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Original article

50S Ribosomal Proteins Family is the Main Target of Cinnamon Extract: a Network Analysis

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Abstract

Background and objectives: It is reported that cinnamon consumption is corelated with improving several diseases and advanced methods are applied to detect the molecular mechanism of this effect. The aim of this study was introducing the main protein targets of cinnamon extract. **Methods**: Among the 100 regulated significant differentially expressed proteins, the recognized individuals by STRING database plus 100 added first neighbors were included in a network and the central nodes of the network were determined and analyzed. **Results**: The queried and added proteins were included in a scale free network. Seven hub nodes as central proteins were determined as critical target proteins. Five hub proteins were members of 50S ribosomal proteins family and others weretsf and dnaK. The last two hubs were related to protein synthesis and protein folding processes, respectively. **Conclusion**: 50S ribosomal proteins family and protein synthesis were identified as the main target of cinnamon and the core affected process, respectively.

Keywords: bioinformatics; cinnamon; hub protein; network analysis; proteomics

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Introduction

Traditional medicine is tied to the advanced methods which are known as a part of medicine today. The borders which separated traditional medicine from modern medicine lose their importance year by year. Chromatography, spectrometry, metabolomics, proteomics, and many other advanced methods are applied to develop field of traditional medicine [1-5]. Proteomics as a high throughput methods is applied to assess the effects of many herbal extracts and astonishing information are obtained [6,7]. Analysis of proteomics finding is an interesting field that has attracted attention of many investigators. In such a study among the large numbers of data, the limited numbers of critical proteins are identified as biomarkers or drug targets. Using interactome analysis which is tied mainly with graph theory, provides useful set of proteins that are discriminated from each other [8,9].

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Based on network analysis, the role of proteins in the network construction is diverse; therefore, topology of network depends to the protein combination. In scale free networks, there are a limited number of nodes that are known as central nodes discriminated from others by the numbers of connection with first neighbors or pattern of participation in the shortest paths [10,11].Hub proteins are known as central proteinsand are characterized by creation of many connections with the first neighbors. Determination of hubs of a network provides a useful information to identify biomarkers [12,13]. Cinnamon is the dried barks of *Cinnamomum zeylanicum* Blume. (Lauraceae). The scientific name *C. verum* Presl has been also attributed to cinnamon. It contains volatile oil, phlobatannins mucilage, calcium oxalate and starch [14]. Cinnamon is used as a tonic and also as a spice for its pleasant aroma [15]. The biological activates of cinnamon have been investigated in many studies showing its antioxidant, anti-inflammatory, antidiabetic, antimicrobial and anticancer effects [16]. In the present work, findings of proteomics investigation of Shunt-Kal Yang et al. that refer to regulation of 100 top significant differentially expressed proteins by cinnamon extract [17] were assessed via network analysis to find the main targets of cinnamon extract.

Material and Methods

Ethical considerations

This project is administrated with IR.SBMU.RES.1399.019 ethic code.

Design of the study

Original data was driven from the report of Shunt-Kal Yang et al. [17], which was published in 2019 in PLOS ONE. In this investigation *klebsiella pneumonia* cells which carrycarbapenemase (KPC-KP cells) (the cells that are resistant to the carbapenem) were treated with 0.08% (v/v) of cinnamon bark essential oil to find the protein expression change pattern. Positive effect of cinnamon bark essential oil on drug resistant cells was detected and alist including 100 top regulated proteins was presented. More details of used material and methods are described in the original published document [17].

Network analysis

Considering Fold change ≥ 1.5, 92 proteins among the 100 introduced proteins were selected for more analysis. Cytoscape 3.7.2 [18] and its applications was applied to create network. The query data were included in network via protein query of STRING database[19]. The nodes were connected by undirected edges with score of 0.4as the default score of software. To find valuable information about the relationship between the query proteins via decrement of numbers of isolated proteins, 100 neighbors from STRING database were added to the recognized proteins and the new network was formed.

"Network Analyzer" application of Cytoscape was used for network analysis. The network was visualized based on degree values and the centrality parameters such as degree (K), betweenness centrality (BC), closeness centrality (CC), and stress were determined for the nodes of the main connected component of the analyzed network. The CC and BC values were normalized by (X-min)/(max-min) which x refers to BC or CC value and max and min refer to maximum and minimum of BC or CC respectively. Top 10% of queried proteins (7 nodes) based on degree value were selected as hub nodes. Since most of the hub nodes belong to 50S ribosomal protein family, the queried hubs and the first neighborproteins which belong to this family were determined.

Results and Discussion

Ninety-two differentially expressed proteins were assessed to construct a network by Cytoscape software 3.7.2 via protein query of STRING database. Database recognized 67 proteins and the network including a main connected component, 4 paired proteins, and 17 isolated individuals were created (Figure 1). The new network including the recognized queried proteins and 100 added proteins is shown in the Figure 2. This network includes a main connected component and five isolated proteins.The main connected component is visualized based on degree value by node size and color.

Seven hub nodes were identified among the queried proteins. The hubs and their centrality parameters are represented in the Table 1. Except tsf and dnaK, the other hup proteins belong to the 50S ribosomal protein family, all members of this family including the queried proteins hubsand the first neighbors are shown in the Table 2.

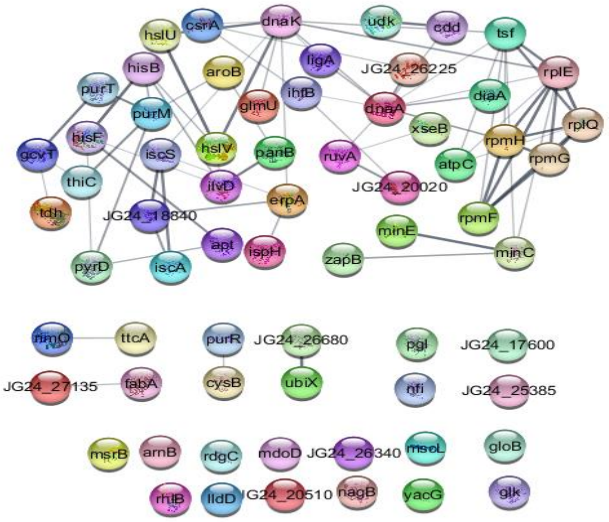

Figure 1. The 67 queried proteins which were recognized by STRING database are included in a network via undirected connections with default score of 0.4

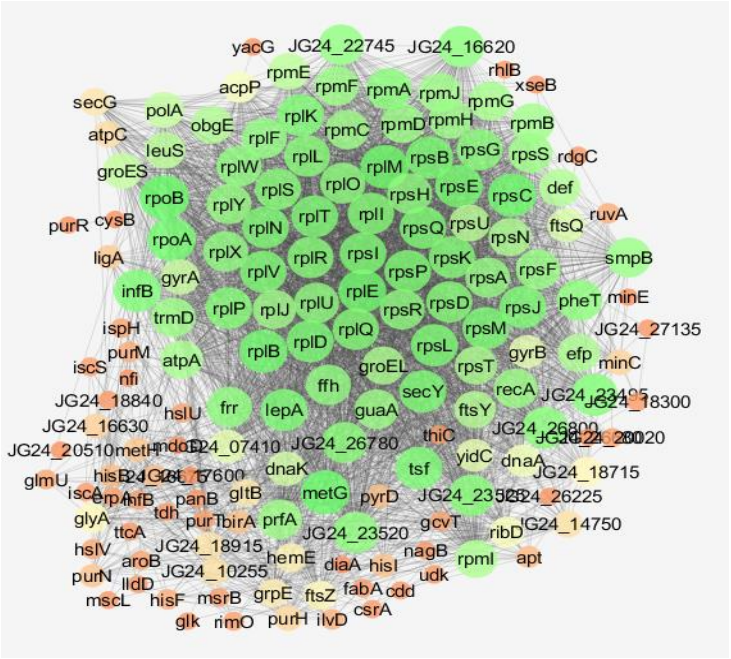

Figure 2. The 67 queried proteins which were recognized by STRING database plus 100 first neighbors from STRING are included in a network via undirected connections whit default score of 0.4. Five isolated proteins were excluded.The nodes are layout by degree value; colors from red to green and size increment are corresponding to the increase of degree value.

Table 1. The hub nodes of the main connected component

No.	Name	Description	K	BC	CC	Stress
1	rplE	50S ribosomal protein L5	93	0.14	1.00	3502
2	tsf	Elongation factor Ts	90	0.39	1.00	4118
3	rplQ	50S ribosomal protein L17	88	0.10	0.79	2574
4	rpmF	50S ribosomal protein L32	81	0.00	0.41	1458
5	rpmH	50S ribosomal protein L34	80	0.04	0.32	1954
6	rpmG	50S ribosomal protein L33	78	0.02	0.35	1616
7	dnaK	Chaperon protein DnaK	64	1.00	0.00	8454

K, BC, and CC refer to degree, betweenness centrality, and closeness centrality, respectively; BC and CC are normalized as described in the methods.

Table 2. The 5 hub nodes (the bolded proteins) that belong to the 50S ribosomal protein family and all first neighbors which are fitted into this family.

No.	Name	K	No.	Name	K
1	rplM	94	17	rplF	84
2	rplB	93	18	rplU	84
3	**rplE**	93	19	rplW	84
4	rplD	90	20	rplX	84
5	rplK	89	21	rplY	83
6	rplN	89	22	**rpmF**	81
7	rplT	89	23	rpmB	80
8	rplV	89	24	rpmC	80
9	rplI	88	25	rpmD	80
10	rplP	88	26	**rpmH**	80
11	**rplQ**	88	27	rpmJ	80
12	rplL	87	28	rplJ	79
13	rplR	87	29	**rpmG**	78
14	rplS	87	30	rpmI	76
15	rpmA	87	31	rpmE	71
16	rplO	85			

K refers to degree value

The effect of cinnamon extract on gene expression has been investigated by researchers. As it is reported, this extract regulates expression of glucose transporters and insulin signaling genes and also HIF-1α, TNF-α, and some other genes [20-22]. In the original study among many genes, 100 top regulated proteins by cinnamon extract were introduced [17]. As it is depicted in Figure 1, 67 proteins among the queried proteins were recognized by STRING database. Analysis revealed that there were not enough connections between the studied proteins to form a scale free network and some proteins remained as isolated individuals and also paired proteins. The added first neighbors to the queried proteins provided the possibility to create a scale free network and also including the paired proteins and some isolated ones in the network. Investigations indicated that the scale free network contained useful information to identify a limited set of proteins among a large numbers of proteins which play critical role in the constructed network [23]. The discriminated nodes based on degree value as an important centrality parameter is displayed in the Figure 2. Seven proteins including rplE, tsf, rplQ, rpmF, rpmH, rpmG, and dnaK are tabulated in Table 1 as the central nodes of the studied network among the queried proteins. A simple analysis revealed that rplE, rplQ, rpmF, rpmH, and rpmGwere members of 50Sribosomal proteins family. An assessment showed that 25 proteins among the added proteins (25% of the added proteins) like these proteins belonged to the 50S ribosomal proteins family. Nenad Ban et al. represented a new system to name ribosomal proteins. Based on this system, the proteins of large ribosomal subunit from bacteria are named as L1-L6, L9-L25, and L27-L36. In this naming system L12 is also known as L7 [24].

Beside some 50S ribosomal protein family tsf and dnaK also appeared as central proteins. As it is reported,dnaK is a member of hsp70 family. It is well known that the members of this heat-shock protein have a prominent role in protein folding, interaction, and translocation, either constitutively or in response to stress. The chaperons bid to unfolded polypeptide parts to promote the mentioned processes [25]. It is found that elongation factor ts (tsf) is an aggregation-resistant protein which is activated in response to a protein denaturant. Experiments indicates that tsf in the presence of strong denaturation environment remains significantly soluble [26].As it is known, the translation elongation factors play prominent roles in protein synthesis which occur in the ribosome [27].

Our findings indicated that ribosome is the main target of cinnamon extract by regulation of some critical members of 50S ribosomal proteins family. Since ribosome is the unique station of protein synthesis in the cell, it can be concluded that cinnamon extract is involved in regulation of many different types of proteins. Regulation of several elements of ribosome can regulate the function of the whole ribosome organelle. This implies the ability of cinnamon in regulation of various processes in the body; however, clear understanding needs wide surveys.

Investigation in traditional medicine revealed prominent role of cinnamon on improvement of respiratory system, digestive and gynecological diseases [28]. Examples includes the study of Shan et al. who confirmed the antibiotic property of cinnamon against foodborne pathogenic bacteria [29]. WhereasFrydman et al. pointed the benefits of cinnamon consumption in reduction of β-amyloid organization in Alzheimer's disease [30]. Since ribosome is involved in nearly all functions of body, possible disadvantages of cinnamon feeding should be considered.

Conclusion

In conclusion, cinnamon extract induces gene expression change in different types of genes. This gross effect is pointed prominently to 50S ribosomal proteins family. The critical proteins which are regulated by cinnamon extract are

related to the protein synthesis processes. It is a rational conclusion that cinnamon consumption is accompanied with wide range of benefits however it needs a scientific protocol for use. Considering the ribosomal regulation, it is possible that cinnamon treatment can affect wide range of physiological activities in body.

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Author contributions

Mostafa Rezaei Tavirani designed and supervised the study; Mahmoud Khodadoust was involved in data collection; Babak Arjmand, Mohamadreza Razzaghi and Alireza Ahmadzadeh were involved in data collection and data analysis; all authors approved the final draft of the manuscript.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations

K: degree; BC: betweenness centrality; CC: closeness centrality