Saudi Journal of Biological Sciences 26 (2019) 660–664

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Screening & analysis of anionic peptides from *Foeniculum vulgare Mill* by mass spectroscopy

Abdullah A. Alyousef [a], Ayesha Mateen [a], Raid Al-Akeel [a], Abdulaziz Alqasim [a], Yazeed Al-Sheikh [a], Mohammed S. Alqahtani [b], Rabbani Syed [a,b,*]

[a] Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Saud University, Riyadh 11451, Saudi Arabia
[b] Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

ARTICLE INFO

Article history:
Received 28 October 2018
Revised 29 November 2018
Accepted 3 December 2018
Available online 7 December 2018

Keywords:
Foeniculum vulgare
Antimicrobial proteins
2-D gel separation and MALDI TOF/TOF
Identification

ABSTRACT

Fennel (*Foeniculum vulgare* Mill.) member from the family *Umbelliferae* (*Apiaceae*) and has been used in Saudi Arabia as an medicine as of the from the tradition. Our previous work with seed extracts of this plant generated DEAE-ion exchange purified proteins that exhibited antibacterial properties. The current study moves this work forward by using 2-D gel separation and MALDI TOF/TOF to identify proteins in this active extract. Fourteen protein spots were excised, digested, and identified. Several putative functions were identified, including: a copper-trans locating ATPase PAA1 chloroplastic-like isoform X1; a cytosolic enolase; a putative pentatricopeptide repeat-containing protein; an NADP-requiring isocitrate dehydrogenase; two proteins annotated as being encoded downstream from Son-like proteins; three probable nuclear proteins 5–1; and four predicted/ unidentified proteins. Future efforts will further characterize their relevant antimicrobial properties with the aim of cloning and high throughput synthesis of the antimicrobial element(s).

1. Introduction

In nature, plants must deal with a range of pathogens and have evolved various defenses like tannins, polyphenolic and phytoalexins compounds (Stintzi et al., 1993). Peptides known to be useful in protecting multi gene families with fascinating evolutionary characteristics, and thus information about these plant proteins/peptides is essential together in applied & basic research. Plant defense systems generally depend of the origin (Marmiroli and Maestri, 2014) and have been documented to contain antimicrobial proteins (AMPs) (Leah et al., 1991; Melchers et al., 1994), defensins (Broekaert et al., 1995; Thomma et al., 2002), lipid transfer proteins (Cammue et al., 1995; Cheng et al., 2004), thionins (Florack and Stiekema, 1994), 2S albumins (Terras et al., 1992; Agizzio et al., 2003) and ribosome-inactivating proteins (Barbieri et al., 1993; Dong et al., 1994; Park et al., 2004).

The current trend and specific interest in AMPs is due to their application in medicine, with the goal being to develop AMPs as novel therapeutic agents as alternatives to common antibiotics. Functionally, AMPs contain β-sheets or α-helixes can create pores in bacterial membrane leading to dysfunction of the membrane (Seo et al., 2012). In our current study we have selected therapeutic plant known as *Foeniculum vulgare* Mill., known to be used in traditional medicine, also having various protective properties like anti-cancer, anti-inflammatory etc. (Badgujar et al., 2014). Mohsenzadeh (2007), in this study against important foodborne pathogens have concluded that *F. vulgare* extracts have antimicrobial property and identification of these proteins is of vital important to analyze their function and also in characterization of these peptides. In methodological aspects, its general but unique procedures like chromatography usually liquid for separation of peptides and identification by tandem mass (Langsdorf et al., 2010). Further, conventional data from mass spectroscopy can be analyzed by bioinformatics for better understanding the identity of the mature peptides and their post translational modifications (Ohyama et al., 2008). Herein, we describe the use of 2-D gel separation and MALDI TOF/TOF identification of proteins followed by bioinformatics approach in active extracts from *F. vulgare* seeds.

* Corresponding author at: Pharmaceutics Department, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.
E-mail address: rsyed@ksu.edu.sa (R. Syed).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2018.12.004
1319-562X/

2. Methods

2.1. Protein extraction and purification

This project is the extension for our previous work. In brief, seeds of *F. vulgare* Mill, were collected and subjected to crude extraction by soaking ground seeds in sodium acetate buffer pH (6.5) and then filtered. Sample were further purified by dialysis (3 kDa cut-off). After dialysis the total protein content was estimated calorimetrically at 280 nm and further anti-microbial aanlysis was carried out on four bacterial strains *E. coli* (ATCC-25922), *P. vulgaris* (ATCC 6380) *P. aeruginosa* (ATCC-27853) and *S. aureus* (ATCC-25923) and compared with the standard antibiotic ciprofloxacin [25mcg/ml] and chloramphenicol (100 mcg/ml). The extract was further purified by DEAE anion chromatography.

2.2. 2-Dimensional electrophoresis

In brief, first dimension of separation was done by IEF strip with PH range 3–10 where the sample was dissolved in rehydration buffer before loading on this strip and second dimension of separation was performed on SDS PAGE (12% agar) after IEF run. we have selected elute 2 after ion exchange for 2D analysis and results shown that pI is 5–8 having molecular wt. of 34.3 & 48 kDa where these spots we further characterized by using tandem Mass.

2.3. Trypsin digestion

Convectional protocol is used as per the manual (Agilent technologies). In brief, MS grade trypsin used in gel digestion to achieve maximum specific proteins from the gel and the procedures goes as per our previous study (Al Akeel et al., 2017).

2.4. Mass spectrometry analysis

Maldi plates was prepared by the 2 *ul* peptides which were mixed in matrix containing α-Cyano-4-hydroxycinnamic acid (HCCA) in 1;1 proportion. After the sample was air dried on the MALDI target plate, the plate was placed in the instrument (Bruker ULTRAFLEX III). The instrument initially calibrated using some peptide standards and the calibration of the standard peptides were saved. Spectra of the sample was then acquired with mass range as 500–4500 Daltons and the spectrum was collected until suitable relative abundance of peptides was reached.

For MS-MS analysis, peptides with more relative abundance within the sample were selected and further MSMS spectrum of those peptides was obtained. After acquiring the spectra for all the samples, they were analyzed in FLEX ANALYSIS SOFTWARE (Mascot v. 2.3.0.1., Matrix Science, London, UK) to assign *m*/*z* values for all the peptides in the spectrum. The assigned masses for the sample were then analyzed by MASCOT SEARCH with the following parameters: Database-NCBI, Taxonomy-Green Plants (Viridiplantae), Enzyme- Trypsin, Missed Cleavages - 1, fixed modification-Caramidomethyl, Variable Modification-Oxidation, Mass tolerance- 100 ppm.

3. Results

We previously reported on the antimicrobial activity of anion exchange chromatography purified seed extracts from *F. vulgare* seed (Al Akeel et al., 2017). Preliminary examination of the active fractions suggested that one particularly potent elute fraction (# 2) was relatively non-complex with respect to the number of proteins involved and therefore appeared to be a good candidate for characterization. The results of 2-D gel electrophoresis verified this to be the case, where only 14 distinct proteins spots were evident having pI values ranging from 5–8 and molecular weights of 34 kDa to 48 kDa. These gel spots were further subjected to trypsinization and characterized by TOF/TOF ULTRAFLEX using Flex software. The result mass achieved in peptide mass fingerprint were later uploaded (May 9, 2015) to Mascot in NCBI database (Protein) against the Viridiplantae (Green Plants). Default search parameters used in this study were: (1) mass protein unrestricted (2) 500 ppm of mass peptide tolerance (3) 2 Da of mass fragment

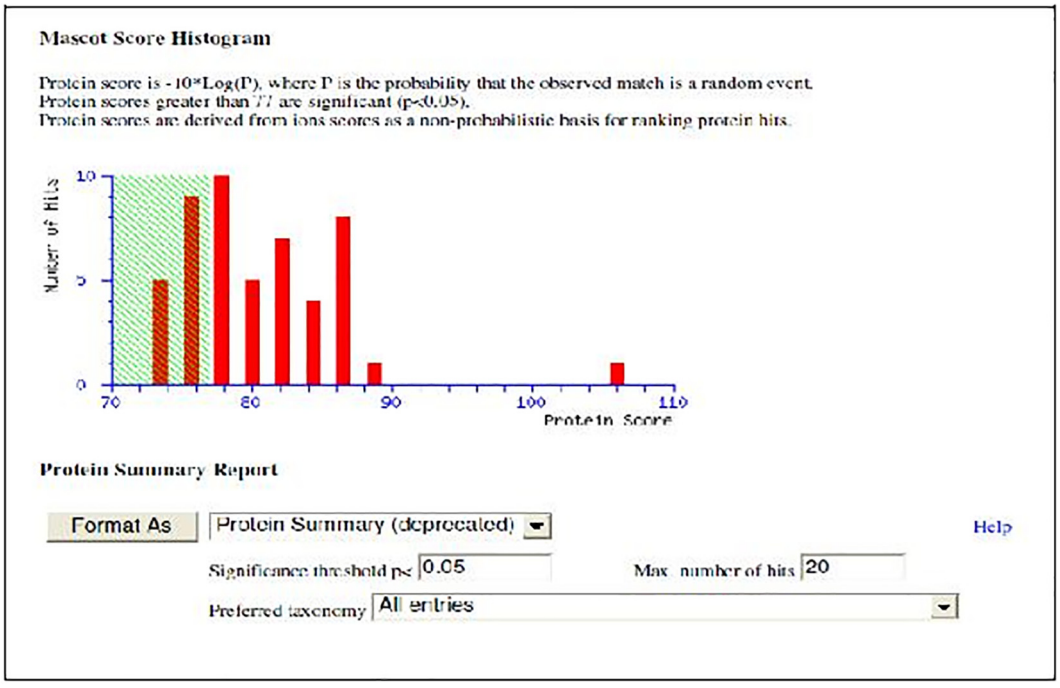

Fig. 1. Represents the histogram of spot 4, showing protein score, significant threshold and maximum number of hits.

MESVSLSRIPLIASSKPLKPSPDYRHLRLPFPLYSSSLKAQCFGSLDSRRS
LDLFSISFGGNILRSSPTAPVSSRFASISNSAAFSGSGGGGDGPPGGGGGG
GGGDYGSSGGEVVVKSVAEESEEVPVLGPDVIVLHVGGMSCGGCAASVKRI
LESQPQVSSATVSLETETAYVRPVPEVKVTQDWQQQLGEKLATHLTTCGFK
SGLKVGEIQSEG

Fig. 2. Amino acid sequence of the predicted Copper Transporting ATPase PAA1, Chloroplast-Like (Spot #2) illustrating the glycine (G) enriched regions of the protein. Total glycine complement = 13%.

Pg-AMP1

RESPS---SSRMECYEQAERY-GYGGYG-------GGRYGGGYGSGRGQPVGQGVE

Spot#2

64 RSSPTAPVSSRFASISNSAAFSGSGGGGDGPPGGGGGGGGGGDYGSSGGEVVVKSVA
120

Fig. 3. Amino acid sequence similarity between an internal region of Spot #2 (aa 64–128) and a known antimicrobial peptide, Pg-AMP1. Amino acid alignments of a partial Copper Transporting ATPase PAA1, Chloroplast-Like (Spot #2) are numbered to provide for orientation of region of interest relative to the entire Pg-AMP1. Red bold letters denote identity whereas residues underscored by * denote similarity.

tolerance; (4) Maximum missed cleavages 1; (5) cysteine modification by carbamidomethyl and methionine oxidation. The peptide match and the strength was based on masses of the peptide obtained and also in masses of fragments obtained from MS/MS scan. An example of a Mascot search result is provided in Figs. 1–3. Protein scores were based on default significance threshold value ($p < 0.05$) and maximum number of hits (20 hits); protein scores greater than 77 are significant.

Spot #4 showed the highest protein score of 106, which matches a protein annotated as a pentatricopeptide repeat-containing protein, putative (Accession number: gi|255572426) (Table 1). Spot #1 also exhibited a good protein score of 101 and was identified as a CARUB_v10005054mg (Accession number: gi|565443868). Protein spots #9, #12 and #13 all matched a probable nuclear protein 5–1, and there were likewise multiple matches to a protein annotated as a protein downstream of a Son-like protein (spots #8 and #10). Protein spots #2, #8 and #11 corresponded to a copper-transporting ATPase PAA1 chloroplastic-like isoform X1, a cytosolic enolase and NADP- isocitrate dehydrogenase, respectively (Table 1). The balance of the protein spots was identified as being predicted (#3, #7) or uncharacterized proteins (#6, #14).

4. Discussion

For centuries indigenous human populations have recognized plants for their medicinal utility (Leonti and Casu, 2013). Recently, however, the use of plants in human health and medicine has broadened and has taken on disease-specific targeting. With the incidence of antibiotic resistance among key human pathogens continuing to rise, there is now greater urgency for the discovery of new antibiotics. Consequently, the interest in, and search for, phytochemicals having such properties has increased significantly and indeed is promoted by the World Health Organization. A variety of antimicrobial metabolites from plants been isolated and identified (Mahabusarakam et al., 2008; Boonnak et al., 2009), with plant seeds now being a popular research target because of the facile nature of working with them and because of reports of antimicrobial proteins/peptides (Ma et al., 2009).

Plants are well known to have evolved defense systems to guard against infection by various bacteria, fungi and viruses. As such and given the stunning diversity of flora in the biosphere, it is arguable that plants should represent a rich source of novel antimicrobials. Relatively recently, plant proteins and peptides have become a focal point for work worldwide (Nawrot et al., 2014), including our research group. The study described herein relates to one of our current research thrusts (Al Akeel et al., 2017), which focuses on the discovery and characterization of novel, plant-derived antibiotics from the plant *F. vulgare*.

Our prior work reported on the antimicrobial activity of partially purified *F. vulgare* seed extracts. The relative low complexity of the protein mixture in an antimicrobial active elute fraction derived from an anion exchange purification strategy suggested it was a good candidate for second-generation studies aimed at identifying the specific antimicrobial agent(s). 2D gel analysis of a selected elute fraction from this study catalogued 14 peptides. Peptide identification illustrated that two spots represented a protein that is significantly similar to a downstream gene neighbor of a Son-like protein in the plant *Pyrus* × *bretschneideri* (spots #5 and #10) (Table 1), and three spots (#9, #12, #13) represent a probable nuclear protein 5–1. Consequently, it is likely that this elute fraction contained only 11distinct proteins and as such helps to focus subsequent analysis efforts. Projecting the basis for how these proteins exert antimicrobial function will require substantial follow-up efforts; however, we offer brief discussion of what is known about these proteins and how they might (or not) function in an antimicrobial context. In assessing the potential antimicrobial basis for the identified proteins, we consider two basic scenarios. First, it is known that large, intact proteins can be taken up by endocytotic type processes (Lodish et al., 2000) and thus intact uptake of these proteins into the cell cytoplasm of the test bacteria must at least be considered. A second scenario would include the

Table 1
Identification information for the protein spots extracted and digested from 2-D gel.

Spot #	NCBI Protein Number	Protein description	Protein		Peptide		Example peptide sequence	Plant species
			Mass	Score	Matches	Ion score		
1	gi|565443868	Hypothetical protein	42640	101	5	42	R.SVGVSAISGAGMDDFFK.S	*Capsello rubella*
2	gi|743801153	Copper transporting ATPase PAA1, chloroplatic-llke	22358	87	8	63	R.SLDLFSISFGGNILR.S	*Elaeis guineenis*
3	gi|326519180	Predicted protein	68861	78	6	42	R.NGSAAHVAAAAAIIGLMALASMAK.L	*Hordeum vulgare, subsp. Vulgare*
4	gi|255572426	Pentatricopeptide repeat-containing	99183	106	12	52	R.GCEPNEFTFGILVR.G	*Ricinus communis*
5	gi|694319765	Protein downstream neighbor of Son-like	68471	91	9	51	R.LSSSSDIDNTPESLLVFSGNK.N	*Pyrus x bretschneideri*
6	gi|719977357	Protein HHL1, chloroplastic	25402	78	9	29	R.LPLSNSRSHEDLLVK.H	*Nelumbo nucifera*
7	gi|168017858	Predicted protein	17739	88	8	15	K.EIHQGPQDSLVNR.V	*Physcomitrella patens*
8	gi|225434716	Cytosolic enolase	52151	85	8	50	K.GMFRASVPSGTPIGMYEAVELR.D	*Vitis vinifera*
9	gi|802757615	Probable nucleolar protein 5-l	63588	89	5	84	K.EASLISMGTEVSEVDLMNIR.E	*Jatropha curcus*
10	gi|694319765	Protein downstream neighbor of Son like	68471	87	11	49	R.LSSSSDIDNTPESLLVFSGNK.N	*Pyrus x bretschneideri*
11	gi|802542207	Isocitrate dehydrogenase	55021	77	11	22	K.SMWRSPNGTIR.N	*Jatropha curcas*
12	gi|802757615	Probable nucleolar protein 5-1	63588	93	5	86	K.EASLISMGTEVSEVDLMNIR.E	*Jatropha curcas*
13	gi|802757615	Probable nucleolar protein 5-l	63588	78	6	69	K.EASLISMGTEVSEVDLMNIR.E	*Jatropha curcas*
14	gi|658005702	Uncharacterized protein LOC104591617	109569	89	12	32	K.DPLVMSWLLNSMER.K	*Malus domestica*

protein acting extracellular, again as a complete protein, but also potentially as a subunit derived from extracellular protease activity from the test pathogen strains.

Isocitrate dehydrogenase (IDH) (Spot #11) catalyzes the oxidative decarboxylation of isocitrate to α-ketoglutarate. Since of these neither metabolites nor NADP were intentionally added to the bioassay agar, appreciable extracellular IDH activity is highly unlikely. In addition, if taken up as an intact protein, additional IDH activity would presumably not be harmful since this is a normal component of the tricarboxylic acid cycle, a central pathway in metabolism. Essentially the same kind of discussion could be presented for enolase (Spot #8), which converts 2-phosphoglycerate to phosphoenolpyruvate. Both IDH and enolase are cytosolic and thus unlikely that they are meaningfully active, but even in the event that they are; it seems difficult to argue how such activity would be toxic to any of the test microorganisms. Consistent with their known cytosolic functions, the GRAVY score for these proteins (−0.247 for each) (Table 1) is consistent with both being hydrophilic and not attracted to or disrupt membranes. Functional information for the downstream neighbor of SON-like protein in plants is not available, but just recently the human homolog (referred to as DONSON) has been described as being a replisome constituent that stabilizes the DNA fork in replication. Mutations or loss of function in this protein leads to DNA damage (Reynolds et al., 2017).

For peptides identified as "predicted" or "hypothetical", the only potential clues relating to their possible antimicrobial function derives from their amino acid sequence. The literature contains many examples of how peptide amino acid composition appears very important in antimicrobial action (Diamond et al., 2009). However, if it is assumed these proteins are in their native state/structure in the bioassays, then it becomes more difficult to suggest how specific amino acid sequences may be active. Hydrophobic regions might be expected to remain internal in the native fold protein, limiting their interaction with the bacterial membranes, although Kyte-Doolittle plots of the proteins represented by Spots #1 and #3 suggest that both of these proteins have hydrophobic runs near the amino terminus (within the first 50 amino acids) and thus extreme regions of these proteins may have a tendency to interact with membranes. If so, then it is at least possible that membrane damage will result.

Glycine is well-known that it restrict the production of a peptidoglycan module of cell wall of bacteria (Hishinuma et al., 1969) and the glycine-rich Pg-AMP1 from *Psidium guajava* is active against Grams −ve bacteria (Pelegrini et al., 2008). In larger proteins such as those identified in the current study, it is not clear if such glycine effects would be important because the 3-D structure is unknown; i.e. the native folding pattern may position key amino acids internally and thus out of contact with otherwise susceptible microbial cellular targets. Regardless, one of the proteins identified in the current study, Spot #2 (Copper Transporting ATPase PAA1, Chloroplast-Like), is of interest in this regard because of its 13% glycine content (Fig. 3). These glycine residues occur as significant runs (see aa 87–112 in Fig. 3) and we are intrigued by the high sequence similarity of the 64–128 aa region of Spot #2 relative to the antimicrobial Pg-AMP1 (31% identity, 42% similarity). BLAST analysis illustrates that significant portions of this G-rich region are highly conserved among several species (e.g., *Elaeis guineensis*, *Phoenix dactylifera*, *Musa acuminata* subsp. *Malaccensis*, *Nelumbo nucifera*) and thus the glycine rich content is not random. Glycine content of the other proteins ranged from 3.9−10.3%, and did not contain any glycine clusters or runs (results not shown).

5. Conclusion

In conclusion of this study, *F. vulgare* has pharmacological and medical value which in future can pay key role in drug discovery. In this study, protein extracts from *F. vulgare* seeds were observed to exhibit growth inhibiting activity against many microorganisms mainly broad spectrum. In one potent antimicrobial partially purified fraction, a total of 14 proteins were separated by 2D gel electrophoresis and further subjected to MS analysis for identification. One particular protein identified as a "Copper Transporting ATPase

PAA1, Chloroplast-Like" protein exhibits traits consistent with it being an antimicrobial. Our next studies will use custom designed sequences to evaluate whether this particular peptide carries antimicrobial features and which residues are important in this regard.

Acknowledgement

The authors extend their appreciation and acknowledge to the Deanship of Scientific Research, College of Applied Medical Sciences at King Saud University, Riyadh, Kingdom of Saudi Arabia for the financial support.

References

Agizzio, A.P., Carvalho, A.O., Suzanna de Fátima, F.R., Machado, O.L., Alves, E.W., Okorokov, L.A., Samarão, S.S., Bloch, C., Prates, M.V., Gomes, V.M., 2003. A 2S albumin-homologous protein from passion fruit seeds inhibits the fungal growth and acidification of the medium by Fusarium oxysporum. Arch. Biochem. Biophys. 416 (2), 188–195.

Al Akeel, R., Mateen, A., Syed, R., Alyousef, A.A., Shaik, M.R., 2017. Screening, purification and characterization of anionic antimicrobial proteins from Foeniculum Vulgare. Molecules 22 (4), 602.

Badgujar, S.B., Patel, V.V., Bandivdekar, A.H., 2014. Foeniculum vulgare Mill: a review of its botany, phytochemistry, pharmacology, contemporary application, and toxicology. BioMed Res. Int.

Barbieri, L., Battelli, M.G., Stirpe, F., 1993. Ribosome-inactivating proteins from plants. Biochim. Biophys. Acta (BBA)-Rev. Biomembr. 1154 (3–4), 237–282.

Boonnak, N., Karalai, C., Chantrapromma, S., Ponglimanont, C., Fun, H.K., Kanjana-Opas, A., Chantrapromma, K., Kato, S., 2009. Anti-Pseudomonas aeruginosa xanthones from the resin and green fruits of Cratoxylum cochinchinense. Tetrahedron 65 (15), 3003–3013.

Broekaert, W.F., Terras, F., Cammue, B., Osborn, R.W., 1995. Plant defensins: novel antimicrobial peptides as components of the host defense system. Plant Physiol. 108 (4), 1353.

Cammue, B.P.A., Thevissen, K., Hendriks, M., Eggermont, K., Goderis, I.J., Proost, P., Van Damme, J., Osborn, R.W., Guerbette, F., Kader, J.C., 1995. A potent antimicrobial protein from onion seeds showing sequence homology to plant lipid transfer proteins. Plant Physiol. 109 (2), 445–455.

Cheng, C.S., Samuel, D., Liu, Y.-J., Shyu, J.C., Lai, S.-M., Lin, K.F., Lyu, P.C., 2004. Binding mechanism of nonspecific lipid transfer proteins and their role in plant defense. Biochemistry 43 (43), 13628–13636.

Diamond, G., Beckloff, N., Weinberg, A., Kisich, K.O., 2009. The roles of antimicrobial peptides in innate host defense. Curr. Pharm. Des. 15 (21), 2377–2392.

Dong, T., Ng, T., Yeung, H., Wong, R., 1994. Isolation and characterization of a novel ribosome-inactivating protein, β-kirilowin, from the seeds of Trichosanthes kirilowii. Biochem. Biophys. Res. Commun. 199 (1), 387–393.

Florack, D., Stiekema, W., 1994. Thionins: properties, possible biological roles and mechanisms of action. Plant Mol. Biol. 26 (1), 25–37.

Hishinuma, F., Izaki, K., Takahashi, H., 1969. Effects of glycine and D-amino acids on growth of various microorganisms. Agric. Biol. Chem. 33 (11), 1577–1586.

Langsdorf, M., Ghassempour, A., Römpp, A., Spengler, B., 2010. Isolation and sequence analysis of peptides from the skin secretion of the Middle East tree frog Hyla savignyi. Anal. Bioanal. Chem. 398 (7–8), 2853–2865.

Leah, R., Tommerup, H., Svendsen, I., Mundy, J., 1991. Biochemical and molecular characterization of three barley seed proteins with antifungal properties. J. Biol. Chem. 266 (3), 1564–1573.

Leonti, M., Casu, L., 2013. Traditional medicines and globalization: current and future perspectives in ethnopharmacology. Frontiers Pharmacol. 4.

Lodish, H., Berk, A., Zipursky, S.L., Matsudaira, P., Baltimore, D., Darnell, J., 2000. Molecular Cell Biology. National Center for Biotechnology InformationÕs Bookshelf.

Ma, D., Wang, H., Ng, T., 2009. A peptide with potent antifungal and antiproliferative activities from Nepalese large red beans. Peptides 30 (12), 2089–2094.

Mahabusarakam, W., Rattanaburi, S., Phongpaichit, S., Kanjana-Opas, A., 2008. Antibacterial and cytotoxic xanthones from Cratoxylum cochinchinense. Phytochem. Lett. 1 (4), 211–214.

Marmiroli, N., Maestri, E., 2014. Plant peptides in defense and signaling. Peptides 56, 30–44.

Melchers, L.S., Groot, M.Ad., Knaap, J.A., Ponstein, A.S., Sela-Buurlage, M.B., Bol, J.F., Cornelissen, B.J., Elzen, P.J., Linthorst, H.J., 1994. A new class of tobacco chitinases homologous to bacterial exo-chitinases displays antifungal activity. Plant J. 5 (4), 469–480.

Mohsenzadeh, M., 2007. Evaluation of antibacterial activity of selected Iranian essential oils against Staphylococcus aureus and Escherichia coli in nutrient broth medium. Pak. J. Biol. Sci. 10 (20), 3693–3697.

Nawrot, R., Barylski, J., Nowicki, G., Broniarczyk, J., Buchwald, W., Goździcka-Józefiak, A., 2014. Plant antimicrobial peptides. Folia Microbiologica. 59 (3), 181–196.

Ohyama, K., Ogawa, M., Matsubayashi, Y., 2008. Identification of a biologically active, small, secreted peptide in Arabidopsis by in silico gene screening, followed by LC-MS-based structure analysis. Plant J. 55 (1), 152–160.

Park, S.-W., Vepachedu, R., Sharma, N., Vivanco, J.M., 2004. Ribosome-inactivating proteins in plant biology. Planta 219 (6), 1093–1096.

Pelegrini, P.B., Murad, A.M., Silva, L.P., Dos Santos, R.C.P., Costa, F.T., Tagliari, P.D., Bloch Jr, C., Noronha, E.F., Miller, R.N.G., Franco, O.L., 2008. Identification of a novel storage glycine-rich peptide from guava (Psidium guajava) seeds with activity against Gram-negative bacteria. Peptides 29 (8), 1271–1279.

Reynolds, J.J., Bicknell, L.S., Carroll, P., Higgs, M.R., Shaheen, R., Murray, J.E., Papadopoulos, D.K., Leitch, A., Murina, O., Tarnauskaitė, Ž., 2017. Mutations in DONSON disrupt replication fork stability and cause microcephalic dwarfism. Nat. Genet. 49 (4), 537–549.

Seo, M.-D., Won, H.-S., Kim, J.-H., Mishig-Ochir, T., Lee, B.-J., 2012. Antimicrobial peptides for therapeutic applications: a review. Molecules 17 (10), 12276–12286.

Stintzi, A., Heitz, T., Prasad, V., Wiedemann-Merdinoglu, S., Kauffmann, S., Geoffroy, P., Legrand, M., Fritig, B., 1993. Plant 'pathogenesis-related'proteins and their role in defense against pathogens. Biochimie. 75 (8), 687–706.

Terras, F., Schoofs, H., De, Bolle M., Van Leuven, F., Rees, S.B., Vanderleyden, J., Cammue, B., Broekaert, W.F., 1992. Analysis of two novel classes of plant antifungal proteins from radish (Raphanus sativus L.) seeds. J. Biol. Chem. 267 (22), 15301–15309.

Thomma, B.P., Cammue, B.P., Thevissen, K., 2002. Plant defensins. Planta 216 (2), 193–202.