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Original article

# Cancer Chemo-Preventive Effects of Red Propolis: a System Biology Approach

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## Abstract

**Background and objectives:** Propolis is a natural product of honey bees that is characterized by therapeutic effects on diverse diseases. To elucidate the molecular mechanism of propolis effects on tumor, protein-protein interaction (PPI) network analysis of proteome data of Hep-2 cells treated with red propolis was conducted. **Methods:** Cytoscape V 3.9.1 and its plug-ins evaluated the differentially expressed proteins (DEPs) in terms of network construction and the corresponding topological features. **Results:** The results implied that six hub-bottlenecks including ACTB, GAPDH, HSP90AA1, HSPA8, HSP90AB1, and HSPA5 were present in the PPI network; however, only the last central protein was among DEPs. ClueGO+ CluePedia identified five related biological processes and three action types of their connections. Results refer to anticancer property of red propolis. **Conclusion:** The proposed crucial proteins and their linked biological processes may represent as key players in the anticancer underlying mechanism of red propolis.

**Keywords:** biological process; cancer; network analysis; propolis; therapeutic

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## Introduction

Cancer with the highest rate of morality in the world is essential to be studied in terms of molecular diagnosis and treatments [1]. On the other hand, propolis is a natural product by honeybees [2] that has shown to be effective in diverse diseases [3]. Many properties have been recognized for this product including regulation of cell proliferation, anti-inflammatory and immune system modulation, antioxidant, anti-microbial activity and blood pressure, and cholesterol level regulation [4-7]. Due to possessing these types of characteristics, propolis has displayed to be effective in cancer treatment, for tooth cavities, diseases linked to gum and microbial biofilms, parasites such as *Leshmonia*, viral diseases, arthritis, obesity, diabetes and for wound healing [7-12]. Furthermore, the native Iranian propolis extract has shown promising in

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diseases that are caused by free radicals due to its antioxidant effects [4]. These features of propolis are correspondent to the bioactive molecules that like flavonoids and phenolic acids, caffeic acid phenethyl ester, and cinnamic acid [13,14]. For instance, in the therapy of type 2 diabetes, some enzymes are inhibited by the chemical composition of propolis including propolins C, D, F, G, H, and solophenol-D [12]. Moreover, red propolis expresses pharmacological properties via bioactive components. It is important to know that based on the plant source that bees feed on, the compounds could vary. However, the main composition of propolis are plant resin, pollen, wax, essential oils, and other organic ingredients. Therefore, the origin of the propolis has an indispensable part in its pharmacological behavior. From a proteomics study by 2DE gel electrophoresis, differentially expressed proteins were identified in the presence of propolis extract. It was indicated that red propolis stimulate late apoptosis in cancerous cells [15]. Pro-inflammatory TNF/NF-κB, pro-proliferative MAPK/ERK pathways, and Nrf2-ARE are some of the biological terms which are targeted by propolis components in cancer fighting process [2]. Consequently, the mechanism by which propolis imposes some anticancer effects is through inhibition of cancer cell cycle and proliferation [3]. What is more, proteomics analysis of cancer cells in the presence of plant extracts can provide fundamental information related to the anticancer mechanism of the treatment. To further assess the anticancer properties of propolis, bioinformatics exploration in terms of protein-protein interaction network could be critical. In this regard, interactome study of Hep-2 cells was analyzed to open new insight of new therapeutic targets for treatment of cancer exposed to red propolis extract. In this view, by the application of functional interaction network analysis of proteome of Hep-2 cells treated with red propolis, it is aimed to better understand the molecular mechanism of this natural source bioactive value.

## Material and Methods

### Ethical considerations

This project was approved by ethical committee of Shahid Beheshti University of medical Sciences (IR.SBMU.RETECH.REC.1401.425).

### Data collection

In the main proteomics study, among 325 detected spots, 177 were identified. Down-regulation was dominant among the identified proteins. Investigation was through the study of cell-based model and by application of two-dimensional gel electrophoresis. Honey bee, *Apis mellifera*, was the source for the production of propolis extract and the cancer cell line was chosen as Hep-2 (laryngeal epidermoid cancer cells) [15]. The extract concentration applied for the proteomics approach was 120 µg/mL and 60 µg/mL. The down-regulated proteins were detected in the highest concentrations whereas the up-regulated proteins were explored at 60 µg/mL. Protein-protein interaction (PPI) network analysis of significant differentially expressed proteins (DEPs) (seven proteins) was handled by the use of Cytoscape software version 3.9.1 (https://cytoscape.org/) [16], and through query from STRING database (http://string-db.org/). STRING plug-in uses four sources for the interaction query including protein query, PubMed query, Disease query, and Stitch. The last database is designed for studying metabolites connections. The cut off score for the interaction study was set as default option [17].

Two networks were constructed via protein query, the first one without additional nodes while the second with the addition of 50 neighbors. NetworkAnalyzer performed the centrality analysis of the second network in terms of two prominent network centrality parameters [18]. Nodes with highest number of linkages (known as degree) are called hubs versus those having highest amount of information flow and serving as bridge in the middle of the network which are called bottlenecks (nodes with high value of betweenness centrality). Nodes with highest values of both are called hub-bottlenecks that play a noteworthy role in the stability of a constructed scale-free network. The hub identification is based on 10% of highest values of degree calculations [1]. After identification of hub-bottlenecks, gene ontology of these elements in terms of biological process was conducted via ClueGO 2.5.9+CluePedia1.5.9 application [19]. In addition, action type analysis of the hub-bottlenecks was also investigated considering kappa statistical analysis by the use of CluePedia. In this application, different edge colors indicate the corresponding action type.

**Statistical analysis**

The cut off score for the interaction study was set as default option of 0.4. Grouping of biological terms was set to 0.5 and protein numbers per term; 2 and percentage of proteins was 3. P-value correction test was also Bonferroni step-down. Kappa score with threshold customized to 0.5 was applied to the action map analysis.

## Result and Discussion

The significant differentially expressed proteins were queried in Cytoscape platform via STRING database considering confidence score cut off=0.4 (Figure1). The first network query of 7 DEPs shows an acceptable consistency between the participating nodes of 7 and links of 6.

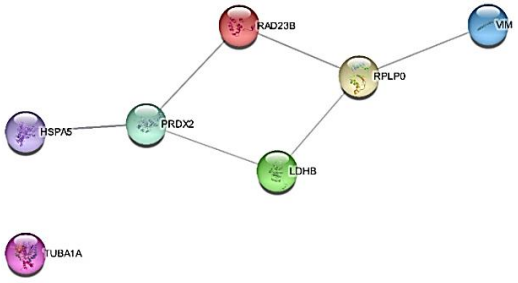


**Figure 1.** Protein-protein interaction network of seven DEPs constructed via STRING plug-in of Cytoscape; cut off= 0.4

Further analysis showed that the second network consisted of 57 nodes and 956 edges (data not shown) and the centrality analysis of this network via two prominent centrality parameters was handled to obtain related structural information. Topological measurements of the PPI network identified the degree (K) and the betweenness centrality (BC). Nodes with highest values of these parameters are more valuable in the network stability (Table 1). The average degree of hub-bottlenecks was 51.83 and the betweenness centrality was 0.02. ACTB was the top ranked hub-bottleneck in the network while HSPA5 was the least ranked node. This protein was among the DEPs of the proteomics study that was down-regulated.

In Figure2, five groups of biological processes which are related to the hub-bottleneck nodes are presented based on the designated statistical thresholds.

The leading groups were response to cocaine, protein folding chaperone, substantia nigra development, and positive regulation of type 1 interferon production. HSP90AA1, HSPA5, and HSPA8 are the most involved proteins in the biological processes in a way that the first protein is present in four groups, the second and third proteins in three groups of biological terms.

**Table 1.** The hub-bottlenecks of the PPI network ranked based on degree (K) value

| Row | Display name | K | BC |
|---|---|---|---|
| 1 | ACTB | 55 | 0.03 |
| 2 | GAPDH | 54 | 0.02 |
| 3 | HSP90AA1 | 53 | 0.02 |
| 4 | HSPA8 | 53 | 0.02 |
| 5 | HSP90AB1 | 50 | 0.02 |
| 6 | HSPA5* | 46 | 0.01 |

*: the query DEPs

The action type investigation expresses that three types of actions including binding, reaction, and catalysis are between the introduced hub-bottlenecks (Figure 3). These interactions are between HSPA8, HSP90AA1, HASA5, and HSP90AB1. The action score cut off was above 0.9 which shows statistically significant amount.

Propolis extract has demonstrated to be an effective natural source for different kinds of diseases including cancer [20]. One of the examined types of propolis is the red one with potential effects on cancer cell line. Red propolis extracts was applied on Hep-2 cell line in a proteomics evaluation at the doses of 120 μg/mL and 60 μg/mL [15]. In the present study, protein interaction characteristic of the DEPs in the original proteomics study was carried out and two networks were obtained. As mentioned before, hubs are nodes with highest value of degree and bottlenecks are nodes with highest amount of betweenness centrality.

The evaluation of hub-bottlenecks adds more information to the study of underlying mechanism of propolis cellular effects. The second analysis indicated that there are six hub-bottlenecks in the PPI network of the treated Hep-2 cells. Besides, analysis of the key nodes of the network, leads to introducing additional information related to network stability.

This exploration discovers the neighbor proteins that may be potential as therapeutic biomarkers and also identifies which extensive examination of these candidates is required. In this sense, the literature review of the hub-bottlenecks could add more information related to the anticancer mechanism of propolis. Actin beta (ACTB) as the first ranked hub-bottleneck is a housekeeping protein [21] that has a concrete link with development of different kinds of cancer.

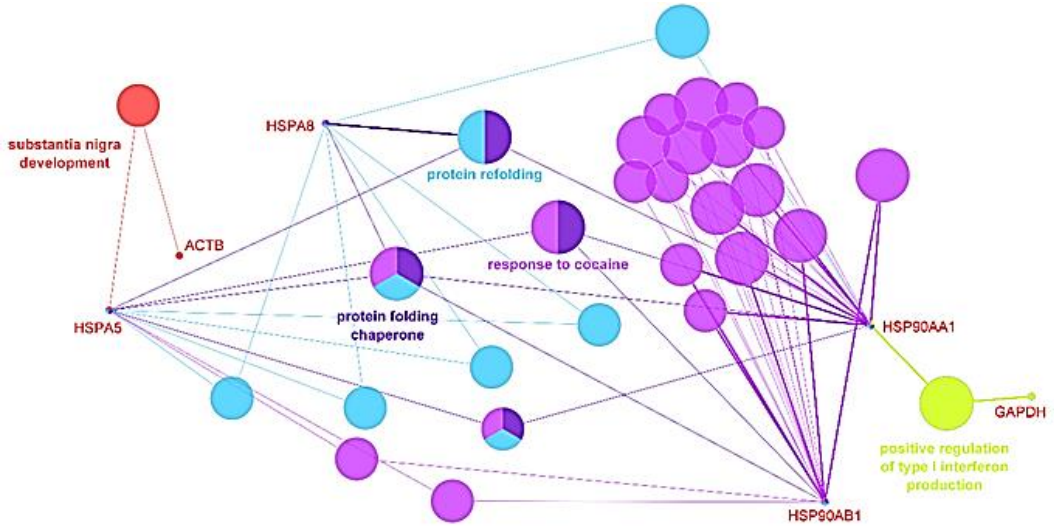


**Figure 2.** Grouping of biological process of six hub-bottlenecks in different colors, grouping score cut off: 0.4; p-value ≤ 0.05; the associated proteins are labeled with display names. Colors of biological terms are corresponded with the colors of group labels.

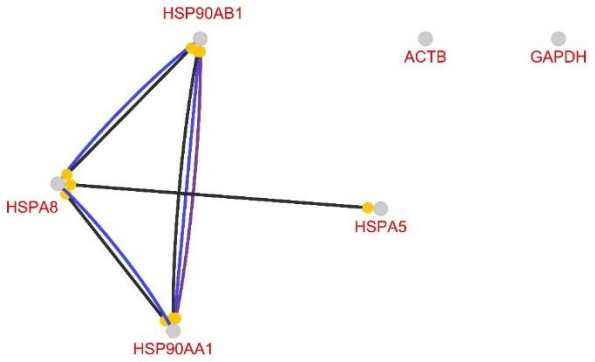


**Figure 3.** Action view of hub-bottlenecks show reaction, catalysis, and binding relationships; blue color: binding; black color: reaction; purple color: catalysis; Kappa score cut off =0.5; ACTB and GAPDH remained isolated.

It is accounted as one of the important participant in the immunomodulatory process of human body [22]; therefore, changes in this protein can have a great impact on the cancer pathogenesis and metastasis. The next key hub-bottleneck is glyceraldehyde-3-phosphate dehydrogenase (GAPDH) that is another housekeeping protein. It is also an important protein in the glycolysis; dysregulation of this key protein has been reported in development of many cancers [23]. In addition, a study by Forma E and Bryś M suggested that propolis extract could have regulatory effects on this protein and promotes its decrement in cancer cells [24]. It has mentioned that ethanol extract of propolis induces up-regulation of p53 and GAPDH mRNA in P19 neuronal cells [25]. Heat shock protein 90α (HSP90AA1) is the third hub-bottleneck. This chaperon plays a regulatory role in cancer [26]. Consequently, targeting this vital protein in cancer can be important in the treatment. The next high ranked chaperon protein is "heat shock protein family A (Hsp70) member 8" (HSPA8) which has regulatory part in biological features of cellular actions [27]. It is also reported as a potential high expressed biomarker of carcinoma [28]. Similarly, other studies presented that the polymorphism of HSPA8 gene is associated with breast cancer [29]. On the other hand, some compounds of plant extract from polyprenylated acylphloroglucinols group (Gt-K and Ob-C) are demonstrated to have potential binding to HSPA8. However, Gt-E and Ob-A that were found in the Brazilian red propolis were not addressed as potential targets of HSPA8 but as a candidate anticancer chemical agents [27].

Heat shock protein 90 alpha family class B member 1 (HSP90AB1) is the fourth ranked node which is highly expressed in cancers such as lung adenocarcinoma [30]. It is also known as the natural product targets including 9s-hydroxy-octadecadienoic acid (9S-HOD). This cytotoxic compound regulates expression of HSP90AB1 [31]. The last hub-bottleneck to be discussed is HSPA5 that is with the lowest centrality value of

the PPI network while being a part of stated DEPs of the original study. This protein is down-regulated in the presence of propolis. Previous studies showed that this protein is highly expressed in the most known tumors [32]. Like HSP90AB1, it is one of the most studied proteins for natural compounds targeting [14]; therefore, this protein has remarkable value for drug targeting in cancer treatment of propolis.

To better understand the biological properties of the recommended hub-bottlenecks, the functional features of top ranked proteins of the PPI was assessed by ClueGO + CluePedia. Protein folding chaperone, substantia nigra development, and positive regulation of type-1 interferon processes are responsible for cocaine production. These biological processes could be a part of mechanism of action of propolis in cancer cells. Additional analysis in terms of action type of edges of hub-bottlenecks, provided more knowledge of these essential elements. It can be inferred that there are statistically significant interactions occurring between the heat shock proteins.

Overall, the central candidates of PPI network could play important roles in the anticancer activity of red propolis. Especially neighbor hub-bottlenecks (ACTB, GAPDH, HSP90AA1, HSPA8, and HSP90AB1) should be studied further to identify their feasible binding affinity to propolis. A limited numbers of queried proteins appeared as central protein, other central proteins (as the first neighbor proteins) are also crucial to be studied since they have key roles in cancer initiations and progressions. Red propolis could be applicable in prevention and treatment of malignancies; yet, for this claim, clinical confirmation studies are required.

## Conclusion

The molecular mechanism whereby propolis provides anticancer properties could be related to the possible regulatory effects on central proteins (ACTB, GAPDH, HSP90AA1, HSPA8, HSP90AB1, and HSPA5) and their corresponding biological processes. Nevertheless, this claim requires more investigations and complementary studies.

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## Author contributions

Mona Zamanian Azodi, Mostafa Rezaei Tavirani, Zahra Razzaghi, Babak Arjmand, Reza Vafaee and Somayeh Esmaeili were involved in project design, data collection and analysis and 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

PPI: protein-protein interaction; DEPs: differentially expressed proteins; K: degree; BC: betweenness centrality; ACTB: actin beta; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; HSP90AA1: heat shock protein 90α; HSP70: heat shock protein family A; HSPA8: heat shock protein family A member 8; HSP90AB1: heat shock protein 90 alpha family class B member 1; 9S-HOD: 9s-hydroxy-octadecadienoic acid