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Research Article

In vitro trypsin inhibitory activities of some plant and fruit extracts and chemical compounds

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ABSTRACT

Trypsin, an enzyme from the serine protease class, is known to be involved in the degradation of proteins. Excessive activity of trypsin is strongly implicated in triggering many diseases, such as acute pancreatitis, inflammation, and tumour. Therefore, this enzyme's regular and balanced activity is necessary for normal physiological functions. Thus, there is a need to develop new trypsin inhibitors from natural sources and chemical compounds. In this study, the inhibitory effects of aqueous extracts prepared from 29 different plants and 10 different chemical compounds were investigated on the activity of trypsin due to its importance in the health sector. The present study's plant extracts and chemical compounds showed trypsin-inhibitory effects. The inhibitory activities of the extracts and chemical compounds increased in a dose-dependent manner. Several plant extracts and chemical compounds that showed high trypsin inhibitory activities may be appropriate for use as trypsin inhibitors to provide additional support to drug treatment in the health field.

Keywords: Trypsin, Plant extract, Enzyme inhibition, Chemical compounds

Introduction

Proteases are important enzymes having proteolytic cleavage activities. Every protease enzyme hydrolyses peptide clusters from different points. They can control some physiologic processes (Cid-Gallegos et al., 2022). For example, the existence of proteases is strongly necessary for the regeneration and refreshing of lung tissue homeostasis (Greene & McElvaney, 2009). In addition, proteases regulate signalling functions (Gräwe et al., 2020). Although their biological necessity for maintaining regular biological activity in organisms is important (Zhang et al., 2021), their inhibitions have been studied for a long time due to specific reasons. Besides, there are many reports about inhibitions of proteases (Mancek-Keber, 2014; Elsässer & Goettig, 2021). The increased need for their inhibition studies has been raised due to their relations with tumour masses, inflammatory conditions, cardiovascular diseases, renal failures, etc. (Regulski et al., 2015; Srikanth & Chen, 2016; Park et al., 2020; Yi et al., 2020). Proteases can be classified according to their many properties. One of the well-known classifications is based on the functional group which exists at their active site. Metalloproteinases, aspartic proteases, cysteine proteases, and serine proteases are the basic sub-classes of this classification.

Trypsin (a digestive enzyme) is a serine protease initially synthesised as an inactive pre-form, called trypsinogen, that is then transformed to its active form, "trypsin", by the proteolysis of eight amino acids from the N-terminal side of peptide structure (Hegyi & Sahin-Tóth, 2017). This enzyme is responsible for the digestion of proteins by cleaving the peptides from their lysine and arginine moieties. Nevertheless, its increased activity must be limited due to its role in acute pancreatitis, cancer, and inflammation (Li et al., 2014). "For this purpose, natural products have been preferred by many researchers due to their lesser side effects and productive inhibition (Navaei-Bonab et al., 2018; Geisslitz et al., 2022). The current study investigated the possible inhibitory effects of various plant extracts and chemical compounds on trypsin activity.

Materials and Methods

Experimental Material and Chemical Compounds

In this study, aqueous extracts of plants, fruits, vegetables, and leaves were used for enzyme inhibition studies. The experimental materials which were used in this study can be named as fruit parts of apple, blueberries, grapefruit, lemon, bitter melon, olive, and pomegranate; leave parts of basil, black cabbage, green tea, fresh dock, lemon balm, mint, nettle, parsley, rocket, senna, smoke tree, thyme, and white cabbage; vegetable parts of faba bean, brussel sprouts, capers, garlic, onion, and radius, and plant parts of clove and fennel. All the plants, fruits and vegetables were purchased from a Local Market in Istanbul. Edible parts of the supplied samples were separated from their roots and stem, passed through distilled water, and dried in room conditions in the shade. Raw fruits were kept in a low-temperature oven for 2-3 days. The dried materials were kept at 4°C until their various extracts were prepared. Caffeine, catechin, epicatechin, gallic acid, glyoxylic acid, lansoprazole sodium, ranitidine, resorcinol, and vitamin U were chosen as chemical compounds. The chemical compounds were analytical grade and obtained from Merck, Sigma–Aldrich, Fluka, and BYK.

Preparation of the Plant, Fruit, and Vegetable Extracts

The plant extracts were prepared by refluxing 20 g of the dried material with 200 ml of distilled water for eight hours, then cooled and filtered through a filter paper at room temperature. The filtrates were evaporated to dryness in a rotary evaporator. The recovered extract residue was placed in an initially tared crucible and then in an oven at 37°C. The extracts were kept at -20°C until the experiments were employed. All the extracts were dissolved in distilled water for experiments. Chemical compounds used were also dissolved in distilled water. Tannic acid was used as a standard and positive control.

In Vitro Trypsin Inhibitory Assay for Extracts and Chemical Compounds

The inhibition of trypsin activity was determined by a spectrophotometric method (Ribeiro et al., 2010). A 10 µL of trypsin enzyme (0.3 mg / mL) dissolved in 1 mM HCl solution was placed into a test tube, and then 120 µL of 2.5 mM HCl solution was added to this enzyme solution. A 50 µL of plant and fruit extracts or chemical compounds (dissolved in distilled water) were added to this mixture, followed by 440 µL 50 mM Tris HCl (pH: 7.5) buffer solution and incubated at 37°C for 10 minutes. To this mixture, 500 µL 2.5 mM BAPNA (N-α-benzoyl-DL-arginine-p-nitroanilide) substrate solution dissolved in 1 mM HCl was added, followed by incubation at 37°C for 15 minutes. To terminate the reaction, a 120 µL of 30% acetic acid solution was added to each test tube, and the absorbance value was read in the spectrophotometer at 410 nm. As blank, 50 mM Tris HCl (pH: 7.5) buffer solution was used instead of sample, substrate, and enzyme solution. The 50 mM Tris HCl (pH: 7.5) buffer solution was used instead of plant extracts in the control solution.

The percent inhibition of trypsin activity was calculated as follows:

Trypsin Inhibitory Activity (%) = (A-B)/(A) x 100

A is the enzyme activity without an inhibitor, and B is the activity in the presence of an inhibitor.

The IC_{50} was determined as the concentration of plant extracts and chemical compounds required to inhibit trypsin activity by 50%.

Percentage enzyme inhibition activities of the inhibitors were used to calculate half maximum inhibitors (IC_{50}) for individual enzymes via regression analysis data.

Results and Discussion

The effect of the aqueous extracts of plants on trypsin activity is presented in Table 1. The enzyme inhibitory activities of the extracts increased in a dose-dependent manner. The higher inhibitory activity is associated with a lower IC_{50} value. Pomegranate was found to have the highest trypsin inhibitor activity with an IC_{50} value of 14.21 ±1.42 µg/mL. On the other hand, Basil, exhibited the lowest trypsin inhibition activity (775.59 ±23.00 µg/mL) (Table 1). Tannic acid showed inhibitor activity with an IC_{50} value of 36.47 ±5.86 µg/mL. According to the results, the trypsin inhibitory activity decreased in order of pomegranate > mint > lemon balm > lemon > tannic acid > clove > bitter melon > fresh dock > senna > black cabbage > radius > thyme > green tea > nettle > brussels sprouts > smoke tree > arugula > faba bean > white cabbage > onion > garlic > grapefruit > parsley > blueberry > capers > fennel > olives > apple > basil (Table 1).

The effect of some chemical compounds on trypsin activity is given in Table 2. The inhibition % values of the chemical compounds on trypsin increased with increasing concentration. Tannic acid showed the highest trypsin inhibitory activity having an IC_{50} value of 21.44 ±3.15 µM, while lansoprazole sodium had the lowest trypsin inhibitor activity (2528.68 ±228.15 µM) (Table 2). Trypsin inhibitory activity of chemical compounds decreased in the order of tannic acid > caffeine > glyoxylic acid > gallic acid > catechin > ranitidine > resorcinol > epicatechin > vitamin U > lansoprazole sodium (Table 2).

Trypsin (E.C. 3.4.21.4) is a serine protease that plays a vital role in the cleavage of peptides from the carboxyl moieties of lysine and arginine. It is secreted from the pancreas to the small intestine as zymogen (trypsinogen). In the small intestine, trypsinogen is converted to its active form, "trypsin", via the activity of enterokinase and/or by a previously activated trypsinogen (as trypsin). Under certain pathological conditions, its activity increases; thus, inhibition is required to prevent deleterious outcomes such as acute pancreatitis. The most crucial issue is preventing the activation of pancreatic enzymes before entering the small intestine to prevent trypsin and other enzymes from digesting the pancreas. When these enzymes are activated within the pancreatic duct, the pancreas can be seriously damaged, or obstruction of the pancreatic duct can occur, leading to acute pancreatitis. This situation has been recognised by researchers (Zhan et al., 2019).

Trypsin contains histidine (His), serine (Ser) and aspartic acid (Asp) in its active site at position 57th, 195th and 102^{nd,} respectively. Ser performs the first nucleophilic attack on the peptide to make the cleaving process of His easier, and then His does the same process for the final step of the cleavage mechanism. In general, the real inhibition process involves targeting the active site of the enzyme via two types of inhibition mechanisms; reversible and irreversible inhibitions (Sultana et al., 2022). In reversible processes, the inhibitor binds to the active site; then, the formed complex (proteinase-inhibitor complex) degrades the proteinases and frees the active inhibitor for new inhibitions. Contrary, in the irreversible mechanism, the inhibitor directly inhibits the activation of these enzymes (Clemente et al., 2019). This is the most popular process for inhibiting proteases (Bateman & James, 2011).

This study studied twenty-nine plant extracts and ten chemical compounds for trypsin inhibition. Tannic acid was used as a positive control for comparing the inhibitory activities of plant extract samples. The IC_{50} value for tannic acid was 36.47 ± 5.86 µg/mL. According to obtained results, all the plant extracts showed trypsin-inhibitory activity. Among them, pomegranate was detected to have the best inhibitory activity corresponding to the lowest IC_{50} value (14.21 ±1.42 µg/mL). In addition to pomegranate, mint (IC_{50} = 17.30 ±1.75 µg/mL), lemon balm (IC_{50} = 20.47 ±2.36 µg/mL) and lemon (IC_{50} = 21.87 ±0.02 µg/mL) extracts had a higher inhibition effect and lower IC_{50} values for trypsin inhibition as compared to tannic acid.

Table 1. Trypsin inhibitory activity of plant, fruit, and vegetable extracts

The name of the plant/Preferred parts of plants	Latin Names of Plants	Concentration (µg/mL)	Inhibition (%)*	IC_{50} (µg/mL)*
Apple / Fruit	*Malus domestica*	10	8.01 ±2.09	251.50 ±15.92
		20	11.61 ±2.76	
		50	15.64 ±1.27	
		100	20.71 ±0.85	
		150	34.43 ±3.39	
Basil / Leaf	*Ocimum basilicum*	50	5.72 ±1.01	775.99 ±23.00
		100	20.53 ±4.29	
		150	22.71 ±2.31	
		250	27.72 ±1.05	
		500	33.52 ±2.27	
Black Cabbage / Leaf	*Brassica oleracea* var. Acephala	5	21.78 ±2.28	86.08 ±1.21
		7.5	28.53 ±4.14	
		25	35.29 ±0.57	
		50	40.52 ±0.00	
		75	45.06 ±1.85	
Bitter Melon / Fruit	*Momordica charantia*	5	3.59 ±0.72	67.42 ±3.08
		10	9.75 ±2.19	
		25	18.36 ±2.75	
		50	43.35 ±4.73	
		75	52.03 ±0.40	
Blueberries / Fruit	*Vaccinium myrtillus*	25	17.01 ±0.00	166.00 ±7.71
		50	23.36 ±2.01	
		75	28.66 ±0.42	
Brussel Sprouts / Vegetable	*Brassica oleracea gemmifera*	10	10.89 ±0.82	99.44 ±13.92
		25	22.61 ±3.59	
		50	32.01 ±6.64	
		75	38.70 ±0.76	
		100	50.46 ±7.16	
Capers / Vegetable	*Capparis spinosa*	10	10.95 ±1.89	177.18 ±14.30
		50	15.77 ±3.81	
		75	26.16 ±3.26	
		100	32.04 ±2.35	
Clove / Plant	*Syzygium aromaticum*	5	17.72 ±4.11	50.54 ±1.45
		10	26.18 ±1.13	
		25	40.48 ±1.87	
		50	50.56 ±2.96	
		75	57.29 ±3.28	
Faba Bean / Vegetable	*Vicia faba* L.	1	6.48 ±2.06	119.24 ±4.21
		5	10.82 ±0.07	
		25	22.64 ±3.73	
		50	28.79 ±0.62	
		75	34.95 ±1.86	
		100	41.54 ±1.24	
Fennel / Plant	*Foeniculum vulgare*	10	10.79 ±2.30	222.10 ±17.27
		50	16.27 ±4.95	

		75	21.51 ±0.96	
		100	27.98 ±3.59	
Fresh Dock / Leaf	*Rumex cristacus* DC	1	7.78 ±0.60	76.51 ±7.00
		5	13.87 ±1.36	
		10	17.72 ±1.96	
		50	39.02 ±2.10	
		75	49.22 ±4.50	
Garlic / Vegetable	*Allium sativum* L.	25	12.95 ±3.79	137.81 ±10.13
		50	19.77 ±1.72	
		75	30.81 ±4.43	
		100	41.13 ±1.34	
		125	43.47 ±1.44	
		150	53.67 ±5.49	
Grapefruit / Fruit	*Citrus paradisi*	10	12.91 ±1.36	140.35 ±49.92
		25	19.04 ±0.91	
		50	21.94 ±1.36	
		75	34.52 ±9.58	
Green Tea / Leaf	*Camellia sinensis*	10	20.09 ±1.85	94.15 ±2.36
		25	23.43 ±2.35	
		75	35.29 ±0.26	
		100	53.12 ±1.33	
		150	60.66 ±0.04	
Lemon / Fruit	*Citrus limon*	1	15.43 ±1.75	21.87 ±0.02
		2.5	21.34 ±3.68	
		5	22.89 ±5.72	
		10	31.21 ±0.46	
Lemon Balm / Leaf	*Melissa officinalis*	2.5	11.08 ±3.82	20.47 ±2.36
		5	23.21 ±3.04	
		7.5	28.11 ±1.65	
		10	30.82 ±1.91	
		15	37.40 ±5.52	
Mint / Leaf	*Mentha piperita* L.	2.5	12.52 ±4.70	17.30 ±1.75
		7.5	17.34 ±2.79	
		10	29.57 ±6.11	
		12.5	35.24 ±1.17	
		15	47.15 ±6.94	
Nettle / Leaf	*Urtica dioica*	10	18.27 ±0.57	94.71 ±8.94
		25	24.77 ±1.31	
		50	36.23 ±2.35	
		75	43.07 ±0.00	
		100	51.02 ±4.97	
Olive / Fruit	*Olea europaea* L.	5	11.61 ±4.47	241.90 ±51.77
		50	15.33 ±0.79	
		75	17.19 ±0.79	
		100	30.22 ±8.68	
Onion / Vegetable	*Allium cepa* L.	10	2.94 ±0.78	124.38 ±10.29
		25	9.25 ±2.71	
		50	22.44 ±5.42	
		75	29.31 ±0.48	
		100	39.80 ±4.46	

Parsley / Leaf	*Petroselinum crispum*	10	16.84 ±4.09	149.15 ±16.60
		50	17.73 ±1.41	
		75	33.67 ±0.28	
		100	44.02 ±0.85	
		150	50.60 ±5.64	
Pomegranate / Fruit	*Punica granatum* L.	1	4.18 ±4.28	14.21 ±1.42
		2.5	8.93 ±6.19	
		5	17.29 ±0.82	
		7.5	23.54 ±8.03	
		10	37.32 ±3.88	
Radius / Vegetable	*Trachystemon orientalis* (L.) *G. Don*	5	10.30 ±6.22	86.39 ±1.66
		25	24.15 ±4.07	
		50	30.68 ±1.03	
		75	47.58 ±2.39	
		100	54.74 ±0.21	
Rocket / Leaf	*Eruca vesicaria*	10	10.07 ±2.91	118.99 ±9.22
		25	13.23 ±0.78	
		50	24.68 ±1.83	
		75	31.82 ±2.14	
		100	44.00 ±3.96	
Senna / Leaf	*Cassia angustifolia*	10	5.92 ±3.83	81.06 ±3.76
		25	11.77 ±0.88	
		50	33.72 ±0.99	
		75	48.17 ±5.83	
		100	59.63 ±0.65	
Smoke Tree / Leaf	*Cotinus coggygria*	10	9.34 ±0.42	116.83 ±3.97
		25	16.50 ±0.47	
		75	31.57 ±0.46	
		100	48.27 ±4.63	
		150	60.71 ±0.01	
Tannic Acid (Positive Control)		17	32.16 ±2.76	36.47 ±5.86
		85	73.10 ±1.65	
		170	77.98 ±1.93	
		425	81.65 ±0.59	
Thyme / Leaf	Thymus vulgaris L.	10	8.04 ±4.00	90.32 ±0.65
		50	31.30 ±0.62	
		75	40.00 ±1.85	
		100	56.74 ±3.38	
		150	64.13 ±3.38	
White Cabbage / Leaf	*Brassica oleracea* var. capitata f. alba	10	10.56 ±1.41	121.62 ±0.79
		25	13.95 ±4.50	
		50	22.34 ±2.29	
		75	36.46 ±1.41	
		100	40.72 ±0.44	

*Mean ± SD

Table 2. Trypsin inhibitory activity of chemical compounds

Chemical Compounds	Concentration (μM)	Inhibition (%)*	IC_{50} (μM)*
Caffeine	20	8.17 ±3.85	122.15 ±4.67
	25	24.93 ±5.27	
	50	26.93 ±3.65	
	75	36.68 ±2.84	
	100	40.26 ±2.23	
Catechin	10	6.16 ±0.20	220.63 ±92.44
	20	8.31 ±2.02	
	25	13.61 ±2.63	
	50	15.33 ±4.26	
Epicatechin	1	6.64 ±2.34	959.22 ±27.86
	5	16.71 ±2.85	
	100	18.31 ±0.92	
	250	21.21 ±1.83	
Gallic Acid	10	15.85 ±0.21	185.17 ±89.81
	20	18.03 ±1.65	
	25	25.00 ±2.05	
	50	30.09 ±7.19	
	100	35.18 ±9.04	
Glyoxylic Acid	10	7.66 ±1.42	174.60 ±17.88
	20	11.42 ±0.21	
	25	13.01 ±0.81	
	50	17.92 ±2.45	
	100	31.36 ±2.66	
Lansoprazole Sodium	10	11.15 ±1.62	2528.68 ±228.15
	100	15.44 ±5.73	
	1000	27.39 ±0.66	
Ranitidine	0.1	5.85 ±0.00	385.00 ±68.09
	5	12.14 ±3.10	
	50	13.74 ±2.06	
Resorcinol	10	10.54 ±8.08	405.15 ±45.28
	100	25.48 ±4.35	
	250	34.55 ±0.62	
Tannic Acid (Positive Control)	10	32.16 ±2.76	21.44 ±3.15
	50	73.10 ±1.65	
	100	77.98 ±1.93	
	250	81.65 ±0.59	
Vitamin U	2.5	3.55 ±1.20	1026.72 ±307.67
	50	13.51 ±0.07	
	100	18.15 ±1.92	
	500	28.90 ±6.41	

*Mean ± SD

The plants explored in the present study have been found to show many protective effects against various diseases. For instance, pomegranate and its bioactive compounds were proven to have a preventive effect on gastric cancer cells (Cheshomi et al., 2022) and a protective effect on different gastric disorders (Abd el-Rady et al., 2021). Mint oils, various extracts of lemon balm and lemon have been reported to protect the gastrointestinal system and the stomach (Saberi et al., 2016; de Mashayekhi-Sardoo et al., 2020; de Oliveira Braga et al., 2022). Moreover, the common feature shared by these plants is their bioactive compounds since they all contain phenolic compounds and flavonoids (Abdellatif et al., 2014; Uçan et al., 2016; Tarantino et al., 2020; Fan et al., 2021). Phenolic compounds and flavonoids effectively inhibit many enzymes (Sacan & Yildiz Turhan, 2014; Dağsuyu & Yanardağ, 2021; Kan et al., 2021). These compounds may directly be related to blocking trypsin's active site by interacting with His, Ser and Asp amino acids. Ghosh et al. (Ghosh et al., 2008) revealed the interactions of some polyphenolic compounds and His-containing enzymes at their active site. They said this interaction changed the histidine stability and affected the carbon-hydrogen chemical shift. Moreover, the inhibitory capacities of polyphenolic compounds and their derivatives on serine proteases are related to the interaction between their polyphenolic skeleton and the hydrophobic region of these enzymes (Viskupicova et al., 2012). Furthermore, most legume plants have been reported to have serine protease inhibition effects (Haq et al., 2004). Based on this information and considering the bioactive components of the plants studied, it can be hypothesised that these plants can be used for effective trypsin inhibition.

The present study evaluated caffeine, catechin, epicatechin, glyoxylic acid, gallic acid, lansoprazole sodium, ranitidine, resorcinol, tannic acid, and vitamin U for trypsin inhibition assays. Tannic acid, considered a standard inhibitor, had the best trypsin inhibition effect due to its lowest IC_{50} value among the chemical substances. The compounds evaluated have phenolic and nonphenolic structures. Caffeine, glyoxylic acid and gallic acid exhibited lower IC_{50} values after tannic acid among the other compounds tested. The trypsin inhibition and inhibiting mechanisms of different synthesised compounds are explained in many research. According to the study of Hovhannisyan et al. (Hovhannisyan et al. 2009) nitrogen associated formyl groups could interact with proteins via hydrogen bonds with Ser residues located at the active site of the enzyme, as well as glutamine residues of substrate binding side. They also revealed that amino groups of different amino acid derivatives could interact with the carboxyl moiety of serine of the substrate binding pocket of trypsin. Based on this approach, their active groups can explain the present findings for caffeine, glyoxylic acid and gallic acid.

It is well-known that polyphenolic compounds consist of hydroxyl groups, which are highly effective and determine the properties of these compounds. Due to this property, phenolic groups can interact with polar portions of this enzyme (Feng et al., 2018). Thus, catechin and epicatechin showed inhibitory activity on trypsin, with the inhibitory efficacy of catechin better than that of epicatechin. The difference between their inhibitory activities can be attributed to the position of the active moieties in their structure.

Ranitidine, resorcinol, and lansoprazole sodium had trypsin inhibitory activity, with the lowest IC_{50} value exhibited by Lansoprazole sodium (IC_{50} = 2528.68 ±228.15 mM). Among these three compounds, ranitidine showed better inhibition activity on trypsin. Ranitidine, a proton pump inhibitor and a histamine blocker, has also been known to have a regulative effect on the gastrointestinal system (Satoh et al., 2014). Its active moieties may be suggested to be responsible for its trypsin inhibitory effect.

Vitamin U, also known as S-methyl methionine sulfonium chloride, is a methionine derivative that has a powerful antioxidant effect. It has protective effects on various toxicity models, regulative effects on antioxidant systems and enzymes, and gastroprotective effects (Turkyilmaz et al., 2019; Topaloglu et al., 2022). According to the study of Dağsuyu and Yanardağ (Dağsuyu & Yanardağ, 2021), sulfur compounds, including Vitamin U, had different trypsin inhibitory activities. They also mentioned that Vitamin U achieved this inhibition property via binding to the serine part of the enzyme's active site. In the present study, sulfur and/or Vitamin U-containing vegetables like Brussels sprouts, white cabbage, onion, etc., showed inhibitory activity on the enzyme trypsin. Therefore, Vitamin U and its food-based sources had trypsin inhibitory activity.

Conclusion

The results of this study show that water extracts of pomegranate, mint and lemon balm exhibit good trypsin inhibitory activities. Phenolic compounds and flavonoids in the plant kingdom are responsible for the enzyme inhibitory activity. It is known that plant extracts with high inhibitory activity on trypsin contain phenolic and flavonoid compounds. Therefore, in our study, extracts of pomegranate, mint, and lemon balm, which inhibit the enzyme at a high rate, can be used in medicine as a source of natural trypsin inhibitor, in the form of raw extracts or as a source of bioactive compounds. There-

fore, these plant extracts can be used as phytochemical compounds in medicine, especially in gastrointestinal tract diseases, and for future therapeutic purposes.

Compliance with Ethical Standards

Conflict of interests: The author(s) declares that for this article, they have no actual, potential, or perceived conflict of interest.

Ethics committee approval: Authors declare that this study includes no experiments with human or animal subjects.

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Disclosure: -

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