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Pomegranate (*Punica granatum*): a natural source for the development of therapeutic compositions of food supplements with anticancer activities based on electron acceptor molecular characteristics

Veljko Veljkovic[1,2], Sanja Glisic[2], Vladimir Perovic[2], Nevena Veljkovic[2], Garth L Nicolson[3]

[1]Biomed Protection, Galveston, TX, USA; [2]Center for Multidisciplinary Research, University of Belgrade, Institute of Nuclear Sciences VINCA, P.O. Box 522, 11001 Belgrade, Serbia; [3]Department of Molecular Pathology, The Institute for Molecular Medicine, Huntington Beach, CA 92647 USA

Corresponding author: Garth L Nicolson, PhD, MD (H), Department of Molecular Pathology, The Institute for Molecular Medicine, Huntington Beach, CA 92647 USA

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ABSTRACT

Background: Numerous in vitro and in vivo studies, in addition to clinical data, demonstrate that pomegranate juice can prevent or slow-down the progression of some types of cancers. Despite the well-documented effect of pomegranate ingredients on neoplastic changes, the molecular mechanism(s) underlying this phenomenon remains elusive.

Methods: For the study of pomegranate ingredients the electron-ion interaction potential (EIIP) and the average quasi valence number (AQVN) were used. These molecular descriptors can be used to describe the long-range intermolecular interactions in biological systems and can identify substances with strong electron-acceptor properties. In this study, candidate human proteins interacting with pomegranate flavonoids have been analyzed by the informational spectrum method (ISM). This represents a virtual spectroscopy method for studying protein molecular interactions.

Results: Our analysis indicates that the anti-cancer properties of pomegranate juice can be ascribed to the strong electron-acceptor properties of its chemical ingredients. This analysis also

suggests that pomegranate flavonoids inhibit the "NF-kappaB" (NF-κB) pathway, which plays a critical role in the pathogenesis of cancer.

Conclusion: The results offer a possible explanation for an important molecular mechanism underlying the anticancer activity of pomegranate ingredients, which could also serve as a basis for the development of new therapeutic compositions of food supplements with pomegranate-like anticancer properties.

Key words: cancer, pomegranate, flavonoids, food supplement, informational spectrum method

BACKGROUND

With more than 10 million new cases world-wide reported each year, cancer is one of the most devastating human diseases [1]. Despite progress in the understanding and treatment of these diseases including significant improvements in diagnostics and therapy based on applications of modern technology, the incidence and cure rates of various cancers, even in highly developed countries, have not improved significantly [2]. For this reason, increased attention has turned to complementary therapies, including natural dietary supplements. Among these natural agents, pomegranate (Punica granatum) juice has drawn a great deal of attention from both the scientific community and the general public due to its demonstrated ability to suppress cancers [3-13]. For example, pomegranate has been found to possess well-documented cancer chemopreventive and chemotherapeutic effects against prostate cancer [10-13]. The results of a Phase II clinical study of patients with rising prostate-specific antigen (PSA) levels following surgery or radiation for prostate cancer demonstrated that pomegranate juice produced statistically significant prolongation of PSA doubling times, suggesting that pomegranate consumption may retard prostate cancer progression [14].

Despite numerous published results of in silico, in vitro and in vivo studies of pomegranate, several questions remain, including which components of this dietary agent are responsible for these anti-cancer activities and by what mechanism do they actually suppress cancers. Analysis of the electronic properties of 101 pomegranate ingredients revealed that nearly 50% of these compounds represent potentially carcinostatic substances with strong electron-acceptor properties. Bioinformatics analysis of human proteins representing candidate targets for flavonoid ingredients of pomegranate was carried out in the present research to identify possible mechanisms through which flavonoids might inhibit the "NF-kappaB" (NF-κB) pathway. The results may aid in the development of new therapeutic compositions of food supplements with pomegranate-like anticancer activities.

METHODS

Databases

For analysis of the distribution of organic molecules according to their electronic properties, the PubChem compound database (PubChem) [15] and the NCI Natural Products Repository Extracts database (NPRE) [16] were used. For bioinformatics analysis of human proteins, the

Universal Protein Resource database (UniProt) was utilized. This database represents the world's most comprehensive catalog of information on proteins, which was created by joining the information contained in Swiss-Prot, TrEMBL, and PIR databases [17].

Electron-ion interaction potential (EIIP) and average quasivalence number (AQVN) concepts

The intermolecular interactions in biological systems encompass two basic steps: (i) specific long-distance targeting of interacting molecules and (ii) chemical bond formation between interacting molecules. The first step is determined by selective long-range forces that are efficient at a distance longer than one linear dimension of the interacting macromolecules (10^2 – 10^3 Å) [18-20]. These forces directly influence the number of productive collisions between interacting molecules. Before chemical bond formation can take place, reacting molecular regions must be positioned close enough (at a distance of ≈ 2Å) and the appropriate atoms must be held in the correct orientation for a successful reaction to follow. Additionally, the attractive forces involved in the recognition and binding of molecules must include all of the weak non-covalent forces (van der Waals, hydrogen bonding, ionic interactions, etc.). For this reason, stereochemical complementarity between interacting molecules is essential for the second step.

It has been proposed that the number of valence electrons and the EIIP representing the main energy term of valence electrons are essential physical parameters that determine the long-range properties of biological molecules [21]. We previously found [21, 22] that EIIP can be determined for organic molecules by the following simple equation derived from the "general model pseudopotential" [23-25],

$$W = 0.25\frac{Z^* \sin(1.04\pi Z^*)}{2\pi} \quad (1)$$

where Z* is the average quasivalence number (AQVN) determined by

$$Z^* = \frac{1}{N}\sum_{i=1}^{m} n_i Z_i \quad (2)$$

where Z_i is the valence number of the *i*-th atomic component, n_i is the number of atoms of the *i*-th component, *m* is the number of atomic components in the molecule, and N is the total number of atoms. The EIIP values calculated according to equations (1) and (2) are in Rydbergs (Ry). A strong connection has been demonstrated between EIIP and AQVN of organic molecules and their biological activity (mutagenicity, carcinogenicity, toxicity, antibiotic, cytostatic, and antiviral activity) [21, 22, 27-29].

Informational spectrum method (ISM)

The informational spectrum method (ISM) is based on a model of the primary structure of a protein using a sequence of numbers, by assigning to each amino acid a defined parameter describing a physico-chemical property involved in the biological activity of the protein [30]. These values correspond to the EIIP [23-25], and this value determines the electronic properties of amino acids that are responsible for their intermolecular interactions [21]. The values of the EIIP for amino acids are given in Table 1.

Table 1. The electron-ion interaction potential (EIIP) used to encode amino acids.

Amino acid	EIIP [Ry]
Leu	0.0000
Ile	0.0000
Asn	0.0036
Gly	0.0050
Glu	0.0057
Val	0.0058
Pro	0.0198
His	0.0242
Lys	0.0371
Ala	0.0373
Tyr	0.0516
Trp	0.0548
Gln	0.0761
Met	0.0823
Ser	0.0829
Cys	0.0829
Thr	0.0941
Phe	0.0946
Arg	0.0959
Asp	0.1263

The calculated numerical sequence, representing the primary structure of a protein, is then subjected to a discrete Fourier transformation, which is defined as follows:

$$X(n) = \sum_{m=1}^{N} x(m) e^{-i2\pi m(m-1)/N}, \quad n = 1,2,\ldots,N/2 \tag{3}$$

where x(m) is the m-th member of a given numerical series, N is the total number of points in this series, and X(n) are discrete Fourier transformation coefficients. These coefficients describe the amplitude, phase, and frequency of sinusoids that comprised the original signal. The absolute value of complex discrete Fourier transformation defines the amplitude spectrum and the phase spectrum. The complete information about the original sequence is contained in both spectral functions. However, in the case of protein analysis, relevant information is presented in an energy density spectrum [30-32], which is defined as follows:

$$S(n) = X(n)X^{*}(n) = |X(n)|^2, \quad n = 1,2,\ldots,N/2 \tag{4}$$

In this way, sequences are analyzed as discrete signals. It is assumed that their points are equidistant, with the distance d = 1. The maximal frequency in a spectrum defined in this way is

F = 1/2d = 0.5. The frequency range is independent of the total number of points in the sequence. The total number of points in a sequence influences only resolution of the spectrum. The resolution of the N-point sequence is 1/n. The n-th point in the spectral function corresponds to a frequency f(n) = nf = n/N. Thus, the initial information defined by the sequence of amino acids can now be presented in the form of the informational spectrum (IS), representing the series of frequencies and their amplitudes.

The IS frequencies correspond to the distribution of structural motifs with defined physico-chemical characteristics and are responsible for the biological functions of a protein. When comparing proteins that share the same biological or biochemical function, the ISM technique allows detection of code/frequency pairs that are specific for their common biological properties or which correlate with their specific interactions. This common informational characteristic of sequences can be determined by cross-spectrum or consensus informational spectrum (CIS) analysis. The CIS of N spectra is obtained by the following equation:

$$C(j) = \prod_{i=1}^{M} S(i, j), \quad j = 1,2,..., N/2 \qquad (5)$$

where S(i,j) is the j-th element of the i-th power spectrum and C(j) is the j-th element of CIS. Thus, the CIS is the Fourier transform of the correlation function for the spectrum. As a result of this method, any spectral component (frequency) not present in all compared ISs is eliminated. Peak frequencies in CIS are common frequency components for the analyzed sequences. A measure of similarity for each peak is a signal-to-noise ratio (S/N), which represents a ratio between signal intensity at one particular frequency and the main value of the whole spectrum. If one calculates a CIS for a group of proteins that have different primary structures and finds strictly defined peak frequencies, it means that the analyzed proteins participate in mutual interactions or have a common biological function.

The ISM was successfully applied in structure-function analyses of protein sequences, as well as in *de novo* design of biologically active peptides [33-58].

RESULTS AND DISCUSSION

Several laboratory and clinical studies have demonstrated the anti-inflammatory, anti-atherosclerotic, anti-cancer, and anti-viral activities of pomegranate juice [3-13, 59-66]. However, these studies still leave the question of whether these extraordinary health effects of pomegranate are the consequence of some unique properties of the pomegranate ingredients open to discussion. In order to answer this question, we compared the electronic properties (represented by AQVN and EIIP) of 101 chemical ingredients of pomegranate with the molecular descriptors calculated for 45010644 randomly selected compounds from the PubChem database (Table 2) [15]. The results of this analysis (Fig. 1) revealed that 37.7% of pomegranate ingredients have AQVN and EIIP values in the intervals 3.2-3.6 and 0.110-0.135 Ry, respectively. In comparison, only 2,207,359 (4.9%) of analyzed compounds from PubChem have AQVN and EIIP values in this interval range.

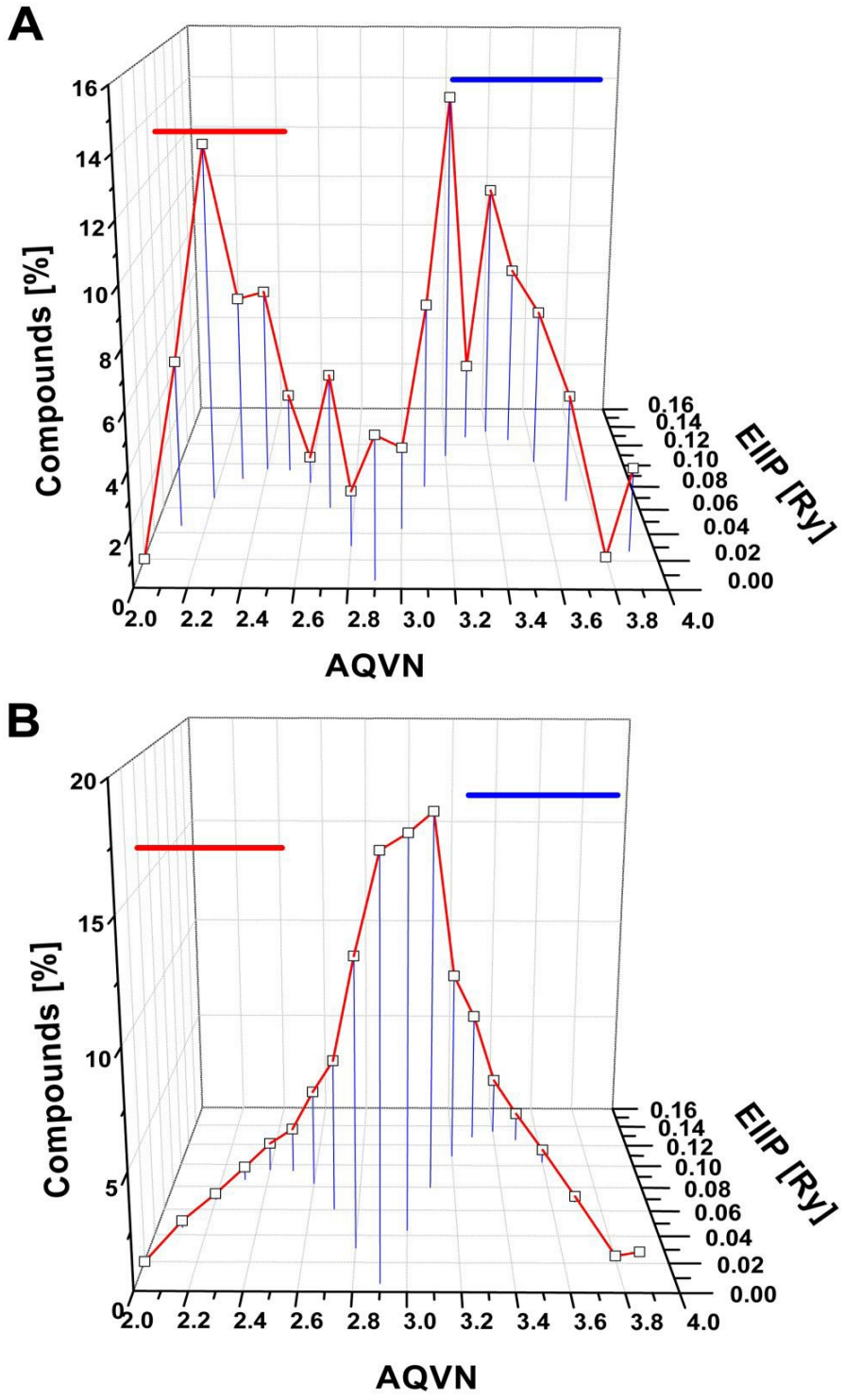

Figure 1. Distribution of chemical compounds in the EIIP/AQVN space: (a) the pomegranate ingredients (Table 2); (b) the compounds from the PubChem database. Domains of the EIIP/AQVN space encompassing electron donors and electron acceptors are marked with red and blue lines, respectively.

Table 2. The EIIP and AQVN values of the pomegranate ingredients [3]

Number	Compound name	AQVN	EIIP [Ry]	Plant part *
1	Glucose	3.000	0.0439	J
2	Fructose	3.000	0.0439	J
3	Sucrose	3.022	0.0523	J
4	Citric acid	3.524	0.1218	J
5	Malic acid	3.467	0.1304	J
6	Tartaric acid	3.625	0.0954	J
7	Fumaric acid	3.667	0.0807	J
8	Succinic acid	3.286	0.1263	J
9	Ascorbic acid	3.400	0.1344	J
10	Gallic acid	3.556	0.1150	J,P,F
11	Ellagic acid	3.929	0.0416	J,P,S
12	3,3'-Di-*O*-methylellagic acid	3.588	0.1066	S
13	3,3',4'-Tri-*O*methylellagic acid	3.460	0.1312	S
14	Caffeic acid	3.238	0.1179	J,P
15	Chlorogenic acid	3.163	0.0993	J
16	*p*-Coumaric acid	3.100	0.0798	P
17	Quinic acid	3.040	0.0588	J,P
18	Brevifolin carboxylic acid 10-monopotassium sulphate	3.938	0.0461	L
19	Flavan-3-ol	2.774	0.0390	J,P
20	Catechin	3.143	0.0934	J,P
21	Epicatechin	3.143	0.0934	J,P
22	Epigallocatechin 3-gallate	3.222	0.1144	J,P
23	Quercetin	3.500	0.1260	J,P
24	Kaempferol	3.419	0.1339	P
25	Rutin	3.206	0.1105	J,P
26	Kaempferol 3-*O*-glycoside	3.269	0.1237	P
27	Kaempferol 3-*O*-rhamnoglycoside	2.975	0.0344	P
28	Luteolin	3.419	0.1339	P
29	Apigenin	3.333	0.1319	L
30	Luteolin 7-*O*-glycoside	3.269	0.1237	P
31	Apigenin 4'-*O*-β-glucopyranoside	3.216	0.1130	L
32	Luteolin 4'-*O*-β-glucopyranoside	3.216	0.1130	L
33	Luteolin 3'-*O*-β-glucopyranoside	3.216	0.1130	L
34	Luteolin 3'-*O*-β- xylopyranoside	3.292	0.1272	L
35	Naringin	3.068	0.0690	P
36	Delphinidin	3.423	0.1337	P
37	Cyanidin	3.334	0.1327	P
38	Pelargonidin	3.258	0.1218	P
39	Cyanidin 3-*O*-glucoside	3.226	0.1154	J
40	Cyanidin 3,5-di-*O*-glucoside	3.176	0.1028	J
41	Delphinidin 3-*O*-glucoside	3.278	0.1251	J
42	Delphinidin 3,5-di-*O*-glucoside	3.213	0.1124	J
43	Pelargonidin 3-*O*-glucoside	3.173	0.10.21	J
44	Pelargonidin 3,5-di-*O*-glucoside	3.137	0.0916	J
45	Punicalin	3.718	0.0602	P,L,B,R
46	Punicalagin	3.774	0.0353	P,L,B,R
47	Corilagin	3.552	0.1158	P,L

Number	Compound name	AQVN	EIIP [Ry]	Plant part *
48	Casuarinin	3.663	0.0820	P
49	Gallagyldilacton	4.037	0.0938	P
50	Pedunculagin	3.650	0.0868	P
51	Tellimagrandin	3.585	0.1074	P
52	Granatin A	3.679	0.0760	P
53	Granatin B	3.688	0.0727	P
54	Punicafolin	3.486	0.1281	L
55	1,2,3-Tri-*O*-galloyl-β- ^{4}C1-glucose	3.478	0.1291	L
56	Punicacortein A	3.552	0.1158	B,R
57	Punicacortein B	3.552	0.1158	B,R
58	Punicacortein C	3.748	0.0472	B,R
59	Punicacortein D	3.798	0.0236	B,R
60	Punigluconin 2,3-di-*O*-galloyl-4,6-(*S*)- hexahydroxydiphenoylgluconic acid	3.411	0.1342	B,R
61	Proline	2.706	0.0594	J
62	Valine	2.706	0.0594	J
63	Methionine	2.700	0.0609	J
64	Tryptamine	2.583	0.0856	J
65	Serotonin	2.720	0.0554	J
66	Melatonin	2.727	0.0534	J
67	Peelletierine	2.320	0.0889	P,B,R
68	*N*-Methylpelletierene	2.286	0.0843	B,R
69	Pseudopelletierene	2.385	0.0947	B,R
70	Norpseudopelletierene	2.435	0.0964	R
71	Sedridine	2.222	0.0733	R
72	2-(2'-Hydroxypropyl)Δ^{1}- piperideine	2.269	0.0817	R
73	2-(2'-Propenyll)Δ^{1}- piperideine	2.273	0.0823	R
74	*N*-(2',5'-Dihydroxyphenyl) pyridium chloride	2.269	0.0817	L
75	Hygrine	2.273	0.0823	R
76	Norhygrine	2.364	0.0932	R
77	Punicic acid (*cis*-9, *trans*-11, *cis*-13 octadecatrienoic acid)	2.280	0.0834	S
78	Linoleic acid	2.231	0.0749	S
79	Oleic acid	2.208	0.0704	S
80	Palmitic acid	2.160	0.0601	S
81	Stearic acid	2.143	0.0561	S
82	Daucosterol	2.283	0.0838	S
83	Camesterol	2.156	0.0591	S
84	Stigmasterol	2.173	0.0630	S
85	β-Sitosterol	2.150	0.0578	S
86	Cholesterol	2.194	0.0677	S
87	17-α-Estradiol	2.455	0.0963	S
88	Estrone	2.524	0.0928	S
89	Testosterone	2.367	0.0935	S
90	Estriol	2.533	0.0919	S
91	γ-Tocopherol	2.205	0.0699	S
92	Ursolic acid	2.296	0.0858	S,F
93	Oleanolic acid	2.308	0.0873	F
94	Maslinic acid	2.354	0.0925	F

Number	Compound name	AQVN	EIIP [Ry]	Plant part *
95	Asiatic acid	2.385	0.0948	F
96	Cerebroside	2.280	0.0834	S
97	Coumestrol	3.500	0.1260	S
98	Coniferyl 9-*O*-[β-D-piofuranosyl (1→6)]-*O*-β-D-glucopyranoside	2.952	0.0258	S
99	Sinapyl 9-*O*-[β-D-apiofuranosyl (1→6)]-*O*-β-D-glucopyranoside	2.955	0.0269	S
100	Phenethyl rutinoside	2.833	0.0188	S
101	Icariside D1	2.877	0.0028	S

* - J: juice, P: leaf, F: flower, L: leaf, S: seed, B: bark of tree, R: bark of tree root

According to the electronic theory of cancer proposed by Albert Szent-Györgyi, cancer is connected with the defective desaturation of the valence band of proteins, and can be inhibited by electron acceptors [67, 68]. Previously, correlations have been reported between EIIP and AQVN of organic molecules and their carcinogenicities [21]. These correlations have been explained by the fact that the electron acceptors are characterized with high EIIP and AQVN values, contrary to the electron donors that have low values for these molecular descriptors [21, 68]. With this perspective, the anti-cancer properties of pomegranate juice could be ascribed to an unusually high content of carcinostaric compounds with high EIIP and AQVN values.

Flavonoids, ellagitannins, anthocyanins, and aliphatic organic acids represent the dominant fraction of pomegranate ingredients with AQVN > 3.2 and EIIP > 0.11Ry. However, the possible molecular mechanisms of action of these compounds must be elucidated before any potential health effects of pomegranate can be reliably predicted. In this study, we performed the ISM analysis on some human proteins representing potential targets of flavonoids. According to the ISM concept, the long-distance recognition and targeting of ligand to a protein is determined by specific information that is encoded in the primary protein structure by the EIIP of their amino acids. This information is represented by the frequencies and corresponding amplitudes in the informational spectrum (IS). Proteins that interact with the same ligand have a common frequency component in their IS.

To identify a characteristic IS frequency representing the information corresponding to specific interactions between flavonoids and proteins, we performed cross-spectral analyses of four quercetin–binding proteins. In Fig. 2a the consensus information spectrum of three flavonol 3-O-methyltransferases 1 from *Chrysosplenium americanum* (OMT1_CHRAE), *Oryza sativa* (OMT1_ORYSA), *Arabidopsis thaliana* (OMT1_ARATH), and flavonol 3-O-methyltransferase 2 from *Chrysosplenium americanum* (OMT2_CHRAE) are presented.

The dominant peak in the presented CIS indicates that the primary structures of the analyzed flavonol 3-O-methyltransferases encode the same information responsible for their interactions with flavonoids, and this can be represented by their IS frequency F(0.289). To demonstrate this, we multiplied the CIS values of flavonoid 3-O-methyltransferases with IS of flavonol 2,3-dioxygenase from *Bacillus subtilis* (QDOI_BACSU). The peak in the frequency F(0.289) in the resulting cross-spectrum (Fig. 2b) strongly indicates that flavonol 2,3-dioxygenase shares common information with flavonol 3-O-methyltransferases. In contrast, multiplying the CIS of flavonoid 3-O-methyltransferases with IS of human tryptophan 2,3-

dioxygenase (BC005355), which does not bind to flavonoids, completely diminished the peak frequency F(0.289), indicating that this human protein does not share common information with these four flavonoid-binding proteins (Fig. 2c). Consequently, the result of the ISM analysis presented in Fig. 2 suggests that the primary structures of proteins interacting with flavonoids encode specific information that corresponds to IS frequency F(0.289).

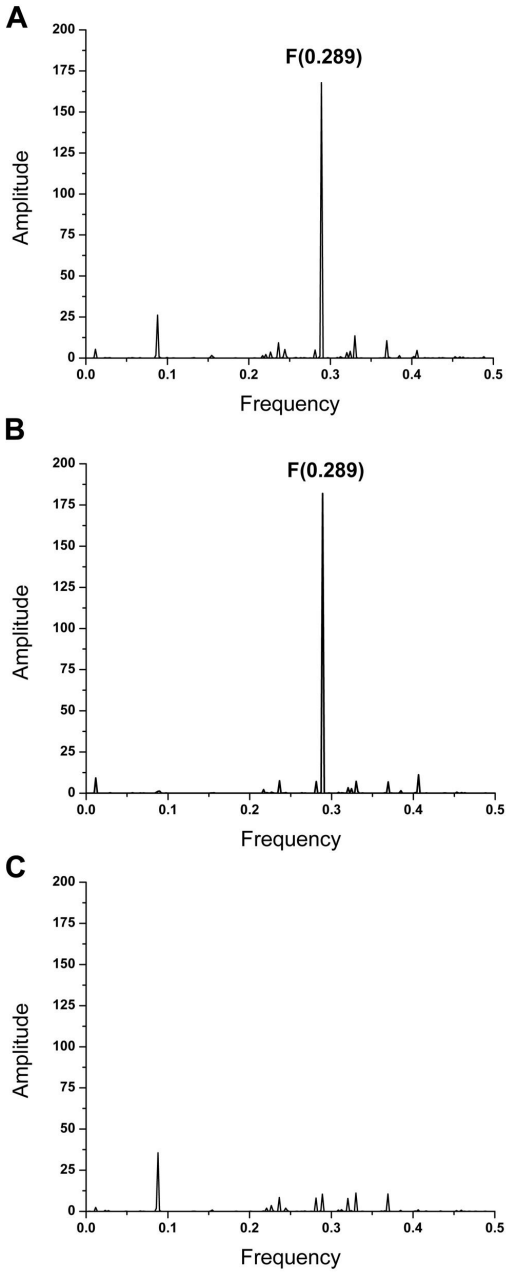

Figure 2. The ISM analysis of flavonoid-binding proteins. (a) CIS of flavonol 3-O-methyltransferases 1. (b) cross-spectrum between CIS of flavonol 3-O-methyltransferases 1 and IS of flavonol 2,3-dioxygenase. (c) cross-spectrum between CIS of flavonol 3-O-methyltransferases 1 and IS tryptophan 2,3-dioxygenase. For each spectrum the abscissa represents the frequencies from the Fourier transform of the sequence of electron-ion interaction potential corresponding to the amino-acid sequence of the protein. The lowest frequency is 0.0 and the highest is 0.5. The ordinate represents amplitudes, in arbitrary units, corresponding to each frequency component in the spectrum.

To identify human proteins that represent the most probable candidates for interactors with flavonoids, we performed ISM analysis of human proteins from the UniProt database. In Table 3 we present 15 human proteins whose IS have a dominant peak on the frequency F(0.289) with the highest amplitude value. Using literature data mined from the PubMed database (www.ncbi.nlm.nih.gov/pubmed), our research revealed that flavonoids are candidates for inhibiting the NF-kappaB (NF-kB) pathway. This finding suggests that among the human proteins that are shown in Table 3, NF-kB represents one of the more likely targets for flavonoids.

Table 3. Human proteins selected as candidate targets of flavonoids.

Protein	ID
Integrator complex subunit 2 (Int2)	INT2_HUMAN
MORC family CW-type zinc finger protein 3	MORC3_HUMAN
Angiogenic factor with G patch and FHA domains 1	AGGF1_HUMAN
Putative RNA methyltransferase NCOA6IP	NC6IP_HUMAN
PR domain zinc finger protein 1	PRDM1_HUMAN
Lethal(3)malignant brain tumor-like protein	LMBTL_HUMAN
Uridine 5'-monophosphate synthase	PYR5_HUMAN
Spastin	PAST_HUMAN
RING finger protein 103 (Zinc finger protein 103 homolog)	RN103_HUMAN
Mucin and cadherin-like protein	MUCDL_HUMAN
Nuclear factor NF-kappa-B p65 subunit	TF65_HUMAN
Zinc finger protein 683	ZN683_HUMAN
Phenylalanyl-tRNA synthetase	SYFA_HUMAN
Oligopeptide transporter, kidney isoform (Peptide transporter 2)	S15A2_HUMAN
Serine/threonine-protein kinase PCTAIRE-2	PCTK2_HUMA

However, the fact that flavonoids are not the natural ligand of NF-kB leaves the question of what the functional role of the dominant frequency information encoded in the primary structure of this protein is still open to discussion. The activity of NF-kB is primarily regulated by its interaction with inhibitory IkB proteins. Therefore, a key step for controlling NF-kB activity is the regulation of the IkB/NF-kB interactions. For this reason, we assumed that the information corresponding to IS frequency F(0.289) is responsible or at least characteristic for the interaction of NF-kB with its principal ligand IkB. To prove this assumption, we performed a cross-spectral analysis of these proteins. As can be seen from results presented in Fig. 3b, the dominant peak in the IkB/NF-kB cross-spectrum is at the frequency F(0.289).

This strongly suggests that the primary structures of IkB and NF-kB encode the same information which is responsible or at least characteristic for the interactions between these proteins. Because the same information is also responsible for or characteristic of protein/flavonoid interactions, both of these human proteins are potential targets for flavonoids. Although inhibition of the NF-kB pathway by flavonoids is well documented [69-73], the molecular target(s) of these compounds had not been established. Newly-synthesized IkB-alpha can enter the nucleus, remove NF-kB from DNA, and thereby result in the export of the complex back to the cytoplasm. In the classical or canonical pathway, the phosphorylation by IkappaB kinase (IKK) of two specific serines near the N-terminus of IkB-alpha targets this protein for

ubiquitination and degradation by the 26S proteasome. It has been demonstrated that flavonoids inhibit the phosphorylation of IkB by IKK [74-76].

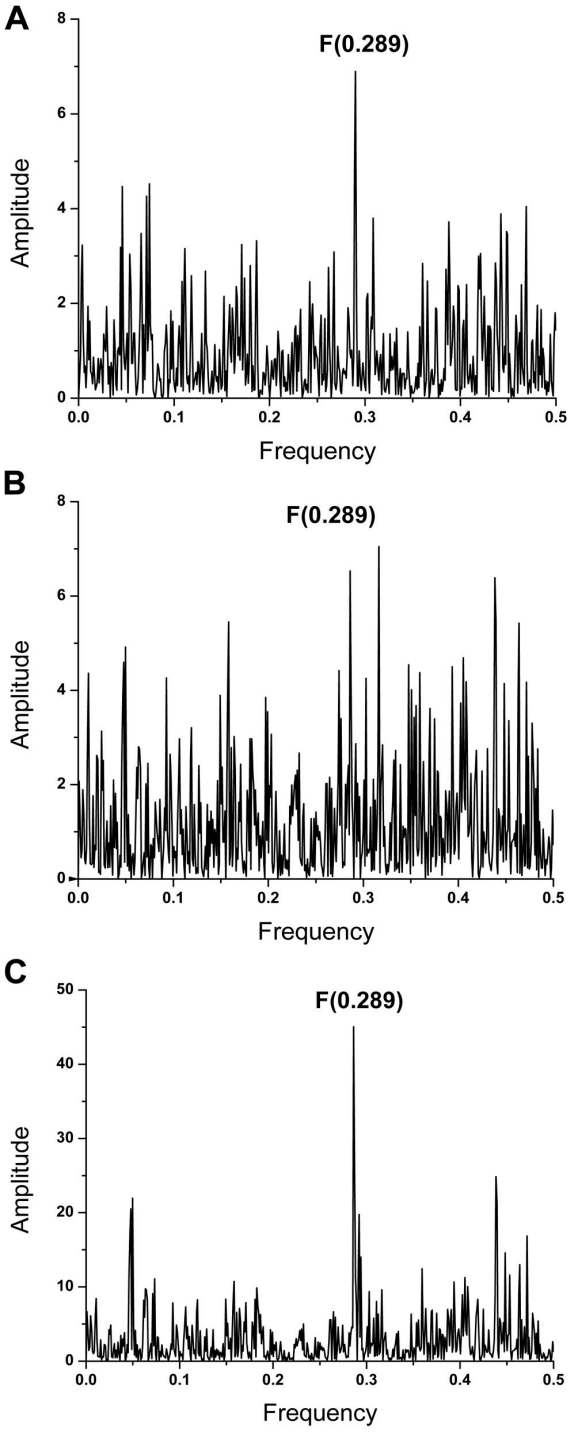

Figure 3. The ISM analysis of NF-kB and IkB-alpha proteins. (a) IS of NF-kB; (b) crosspectrum of NF-kB and IkB-alpha

We hypothesized that this inhibition could be the consequence of the binding of flavonoids to the phosphorylation site of IkB-alpha. A computer scanning survey of the IkB-

alpha primary structure revealed that the N-terminus of this protein, encompassing residues 16-41, is essential for information represented by the IS frequency F(0.289) (Fig. 3c). It is of interest that this domain contains S32 and S38, which are phosphorylated by IKK. Additionally, it was previously demonstrated that domains which are essential for information determining the long-distance recognition and targeting between a protein and its ligand usually are situated close to their chemical binding sites [33, 34]. This points out the possibility that flavonoids inhibit the NF-kB pathway by preventing IkB phosphorylation by IKK.

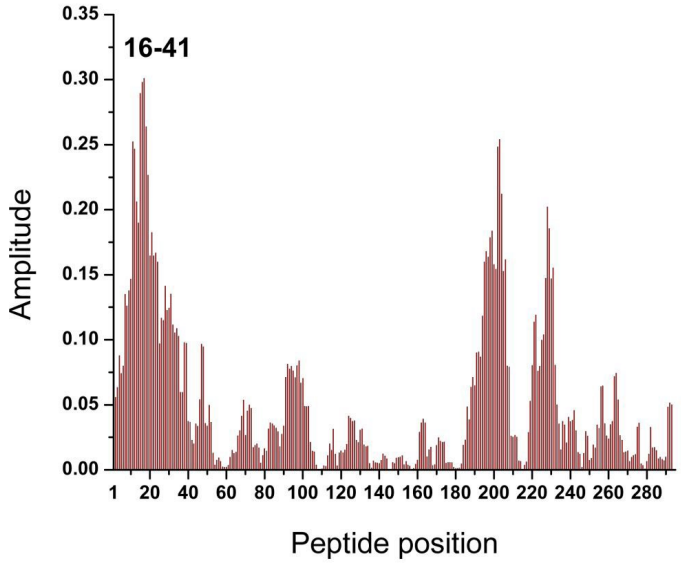

Figure 4. Mapping of the region in IkB interacting with NF-kB

The NF-kB proteins comprise a family of structurally related eukaryotic transcription factors that are involved in the control of a large number of normal cellular processes, such as immune and inflammatory responses, developmental processes, cellular growth, and apoptosis. Additionally, these transcription factors are persistently active in a number of disease states, including cancers, rheumatoid arthritis, chronic inflammation, asthma, neurodegenerative diseases, and heart disease (for a review see Ref. 77). For these reasons, modulation of the NF-kB pathway through inhibition of IkB phosphorylation by flavonoids represents an important potential mode of action of pomegranate ingredients. Inhibition of IkB/IKK interaction by flavonoids provides a rationale framework for designing potent inhibitors of the IkB phosphorylation site, which is an attractive drug target for inflammatory diseases and cancer.

CONCLUSIONS

The analysis presented here predicts that flavonoids inhibit the NF-kB pathway by prevention of IkB-alpha phosphorylation. This provides a rationale framework for designing potent new therapeutic compositions of food supplements that target the IkB-alpha phosphorylation site, which is an attractive drug target for inflammatory diseases and cancer. Our results also point out the strong electron-acceptor properties of pomegranate ingredients as an important factor for the anti-cancer activities of pomegranate. We have recently used this type of analysis of electron donor-acceptor characteristics to select potentially active antibiotics for use against multi-drug-

resistant bacteria [78]. As a result, there appears to be other potential uses of this type of analysis for the development of various new therapeutics.

List of Abbreviations: EIIP, electron-ion interaction potential; AQVN, average quasi valence number; ISM, informational spectrum method; IS, informational spectrum; CS, cross-spectrum; CIS, consensus informational spectrum.

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