml)	% of Inhibition on Membrane Stabilization	IC ₅₀ value
20	56.28	
40	60.14	-
60	67.49	0.592
80	72.78	-
100	78.69	_
	20 40 60 80 100	20 56.28 40 60.14 60 67.49 80 72.78 100 78.69

Table 3: In-vitro Anti-Arthritic Activity Bromelain on Egg Albumin Denaturation Method

Sample	Concentration (µg/ml)	% of Inhibition	IC ₅₀ value
	10	32.29	
	20	38.54	
Bromelain	30	46.87	34.40
—	40	52.08	
	50	63.54	

Table 4: In-vitro Anti-Arthritic Activity	of Diclofenc sodium	(Standard)	on Egg	Albumin	Denaturation
Method					

Standard	Concentration (µg/ml)	% of Inhibition	IC ₅₀ value
	20	47.76	
Diclofenac Na	40	57.71	
	60	63.89	25.31
	80	75.87	
	100	84.81	

Bromelain Concentration (µg/ml)	% Inhibition	Ascorbic acid Concentration (µg/ml)	% Inhibition
10	39.04	50	57.68
20	47.02	100	66.07
30	50.99	150	74.58
40	57.05	200	81.44
50	60.26	250	94.56
IC ₅₀	28.37	IC ₅₀	10.56

 Table 5: DPPH radical scavenging activity of bromelain at and standard at different concentrations

4. DISCUSSION

Bromelain has remarkable anti-inflammatory activity [14], this work was aimed at evaluating the *in-vitro* antiinflammatory activity of bromelain by protein denaturation method. The data of our studies suggests that bromelain supplement showed significant antiinflammatory activity. Denaturation of proteins is a well documented cause of inflammation and rheumatoid arthritis [15]. Auto-antigens productions in some arthritic diseases may be due to denaturation of proteins, therefore bromelain would be worthwhile for anti-inflammatory drug development [16-18]. Nonsteroidal drugs have been the primary therapy for controlling RA with severe side effects [19]. Compared with synthetic drugs, herbal formulations are considered to be holistic and rather safe and therefore are considered more suitable for long-term treatments [20]. Protein denaturation and stabilization of human red blood cell membranes were studied to further establish the mechanism of anti-inflammatory action of bromelain. Inflammation is usually associated with the denaturation of proteins. Bromelain containing variety of proteolytic enzymes, derived from pineapple. Bromealin stimulates fibrinolysis by increasing plasmin, inhibit platelet aggregation, enhance absorption of other drugs, enhance wound healing, help in swelling and as digestive assistance [21]. Bromelain is used to treat a variety of pain and inflammatory conditions. When taken in between meals, the anti-inflammatory properties of bromelain may help to reduce swollen, sinuses, providing relief to people who have sinus infections. When taken with meals, bromelain promotes digestive health by supporting the body to digest proteins and absorb nutrients faster [22]. Results from the present study revealed that bromelain significantly inhibited protein/ albumin denaturation. Bromelain was also effective in stabilizing RBC membranes or inhibiting the heatinduced hemolysis at different concentrations. Studies by Pavan et al., and Tochi et al., supported the current work [8, 23]. The present study provides evidence for membrane stabilization was an additional mechanism of anti-inflammatory activity of *bromelain*. Antioxidation and anti-inflammatory properties of natural products are continuously investigated and considered as primary health benefits. Also, antioxidative agents prevent the formation of a many diseases including inflammatory diseases [24]. Finally, from the analysis of commercially available natural *bromelain* product has potential to be used for arthritic patients as a supplement.

5. CONCLUSION

It is concluded that *bromelain* with anti-inflammatory, anti-arthritic and anti-oxidant potential. These properties have been evaluated by *in vitro* studies. *In vitro* studies have shown that *bromelain* has a very strong anti-inflammatory property by human RBCs membrane stabilization method and *in vitro* studies that have shown anti-arthritis activity of *bromelain* on an egg albumin denaturation method. These results confirm the utilization of *bromelain* in the treatment of chronic inflammatory diseases and consider it a potential candidate for the isolation of new anti-inflammatory and anti-arthritic compounds.

Conflict of interest

The authors wish to declare that there are no conflicts of interest associated with this research work and similarly there has been no financial support involved in this work that could affect its outcome.

6. REFERENCES

- Anonymous, 2005. New medical dictionary. 2nd ed. New Delhi: Oxford and IBH Publishing Co. Pvt. Ltd.
- 2. Libby P. Am J Med, 2008; 121: S21-S3.
- Bharathi D, Hiradeve, SM. J Adv Sci Res, 2020; 11:153-163.
- Tripathi KD. Essentials of Medical Pharmacology. 6th Ed. Jaypee Brother's Medical Publishers (P) Ltd. New Delhi; 2008.

- 5. Bennett PN, Brown MJ. Clinical pharmacology, New Delhi: Churchill Livingstone; 2005.
- 6. Setiasih S, Adimas Ch D A, Dzikria V, Hudiyono S. IOP Conf. Ser.: *Mater Sci Eng*, 2018; 299: 012-016.
- 7. Bala M, Ismail NA, Mel M, Jami MS, Salleh M, Amid A. *Arch Des Sci*, 2012; **65**:369-399.
- Pavan R, Jain S, Shraddha, Kumar A. Biotechnol Res Int, 2012; 2012: 976203.
- 9. Rathnavelu V, Alitheen NB, Sohila S, Kanagesan S, Ramesh R. *Biomed Reports*, 2016; **5**:283-288.
- Sadique J, Al-Rqobahs WA, Bughaith, EIGindi AR. *Fitoterapia*, 1989; 60:525-532.
- 11. Shinde UA, Phadke AS, Nari AM, Mungantiwar AA, et al. *Fitoterapia*, 1999; **70**: 251-257.
- 12. Rahman H, Eswaraiah MC, Dutta AM. Am-Euras. J Agric Environ Sci, 2015; 15:115-120.
- 13. Jain PK, Agrawal RK. Asian J Exp Sci, 2008; 22:213-220.
- 14. Kothare A, Pardhi V, Chivte P, Muley S, Shanbhag T. Int J Pharm Biol Sci, 2017; 7:1-9.
- 15. Karthik K, Kumar BRP, Priya VR, Kumar SK,

Rathore RSB. Ind J Res Pharm Biotechn, 2013; 1:729-731.

- 16. Opie EL. J Exp Med, 1962; 115:597-608.
- Umapathy E, Ndebia EJ, Meeme A, Adam B, Menziwa P, Nkeh-Chungag BN, Iputo JE. J Med Plants Res, 2010; 4:789-795.
- 18. Chandra S, Chatterjee P, Dey P, Bhattacharya S. *Phcog J*, 2012; 4:47-49.
- 19. Kremer JM. Ann Intern Med, 2001; 134:695-706.
- 20. Karimi A, Majlesi M, Rafieian-Kopaei MJ. Nephro pharmacol, 2015; 4:27-30.
- 21. Dubey R, Reddy S, Murthy NYS. Asian J Chem, 2012; 24:1435-1438.
- Andrea H. Zengion ND, MSAOM, Eric Yarnell ND. Herbal and Nutritional Supplements for Painful Conditions in Pain Procedures in Clinical Practice (3rd Edition), 2011; 187-204.
- Tochi BN, Wang Z , Xu S-Y, Zhang W. Pak J Nut, 2008; 7:513-520.
- Kapewangolo P, Omolo JJ, Bruwer R, Fonteh P, Meyer D. J Inflamm, 2015; 12:1-13.