GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

Check for updates

Immuno-compatibility assessment of phytal-proteins of *Zingiber zerumbet* and serum gamma globulins of rheumatoid arthritis disease subjects

Rupal Hemantkumar Desai and Jayaprada Rao Chunduri *

Department of Biotechnology, S.V.K.M'S Mithibai College of Arts, Chauhan Institute of Science, Amrutben Jivanlal College of Commerce and Economics,, Vile Parle (W),Mumbai, India.

GSC Biological and Pharmaceutical Sciences, 2023, 23(01), 168–173

Publication history: Received on 27 February 2023; revised on 10 April 2023; accepted on 13 April 2023

Article DOI: https://doi.org/10.30574/gscbps.2023.23.1.0152

Abstract

Phytal proteins are of great importance as they exhibit unique characteristics as immune modulators. *Zingiber zerumbet* family plants are used in various ways, including as food, beverages, and ornaments. The metabolites and extracts of these plants indicated an anti-viral, anti-cancer, anti-diabetic, and anti-inflammatory therapeutic characteristics features. Rheumatoid arthritis (RA) is an auto-immune condition that causes joint inflammation and malformations. The up- and down-regulation of certain rheumatoid arthritis proteins result in serious side effects. Ayurveda and other naturopathy treatments offer relief to a certain extent, while other pharmaceutical therapy is topical and temporary. The current study is aimed to comprehend the interaction of gamma-globulin proteins of RA patients with phytal proteins from leaves of the *Zingiber zerumbet* plant. The immunoassays, and MS orbitrap studies revealed the binding pattern and composition of the proteins. The protein- protein docking studies supported the method of binding pattern and these phytal proteins can be considered in the future for therapeutical or diagnostic purposes.

Keywords: *Zingiber zerumbet*; Gamma globulin; Immune-binding; Phytal proteins; Protein- protein docking

1. Introduction

141 species of *Zingiber* plants are found in Asia, especially in Asia's tropics, Malaysia, and the Pacific Islands. *Zingiber zerumbet* is commonly known as Pinecone or shampoo ginger as well as goes by a number of names depending on the geographical region (Al-Nahain A.,2014). This herb is popularly considered as an indigenous species to the Malaysian Peninsula and India. Its' leaves are small, about 25 to 35 cm in length. Rhizomes are the most significant component of plants and have been linked to all of the purported therapeutic benefits (Koga.,2016 and Agrawal.,1999). The compound Zerumbone isolated from *Zingiber zerumbet* has indicated various pharmacological activities such as anti-oxidant, anti-bacterial, anti-pyretic, anti-inflammatory, and immunomodulatory, as well as anti-neoplastic. It can suppress the proliferation, survival, angiogenesis, invasion, and metastasis levels of cancer by modulating cancer pathways and the downstream target proteins at the molecular level (Girisa et.al.,2019)

The immune system helps protect the organism from microorganisms, chemicals, viruses, and fungus. A function based on the recognition of foreign body and reaction activity is considered as an immune response. The immune system's numerous effector molecules engage in a complex series of events that respond to an antigen or allergen. One of the most common autoimmune diseases, rheumatoid arthritis, affects more women than males, and can have catastrophic side effects if it is not detected at an early stage. An increase in mortality and morbidity can result from chronic disease. Many dysregulated proteins linked with the disease can be controlled and treated with phytal proteins without generating any side effects (Mun. S.,2018). The objective of the current study is to evaluate the protein-protein interactions between RA subjects' gamma globulin and the protein found in *Zingiber* plant leaves.

* Corresponding author: Jayaprada Rao Chunduri

2. Material and methods

2.1. Sample collection and preparation

Samples of leaves from the *Zingiber zerumbet* plant were gathered in Mumbai's western suburbs (India). Younger plant leaves were considered because they contain more protein than the elder leaves do. Soon after the washing of the leaves with distilled water, they were air dried in a shade and then ground into a fine powder. The Gbadamosi et al. method was adapted to extract the protein. The protein was isolated using 1D native PAGE. The protein bands eluted in Phosphate buffer saline (PBS) were used for immunoassays.

2.2. Serum samples from RA –affected subjects

Blood samples obtained using conventional blood collection methods from RA affected subjects categorized based on 2010 ACR/EULAR scores of more than six. The consent was taken from the subjects, and serum was isolated using the accepted method.

2.3. Serum gel electrophoresis

Horizontal gel electrophoresis was considered to isolate the Gamma globulin bands from the selected serum samples with high Rheumatic factor, anti-CCP, and CRP values. The gamma globulin band was separated on 1 percent Agarose gel prepared in Tris-Glycine buffer as per the standard protocol. The gel was stained with Coomassie Brilliant Blue stain (Csako, G. ,2019) to identify and quantify the protein bands.

2.4. Immunological assays of eluted proteins

The ability of binding of isolated proteins from plant leaves to human gamma globulin of rheumatoid –affected subjects was assessed (Mayer and Walker,1987). According to accepted practices, the immunoassays were reproduced three times and standardized (Collins et.al.,2004). The phytalprotein extracts as viable sources for future medications were evaluated based on the binding effectiveness with the protein components.

2.4.1. Double diffusion assay

The Collins and Lyne standard technique for the double diffusion assay was followed (2004). In this Ouchterlony method**,** 1.5 % agar prepared in the borate buffer was used for the assay. 10 µl each of plant protein and Globulin antibody were added in separate wells and allowed to diffuse by keeping in a moist chamber at 37^0c for 24 to 48 hours. The presence of a visible precipitin line noticed at the point of equivalence between the concentration gradients indicating the relationship between phytal proteins and gamma globulin systems.

2.4.2. Rocket immuno-electrophoresis

The standard protocol of Csako (2019) was followed for the rocket immune-electrophoresis. 1% agarose in TBE buffer (pH 8) and 350 µL gamma-globulin protein was used to coat the glass slide. Wells were loaded with an eluted plant protein sample and subjected to electrophoresis at a steady current of 100 V. Standard CBB staining technique was carried out for 45 min followed by de-staining for 2-3 h.

2.4.3. LC-MS

The interaction bands that represented the bound gamma globulin and leaf extract proteins were sent for Orbitrap liquid chromatography mass spectrometry to IIT –SAIF(Sophisticated Analytical Instrument Facility). The chromatograms were further analyzed using Thermo proteome discoverer 2.2, Homosapiens and *Zingiber* plant data bases to identify the proteins involved in the binding.

2.4.4. Bioinformatics study

The proteins identified using LC –MS were extensively studied to understand the protein-protein interaction using Hawk Dock software and verified using PDBsum analysis software.

3. Results and discussion

Leaf protein was extracted and measured using the Bradford assay. The protein concentration was found to be in a range of 0.55 mg to 0.92 mg. Serum gel electrophoresis showed variation in the concentration of gamma globulin from 0.84 gm/dl to 2.8 gm/dl.

3.1. Immuno-assays

Immuno-diffusion procedures are one of the crucial techniques in immunology. These potent assays rely on the principle that antigen and antibody reactions are based on the concepts of zone electrophoresis and immunodiffusion. These tests have been carried out to determine how well plant proteins bind to antigens or antibodies and to assess the most effective interactions.

In the immunodiffusion process, soluble proteins (antigen or antibody) diffuse through a gel matrix toward one another and create an immunological complex (Kanwar and Verma, 2006). Prior until now, immune-binding experiments have taken into consideration to evaluate the vaccine's capacity to bind virus particles (Ullman et al., 1989). In the current investigation, isolated gamma globulin band proteins are permitted to interact with proteins from plant leaves that may create an immunological complex.

The Double diffusion indicated a good interaction between plant leaves protein and gamma globulin protein. (Fig1a). The Rocket immuno-electrophoresis confirmed antigen-antibody concentration to initiate a serological reaction (Hornok and Szecsi, 1977).(Fig.1b)

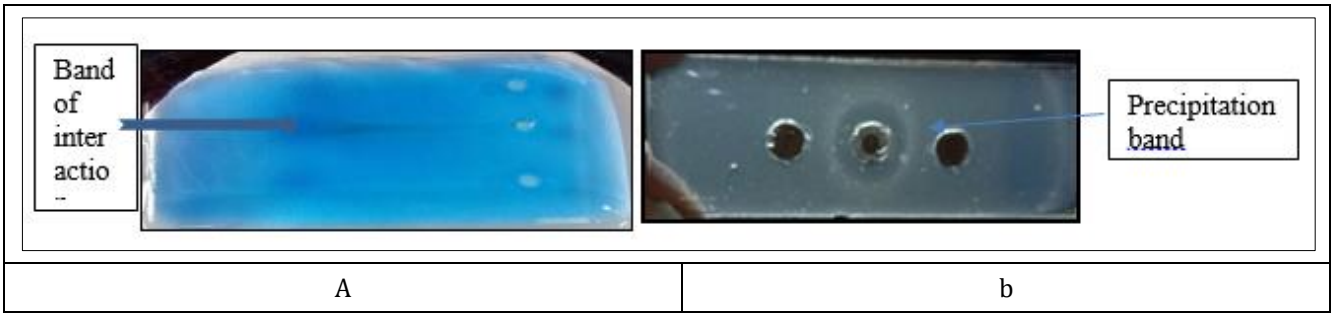


Figure 1 (a) and (b) Rocket and Double diffusion results

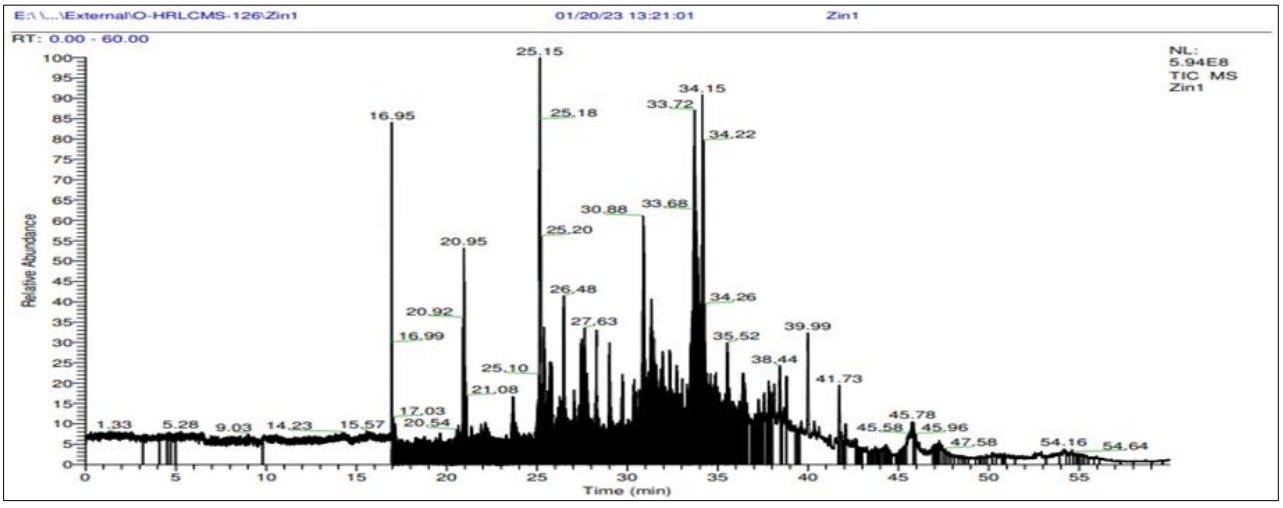


Figure 2 LC-MS chromatogram of precipitin band of *Zingiber zerumbet* phytoprotein and gamma globulin interaction

LC-MS analysis of precipitin band has showed 10 proteins from *Zingiber zerumbet* plant and 50 proteins from gamma globulin RA -affected subjects. The protein-protein docking results has showed good results between protein S100 A7

and plant proteins Ribulose biphosphate carboxylase large chain, NAD(P)H-quinone oxidoreductase, and Photosystem II D2 protein (Table-1)

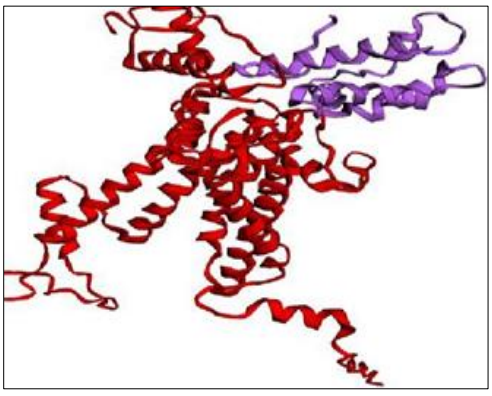
Table 1 Protein -protein docking results

Sr.no	Gamma-globulin protein	*Zingiber zerumbet* protein	Binding energy (kcal/mol)	Hydrogen bond	Salt bridge	Non bonded contact
1	S100 A7	Ribulose biphosphate carboxylase	-4634.07	04	03	134
2	S100 A7	NAD(P) H-quinone oxidoreductase	-4442.97	02	01	196
3	S100 A7	Photosystem II D2	-4721.34	08	00	106

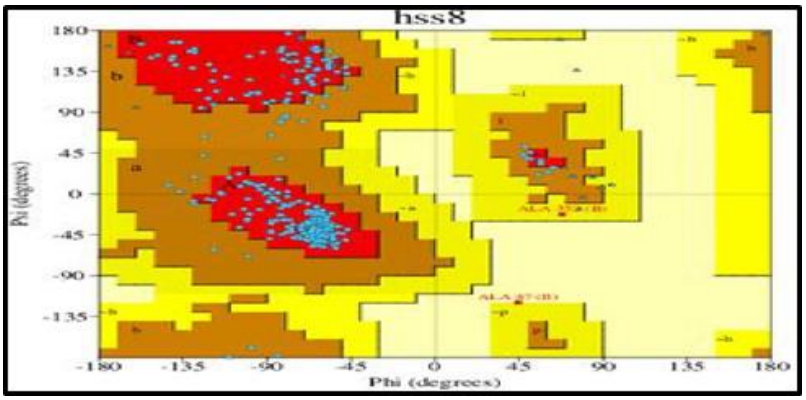
More than 20 S100 protein family's association with the auto immune disorder of RA has been confirmed, including S100A1 to S100A18, S100B, S100P, S100Z, and S100G. The S100 protein family includes calcium-binding proteins with modest molecular weights (9000–14,000 Da) and highly conserved amino acid sequences. Some S100 family members are released from the cells, controlling the activity of macrophages, leukocytes, and inflammatory cells that create cytokines or MMPs from synovial fibroblasts and the proliferation of target cells (Wu.YY.,20220, Porter.,2019). Very few studies were conducted to understand the phytal protein and S 100 family proteins previously. The current study has been conducted to find the interaction among gamma globulin protein and plant *Zingiber zerumbet* leaves protein extract. It is important to consider various factors such as the number and type of intermolecular bonds, the bond energy, the specificity and affinity of the interaction, and the biological relevance of the interacting proteins to prioritize and assess the relevance of protein-protein interaction.

Protein S100 A7 constituted 101 amino acids while, Ribulose bi-phosphate carboxylase with 480 amino acids. The interaction between these two proteins was represented with 3 salt bridges,4 hydrogen bonds and 134 non bonded contacts. The docking process indicated the presence of Hydrogen bonds between Ser 77 - Asn 184, Gln101- Arg187, and Ser 90-Trp411 amino acid and, Salt bridges between among Asp81- Arg187, Glu7-Lys334, and His87-Glu191 amino acids. The Binding energy found to be -4634.07 kcal/mol.

Protein S100 A7 and NAD(P)H-quinone oxidoreductase interactions resulted in Binding energy of -4442.97 kcal/mol**.** Protein S100 A constituted with 101 and NAD(P)H-quinone oxidoreductase with 393 amino acids. The protein -protein interaction indicated 1 salt bridge, 2 hydrogen bonds and 196 non-bonded contacts.



a



b

Figure 3 a and b: S100 A7 and photosystem II D2 interaction and Ramachandran plot

Protein S100 A7 interaction with the Photosystem II D2 protein indicated a Binding energy of -4721.34 kcal/mol. The photosystem II D2 protein has 353 amino-acids. Protein interaction has shown 8 hydrogen bonds and 106 non bonded contacts. The hydrogen bond interaction seen between Ser9-Ser85, Gln5-Thr61, Arg8- Tyr60, Asn42-Leu163, Asn42-Gln165, Asn293-Gln89, and Gln89-Arg295. (Fig.3a ,b,c)

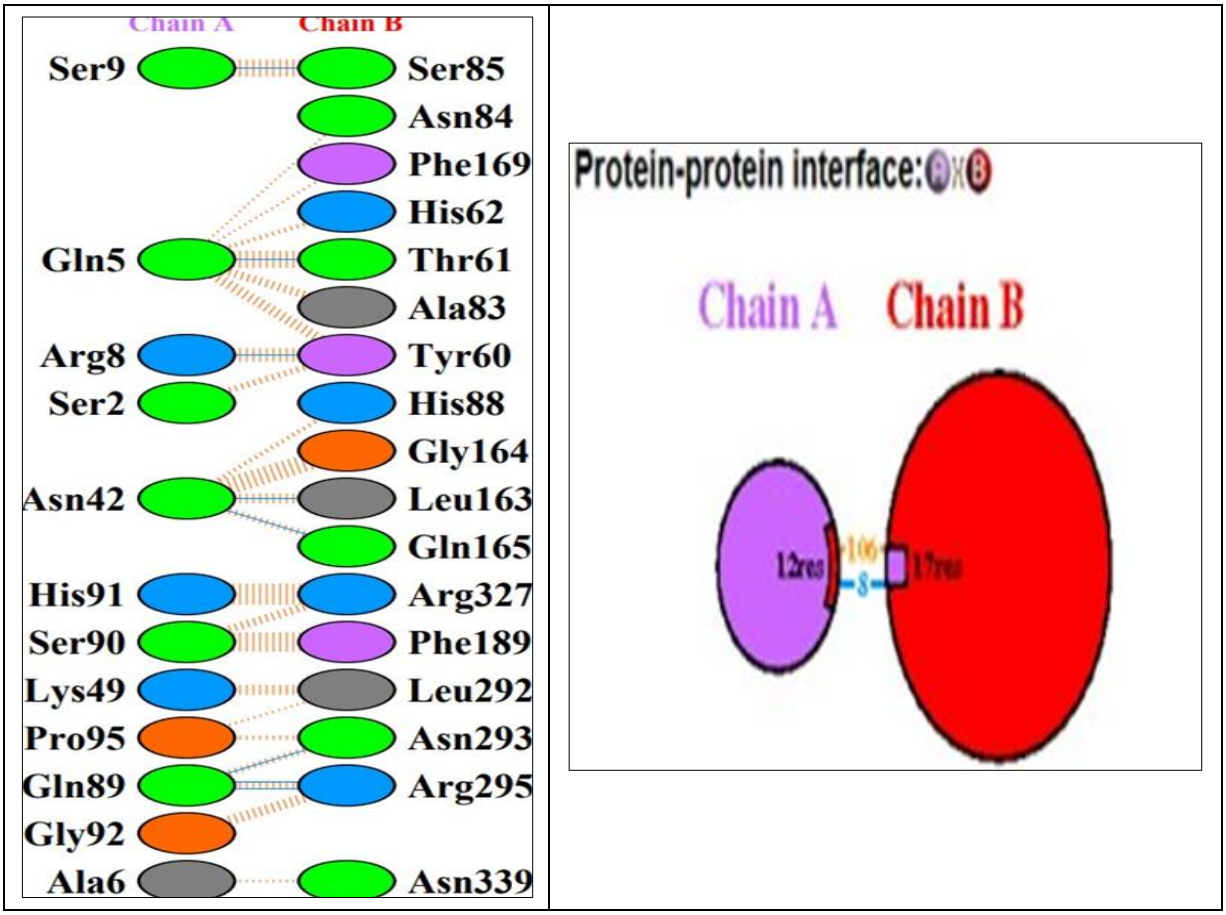


Figure 3c Protein-protein interface and detail interaction view

The overall study indicated among the three protein interactions, the Protein S100 A7 and Photosystem II D2 protein interaction were found to be stable. The reasons could be attributed to the greater number of hydrogen bonds (8), characterized by typically stronger and more directional than s-bonds; In addition to the bond energy of the interaction (4721), was higher than the other two. The evaluation of protein-protein interaction relied on the important parameters such as the number and types of intermolecular bonds, the specificity and affinity of the interaction, as well as the biological relevance of the interacting proteins.

4. Conclusion

The studies indicated that the activity of the Family of Protein S100 can be controlled with *Zingiber zerumbet* plant protein. Phytal extract protein of *Zingiber zerumbet* could be a cheap and safe option remedy to treat RA -affected subjects. Further in vivo studies can be useful for studying therapeutic aspects of zingiber leaf proteins in detail in compliance with ethical standards

Compliance with ethical standard

Acknowledgments

We thank the SVKM management for providing good working conditions to carry this research project.

Disclosure of conflict of interest

No conflict of interest

Statement of ethical approval

'The present research work does not contain any studies performed on animals/humans subjects by any of the authors. However, consent of the subjects was taken while collecting the samples as per the RRC guidelines.

Statement of informed consent

Informed consent was obtained from all the individual participants included in the study.

References

[1] Li Q, Zhang C, Tan W, Gu G, Guo Z. Novel amino-pyridine functionalized chitosan quaternary ammonium derivatives: Design, synthesis, and antioxidant activity. Molecules. 2017 Jan 18, 22(1):156.

[2] Al-Nahain A, Jahan R, Rahmatullah M. Zingiber officinale: A potential plant against rheumatoid arthritis. Arthritis. 2014, 2014.

[3] Koga AY, Beltrame FL, Pereira AV. Several aspects of Zingiber zerumbet: a review. Revista Brasileira de Farmacognosia. 2016 May, 26:385-91.

[4] Agarwal SS, Singh VK. Immunomodulators: A review of studies on Indian medicinal plants and synthetic peptides. Part-I: Medicinal plants. Proceedings of the Indian National Science Academy-Part B: Biological Sciences. 1999, 65(3-4):179-204.

[5] Girisa S, Shabnam B, Monisha J, Fan L, Halim CE, Arfuso F, Ahn KS, Sethi G, Kunnumakkara AB. Potential of zerumbone as an anti-cancer agent. Molecules. 2019 Feb 18;24(4):734.

[6] Mun S, Lee J, Lim MK, Lee YR, Ihm C, Lee SH, Kang HG. Development of a novel diagnostic biomarker set for rheumatoid arthritis using a proteomics approach. BioMed research international. 2018 Nov 26, 2018.

[7] Csako G. Immunoelectrophoresis: a method with many faces. Protein Electrophoresis: Methods and Protocols. 2012:339-59.

[8] Mayer RJ, Walker JH. Immunochemical methods in cell and molecular biology.

[9] Grange J, Lyne P. Collins and Lyne's Microbiological Methods. Hodder Education, 2004.

[10] Kanwar SS, Verma ML. Principles and applications of Immuno-diffusion, immuno-electrophoresis, immuno-fluorescence, ELISA, Western blotting, Minimal Inhibitory Concentration (MIC), Kirby-Bauer method and Widal test.

[11] Ullman DE, German TL, Gunasinghe UB, Ebesu RH. Serology of a closteroviruslike particle associated with mealybug wilt of pineapple. Phytopathology. 1989 Dec 1, 79(12):1341-5.

[12] Hornok L, Szécsi Á. Demonstration of antifungal antibodies in hyperimmune mouse ascitic fluid by means of crossed immunoelectrophoresis. Microbiology. 1977 May, 100(1):213-6.

[13] Wu YY, Li XF, Wu S, Niu XN, Yin SQ, Huang C, Li J. Role of the S100 protein family in rheumatoid arthritis. Arthritis Research & Therapy. 2022 Dec, 24(1):1-9.

[14] Porter KA, Desta I, Kozakov D, Vajda S. What method to use for protein–protein docking?. Current Opinion in Structural Biology. 2019 Apr 1, 55:1-7.