JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal
Published by Faculty of Biotechnology and Food Sciences

MOLECULAR DOCKING ANALYSIS OF ORGANIC ACIDS (OA) FROM HONEY AS MODULATORS OF HUMAN FERRITIN, TRANSFERRIN, AND HEPCIDIN

Srinivasan Kumaraswamy[1], *Geetha Arumugam*[2], *Ashok Kumar Pandurangan*[3], *Vasantha Srinivasan Prabhakaran*[4], *Radhakrishnan Narayanaswamy**[5]

Address(es):
[1]Department of Biochemistry, St. Peter's Institute of Higher Education and Research (SPIHER), Avadi, 600 054, Chennai, Tamil Nadu, India.
[2]Department of Biochemistry, Chennai National Arts and Science College (CNC), Avadi, 600 054, Chennai, Tamil Nadu, India.
[3]School of Life Science, B.S. Abdur Rahman Crescent Institute of Science and Technology, Vandalur, 600 048, Chennai, Tamil Nadu, India.
[4]Department of Bioinformatics, Saveetha School of Engineering, SIMATS (Deemed to be University), Thandalam, 602 105, Chennai, Tamil Nadu, India.
[5]Department of Biochemistry, Saveetha Medical College and Hospital, SIMATS (Deemed to be University), Thandalam, 602 105, Chennai, Tamil Nadu, India.

*Corresponding author: nrkishnan@gmail.com

https://doi.org/10.55251/jmbfs.5743

ARTICLE INFO

Received 15. 1. 2022
Revised 21. 1. 2023
Accepted 23. 1. 2023
Published 1. 4. 2023

Regular article

OPEN ACCESS

ABSTRACT

Organic acids (OA) have been known to regulate both the availability and absorption of non-heme iron. Honey contains 0.57 % of organic acids (OA). These prompt us to carry out the present study, where fifty-three selected (honey) ligands were assessed on the docking behavior of human Ferritin, Transferrin, and Hepcidin using the PatchDock method. Furthermore, Molecular physicochemical and Absorption, Distribution, Metabolism, and Excretion (ADME) analyses were also carried out using Molinspiration and Swiss ADME online servers, respectively. Molecular Physico-chemical analysis has shown that all the ligands (except folic acid, gluconic acid, and lactobionic acid) have obeyed Lipinski's Rule of five. With regard to ADME analysis, two ligands (D-galacturonic acid and quinic acid) have been predicted to possess P-glycoprotein (P-gp) inhibition effect. In addition, the docking studies revealed that kynurenic acid showed the highest atomic contact energy for all the target proteins human Ferritin (-133.39 kcal/mol), Transferrin (-102.84 kcal/mol), and Hepcidin (-197.64 kcal/mol), respectively. Thus the present findings provide a new insight in understanding fifty-three selected (honey) ligands as modulators of human Ferritin, Transferrin, and Hepcidin.

Keywords: Molecular docking, honey, organic acids, Ferritin, Transferrin, Hepicidin, kynurenic acid

INTRODUCTION

Iron is one of the important micronutrients for human life. It plays a vital physiological role in transporting oxygen (O_2) via hemoglobin (Hb) to all tissues. Similarly, myoglobin (Mb) needs iron, which transports oxygen (O_2) to the muscle cells (myocytes). In addition to these, iron also plays a significant role in many biochemical reactions (rxns) that produce energy in the human body. Iron demand is more during i) child growth development, ii) gestation and similarly more loss during i) teenage and ii) reproductive phases in females. These above physiological conditions of iron initially cause a risk of iron deficiency and, if not cared for properly, they may result in iron deficiency anemia (**Nair, 2019**). Ferritin, transferrin and hepcidin levels are used as biomarkers to assess iron deficiency anemia (**Bermejo & García-López. 2009; Nair & Iyengar, 2009**).

Organic acids (OA) have been known to enhance (non-heme) iron bioavailability owing to their unique characteristic nature of more stability and less chemical reactive, particularly in certain food applications. Thus organic acids (OA) have gained much attention among researchers. In addition, organic acids (OA) are known to prevent lipid peroxidation (LPO) in foodstuffs and enhance the solubility of both ferrous (Fe^{2+}) and ferric (Fe^{3+}) iron by binding with carboxyl (COOH) and hydroxyl (OH) groups. **Prica & coworkers (2014)** had reported that honey contains 0.57% of organic acids (OA), which is mainly responsible for the chemical, physical and organoleptic properties of honey (**Mato *et al*., 2006**). In addition, organic acids (OA) have been used as an analytical tool to distinguish between different honey types and sources.

Nelson & Mottern (1931) had reported that honey contains the following organic acids (OA) such as i) lactic acid, ii) malic acid, iii) tartaric acid, iv) oxalic acid, and v) succinic acid. According to author **White Jr (1978)**, several organic acids (OA) have been reported in honey, which include i) acetic acid, ii) butyric acid, iii) citric acid, iv) formic acid, v) gluconic acid, vi) lactic acid, vii) malic acid, viii) pyroglutamic acid, and ix) succinic acid. **Sabatini & coworkers (1994)** had reported that formic acid contents served as an important tool to distinguish the light and dark kinds of honey.

Thus earlier reports engaged us to carry out the present study on selected organic acids (OA) which includes acetic acid, alpha-ketoglutaric acid, anthranilic acid, ascorbic acid, benzoic acid, butyric acid, cis-aconitic acid, citramalic acid, citric acid, 2,4-dihydroxy benzoic acid, 2,6-dihydroxybenzoic acid, 3,4-dihydroxy benzoic acid, erythorbic acid, folic acid, formic acid, fumarate, fumaric acid, D-galacturonic acid, gluconic acid, glutamic acid, glutaric acid, glyoxylic acid, hexanoic acid, 3-hydroxy anthranilic acid, 4-hydroxybenzoic acid, 2-hydroxybutyric acid, 2-hydroxyglutaric acid, 4-hydroxy phenylacetic acid, isobutyric acid, isocitric acid, kojic acid, kynurenic acid, lactic acid, lactobionic acid, malic acid, malonic acid, mandelic acid, (2R)-2-methoxybutanedioic acid, methylmalonic acid, oxalic acid, 2-oxopentanoic acid, phenylacetic acid, 3-phenyl lactic acid, picolinic acid, propionic acid, DL-pyroglutamic acid, pyruvic acid, quinic acid, shikimic acid, succinate, succinic acid, tartaric acid and valeric acid. These above stated organic acids (OA) were studied on the docking analysis of human Ferritin, Transferrin, and Hepcidin by employing the PatchDock method. In addition, molecular Physico-chemical, bioactivity score, Absorption, Distribution, Metabolism and Excretion (ADME), and toxicity analyses were also studied using Molinspiration, Swiss ADME, and ProTox II methods, respectively.

COMPUTATIONAL METHOD

Chemical structures of 53 ligands namely 1) Acetic acid [Pubchem CID 176]; 2) Alpha keto glutaric acid [Pubchem CID 51]; 3) Anthranilic acid [Pubchem CID 227]; 4) Ascorbic acid [Pubchem CID 54670067]; 5) Benzoic acid [Pubchem CID 243]; 6) Butyric acid [Pubchem CID 264]; 7) Cis aconitic acid [Pubchem CID 643757]; 8) Citramalic acid [Pubchem CID 1081]; 9) Citric acid [Pubchem CID 311]; 10) 2,4-Dihyroxybenzoic acid [Pubchem CID 1491]; 11) 2,6-Dihydroxybenzoic acid [Pubchem CID 9338]; 12) 3,4-Dihyroxybenzoic acid [Pubchem CID 72]; 13) Erythorbic acid [Pubchem CID 54675810]; 14) Folic acid [Pubchem CID 135398658]; 15) Formic acid [Pubchem CID 284]; 16) Fumarate [Pubchem CID 5460307]; 17) Fumaric acid [Pubchem CID 444972]; 18) D-Galacturonic acid [Pubchem CID 439215]; 19) Gluconic acid [Pubchem CID 10690]; 20) Glutamic acid [Pubchem CID 33032]; 21) Glutaric acid [Pubchem CID 743]; 22) Glyoxylic acid [Pubchem CID 760]; 23) Hexanoic acid [Pubchem CID 8892]; 24) 3-Hydroxyanthranilic acid [Pubchem CID 86]; 25) 4-Hydroxybenzoic acid [Pubchem CID 135]; 26) 2-Hydroxybutyric acid [Pubchem CID 11266]; 27) 2-Hydroxyglutaric acid [Pubchem CID 43]; 28) 4-Hydroxyphenylacetic acid [Pubchem CID 127]; 29) Isobutyric acid [Pubchem CID

6590]; 30) Isocitric acid [Pubchem CID 1198]; 31) Kojic acid [Pubchem CID 3840]; 32) Kynurenic acid [Pubchem CID 3845]; 33) Lactic acid [Pubchem CID 612]; 34) Lactobionic acid [Pubchem CID 7314]; 35) Malic acid [Pubchem CID 525]; 36) Malonic acid [Pubchem CID 867]; 37) Mandelic acid [Pubchem CID 1292]; 38) (2R)-2-Methoxybutanedioic acid [Pubchem CID 5325475]; 39) Methylmalonic acid [Pubchem CID 487]; 40) Oxalic acid [Pubchem CID 971]; 41) 2-Oxopentanoic acid [Pubchem CID 74563]; 42) Phenylacetic acid [Pubchem CID 999]; 43) 3-Phenyllactic acid [Pubchem CID 3848]; 44) Picolinic acid [Pubchem CID 1018]; 45) Propionic acid [Pubchem CID 1032]; 46) DL-Pyroglutamic acid [Pubchem CID 499]; 47) Pyruvic acid [Pubchem CID 1060]; 48) Quinic acid [Pubchem CID 6508]; 49) Shikimic acid [Pubchem CID 8742]; 50) Succinate [Pubchem CID 160419]; 51) Succinic acid [Pubchem CID 1110]; 52) Tartaric acid [Pubchem CID 875] and 53) Valeric acid [Pubchem CID 7991] were downloaded from PubChem database. The energy minimized three dimensional (3D) chemical structures were further used for Patch Dock study.

The three-dimensional (3D) structure of the human Ferritin (PDB ID: 3AJO with a resolution of 1.52 Å), human Transferrin (PDB ID: 1A8F with a resolution of 1.80 Å), and human Hepcidin (PDB ID: 3H0T with a resolution of 1.89 Å) was down loaded from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (www.rcsb.org). A chain of human Ferritin and Transferrin (C chain of human Hepcidin) was prepared by removing ligands in addition to the crystallographically observed water particles (water without hydrogen bonds). All the proteins mentioned above were processed using UCSF Chimera software (www.cgi.ucsf.edu/chimera).

Molecular Physico-chemical and bioactivity score analyses were performed for fifty-three selected (honey) ligands using the Molinspiration online tool (**Christina *et al*., 2021**).

Absorption, Distribution, Metabolism, and Excretion (ADME) analysis was performed for fifty-three selected (honey) ligands using the SwissADME analysis method (**Solomon *et al*., 2021**).

Toxicity analysis was carried out for fifty-three selected (honey) ligands using the Pro Tox II webserver (**Banerjee *et al*., 2018**).

Docking studies were performed by the PatchDock (**Sangeetha *et al*., 2021**) online server (http://bioinfo3d.cs.tau.ac.il/PatchDock). PatchDock adopts a geometry-based molecular docking algorithm method that was utilized to recognize the binding scores, binding residues, and atomic contact energy (ACE) of the given ligands (53 ligands). Further, the binding site analysis was performed by PyMOL software (www.pymol.org).

RESULTS AND DISCUSSION

Organic acids (OA) are chemical substances that find wide applications in the chemical, food, and pharmaceutical fields (**Tran & Zhao, 2021**). Organic acids (OA) have been recognized by US-FDA as GRAS (Generally Recognized as Safe) substances that engage wide range of food and cosmetics applications. Organic acids (OA) have been known to possess anti-microbial activity, which attracts applications in the preparation of animal (poultry) feed (**El Baaboua *et al*., 2018**). **Gillooly & coworker (1983)** have reported that ascorbic acid, citric acid, malic acid, and tartaric acid drastically increase the intestinal iron absorption from a rice meal. Similarly, **Hazell & Johnson (1987)** had reported that ascorbic acid, citric acid, and malic acid significantly increase the diffusible iron from white wheat flour. Thus, organic acids (OA) from honey were selected for the present study.

Ferritin (Ft) is an iron-binding protein that regulates the storage, sequestration of excess ferrous (Fe^{2+}) iron and iron levels in humans (**Shamsi *et al*., 2021**). Similarly, plant-ferritin plays a defensive role against plant pathogens (**Zielińska-Dawidziak, 2015**). Thus human Ferritin was chosen as one of the target proteins for the present study.

Table 1 Represents the simplified molecular input line entry specification (SMILES) of fifty-three selected (honey) ligands

S.No	Ligands	Simplified Molecular Input Line Entry Specification (SMILES)
1	Acetic acid	CC(=O)O
2	Alpha ketoglutaric acid	C(CC(=O)O)C(=O)C(=O)O
3	Anthranilic acid	C1=CC=C(C(=C1)C(=O)O)N
4	Ascorbic acid	C(C(C1C(=C(C(=O)O1)O)O)O)O
5	Benzoic acid	C1=CC=C(C=C1)C(=O)O
6	Butyric acid	CCCC(=O)O
7	Cis aconitic acid	C(C(=CC(=O)O)C(=O)O)C(=O)O
8	Citramalic acid	CC(CC(=O)O)(C(=O)O)O
9	Citric acid	C(C(=O)O)C(CC(=O)O)(C(=O)O)O
10	2,4-Dihydroxybenzoic acid	C1=CC(=C(C=C1O)O)C(=O)O
11	2,6-Dihydroxybenzoic acid	C1=CC(=C(C(=C1)O)C(=O)O)O
12	3,4-Dihydroxybenzoic acid	C1=CC(=C(C=C1C(=O)O)O)O
13	Erythorbic acid	C(C(C1C(=C(C(=O)O1)O)O)O)O
14	Folic acid	C1=CC(=CC=C1C(=O)NC(CCC(=O)O)C(=O)O)NCC2=CN=C3C(=N2)C(=O)NC(=N3)N
15	Formic acid	C(=O)O
16	Fumarate	C(=CC(=O)[O-])C(=O)[O-]
17	Fumaric acid	C(=CC(=O)O)C(=O)O
18	D-Galacturonic acid	C1(C(C(OC(C1O)O)C(=O)O)O)O
19	Gluconic acid	C(C(C(C(C(C(=O)O)O)O)O)O)O
20	Glutamic acid	C(CC(=O)O)C(C(=O)O)N
21	Glutaric acid	C(CC(=O)O)CC(=O)O
22	Glyoxalic acid	C(=O)C(=O)O
23	Hexanoic acid	CCCCCC(=O)O
24	3-hydroxy anthranilic acid	C1=CC(=C(C(=C1)O)N)C(=O)O
25	4-Hydroxybenzoic acid	C1=CC(=CC=C1C(=O)O)O
26	2-Hydroxybutyric acid	CCC(C(=O)O)O
27	2-Hydroxyglutaric acid	C(CC(=O)O)C(C(=O)O)O
28	4-Hydroxyphenylacetic acid	C1=CC(=CC=C1CC(=O)O)O
29	Isobutyric acid	CC(C)C(=O)O
30	Isocitric acid	C(C(C(C(=O)O)O)C(=O)O)C(=O)O
31	Kojic acid	C1=C(OC=C(C1=O)O)CO
32	Kynurenic acid	C1=CC=C2C(=C1)C(=O)C=C(N2)C(=O)O
33	Lactic acid	CC(C(=O)O)O
34	Lactobionic acid	C(C1C(C(C(C(O1)OC(C(CO)O)C(C(C(=O)O)O)O)O)O)O)O
35	Malic acid	C(C(C(=O)O)O)C(=O)O
36	Malonic acid	C(C(=O)O)C(=O)O
37	Mandelic acid	C1=CC=C(C=C1)C(C(=O)O)O
38	(2R)-2-Methoxybutanedioic acid	COC(CC(=O)O)C(=O)O
39	Methylmalonic acid	CC(C(=O)O)C(=O)O
40	Oxalic acid	C(=O)(C(=O)O)O
41	2-oxopentanoic acid	CCCC(=O)C(=O)O
42	Phenylacetic acid	C1=CC=C(C=C1)CC(=O)O
43	3-Phenyllactic acid	C1=CC=C(C=C1)CC(C(=O)O)O
44	Picolinic acid	C1=CC=NC(=C1)C(=O)O
45	Propionic acid	CCC(=O)O

46	DL-Pyroglutamic acid	C1CC(=O)NC1C(=O)O
47	Pyruvic acid	CC(=O)C(=O)O
48	Quinic acid	C1C(C(C(CC1(C(=O)O)O)O)O)O)O
49	Shikimic acid	C1C(C(C(C=C1C(=O)O)O)O)O)O
50	Succinate	C(CC(=O)[O-])C(=O)[O-]
51	Succinic acid	C(CC(=O)O)C(=O)O
52	Tartaric acid	C(C(C(=O)O)O)(C(=O)O)O
53	Valeric acid	CCCCC(=O)O

Transferrin (Tf) is a glycoprotein that helps in iron solubilization, prevents iron-based free radical toxicity, and assists in iron transport (**Soldin *et al.*, 2004**). Therefore human Transferrin was chosen as one of the target proteins for the present study.

Hepcidin (Hep-25- a peptide hormone) has known to be the chief regulator of iron metabolism. It assists in both the absorption of dietary iron as well the distribution of iron (**Drakesmith & Prentice, 2012**), which has unlocked a new approach to target iron deficiency anemia (**Nair & Iyengar, 2009**). **Table 1** represents the simplified molecular input line entry specification (SMILES) of 53 selected ligands, of which 33 (62.26%) are organic acids (OA); 6 (11.32%) are fatty acids; 3 (5.66%) are vitamins; 3 (5.66%) are phenolic acids; 2 (3.77%) are amino acids; 2 (3.77%) are heterocyclic compounds and one from each aromatic acid (1.89%), carbohydrate (1.89%), cyclic polyol (1.89%), and sugar-acid (1.89%) respectively (as shown in **Table 2**).

Table 2 Represents the chemical nature, source and related literature reference of fifty-three selected (honey) ligands

Ligand name	Chemical nature	Source	Reference
Organic acids			
Acetic acid	Monocarboxylic acid	Honey	**Cianciosi *et al*., 2018**
Alpha ketoglutaric acid	Dicarboxylic acid	Honey	**Cianciosi *et al*., 2018**
Anthranilic acid	Monocarboxylic acid	-	**Chobot *et al*., 2015**
Benzoic acid	Monocarboxylic acid	Honey	**Verzera *et al.*, 2001**
Cis aconitic acid	Tricarboxylic acid	Honey	**Echigo & Takenaka, 1974**
Citramalic acid	Dicarboxylic acid	Honey	**Nozal *et al.*, 2003**
Citric acid	Tricarboxylic acid	Honey	**Cianciosi *et al*., 2018**
Formic acid	Monocarboxylic acid	Honey	**Cianciosi *et al*., 2018**
Fumarate	Dicarboxylic acid	-	-
Fumaric acid	Dicarboxylic acid	Honey	**Cianciosi *et al*., 2018**
Gluconic acid	Monocarboxylic acid	Honey	**Cianciosi *et al*., 2018**
Glutaric acid	Dicarboxylic acid	Honey	**Cianciosi *et al*., 2018**
Glyoxylic acid	Monocarboxylic acid	Honey	**Cianciosi *et al*., 2018**
3-hydroxy anthranilic acid	Monocarboxylic acid	-	**Chobot *et al*., 2015**
4-Hydroxybenzoic acid	Monocarboxylic acid	Honey	**Steeg & Montag, 1988**
2-Hydroxybutyric acid	Monocarboxylic acid	Honey	**Cianciosi *et al*., 2018**
Hydroxyglutaric acid	Dicarboxylic acid	Honey	**Cianciosi *et al*., 2018**
4-Hydroxyphenylacetic acid	Monocarboxylic acid	Honey	**Steeg & Montag, 1988**
Isocitric acid	Tricarboxylic acid	Honey	**Horváth & Molnár-Perl, 1998**
Kynurenic acid	Monocarboxylic acid	Honey	**Beretta *et al*., 2007**
Lactic acid	Monocarboxylic acid	Honey	**Nozal *et al.*, 2003**
Malic acid	Dicarboxylic acid	Honey	**Nozal *et al.*, 2003**
Malonic acid	Dicarboxylic acid	Honey	**Echigo & Takenaka, 1974**
Mandelic acid	Monocarboxylic acid	Honey	**Steeg & Montag, 1988**
2-Methoxybutanedioic acid	Dicarboxylic acid	Honey	**Tan *et al*., 1988**
Methylmalonic acid	Dicarboxylic acid	Honey	**Cianciosi *et al*., 2018**
Oxalic acid	Dicarboxylic acid	Honey	**Nozal *et al.*, 2003**
Phenylacetic acid	Monocarboxylic acid	Honey	**Speer & Montag, 1985**
3-Phenyllactic acid	Monocarboxylic acid	Honey	**Narayanaswamy *et al*., 2015**
Pyruvic acid	Monocarboxylic acid	Honey	**Nozal *et al.*, 2003**
Succinate	Dicarboxylic acid	-	-
Succinic acid	Dicarboxylic acid	Honey	**Cianciosi *et al*., 2018**
Tartaric acid	Dicarboxylic acid	Honey	**Cianciosi *et al*., 2018**
Amino acid			
Glutamic acid	Dicarboxylic acid	Honey	**Cianciosi *et al*., 2018**
Pyroglutamic acid	Monocarboxylic acid	Honey	**Puścion-Jakubik *et al*., 2020**
Aromatic acid			
Shikimic acid	Monocarboxylic acid	Honey	**Cianciosi *et al*., 2018**
Carbohydrates			
Galacturonic acid	Monocarboxylic acid	Honey	**Cianciosi *et al*., 2018**
Cyclic polyol			
Quinic acid	Monocarboxylic acid	Honey	**Cianciosi *et al*., 2018**
Fatty acids			
Butyric acid	Monocarboxylic acid	Honey	**Cianciosi *et al*., 2018**
Hexanoic acid	Monocarboxylic acid	Honey	**Starowicz *et al*., 2021**
Isobutyric acid	Monocarboxylic acid	Honey	**Prica *et al*., 2014**
2-oxopentanoic acid	Monocarboxylic acid	Honey	**Cianciosi *et al*., 2018**
Propionic acid	Monocarboxylic acid	Honey	**Cianciosi *et al*., 2018**
Valeric acid	Monocarboxylic acid	Honey	**Derewiaka *et al*., 2021**
Heterocyclic compounds			
Kojic acid	Pyranone	Honey	**Narayanaswamy *et al*., 2015**
Picolinic acid	Monocarboxylic acid	-	**Testa *et al*., 1985**
Phenolic acids			
2,4-Dihydroxybenzoic acid	Monocarboxylic acid	Honey	**Anand *et al*., 2019**
2,6-Dihydroxybenzoic acid	Monocarboxylic acid	Honey	**Seraglio *et al*., 2019**
3,4-Dihydroxybenzoic acid	Monocarboxylic acid	Honey	**Koulis *et al*., 2021**

Sugar acid			
Lactobionic acid	Monocarboxylic acid	-	**Escandar *et al*., 1994**
Vitamins			
Ascorbic acid	Vitamin C	Honey	**Ciulu *et al*., 2011**
Erythrobic acid	the stereoisomer of vitamin C	-	**Fidler *et al*., 2004**
Folic acid	Vitamin B9	Honey	**Ciulu *et al*., 2011**

Lipinski's Rule of Five (Thumb of Five) is utilized by the researchers as a vital tool to know the drugability nature of new molecular entities (**Narayanaswamy *et al.*, 2015)**. In the present study, molecular Physico-chemical analysis has shown that all the ligands (except folic acid, gluconic acid, and lactobionic acid) have obeyed Lipinski's Rule of Five (**Table 3**).

Table 3 Molecular Physico-chemical analysis of fifty-three selected (honey) ligands using Molinspiration online server

Ligands	**LogP[1]**	**TPSA[2]**	**Natoms[3]**	**MW[4]**	**nON[5]**	**nOHNH[6]**	**Nviolation[7]**	**Nrotb[8]**	**Volume[9]**
Acetic acid	-0.23	37.30	4	60.05	2	1	0	0	56.20
Alpha ketoglutaric acid	-1.49	91.67	10	146.10	5	2	0	4	119.22
Anthranilic acid	1.46	63.32	10	137.14	3	3	0	1	122.33
Ascorbic acid	-1.40	107.22	12	176.12	6	4	0	2	139.71
Benzoic acid	1.85	37.30	9	122.12	2	1	0	1	111.05
Butyric acid	1.00	37.30	6	88.11	2	1	0	2	89.80
Cis Aconitic acid	-1.22	111.90	12	174.11	6	3	0	4	137.86
Citramalic acid	-1.12	94.83	10	148.11	5	3	0	3	124.52
Citric acid	-1.98	132.12	13	192.12	7	4	0	5	151.76
2,4-Dihydroxybenzoic acid	1.37	77.75	11	154.12	4	3	0	1	127.08
2,6-Dihydroxybenzoic acid	1.39	77.75	11	154.12	4	3	0	1	127.08
3,4-Dihydroxybenzoic acid	0.88	77.75	11	154.12	4	3	0	1	127.08
Erythorbic acid	-1.40	107.22	12	176.12	6	4	0	2	139.71
Folic acid	-2.37	213.28	32	441.40	13	7	2	9	367.26
Formic acid	-0.51	37.30	3	46.02	2	1	0	0	39.64
Fumarate	-3.24	80.26	8	114.06	4	0	0	2	88.57
Fumaric acid	-0.68	74.60	8	116.07	4	2	0	2	94.05
D-Galacturonic acid	-2.77	127.44	13	194.14	7	5	0	1	153.99
Gluconic acid	-3.22	138.44	13	196.16	7	6	1	5	163.84
Glutamic acid	-3.25	100.62	10	147.13	5	4	0	4	128.36
Glutaric acid	-0.15	74.60	9	132.12	4	2	0	4	117.04
Glyoxalic acid	-0.62	54.37	5	74.03	3	1	0	1	58.62
Hexanoic acid	2.01	37.30	8	116.16	2	1	0	4	123.40
3-Hydroxyanthranilic acid	1.20	83.55	11	153.14	4	4	0	1	130.35
2-Hydroxybutyric acid	-0.21	57.53	7	104.11	3	2	0	2	97.84
2-Hydroxyglutaric acid	-1.30	94.83	10	148.11	5	3	0	4	125.09
4-Hydroxyphenylacetic acid	0.88	57.53	11	152.15	3	2	0	2	135.86
Isobutyric acid	0.68	37.30	6	88.11	2	1	0	1	89.59
Isocitric acid	-1.95	132.12	13	192.12	7	4	0	5	152.11
Kynurenic acid	0.68	70.16	14	189.17	4	2	0	1	159.01
Lactic acid	-0.71	57.53	6	90.08	3	2	0	1	81.04
Lactobionic acid	-4.76	217.59	24	358.30	12	9	2	8	295.96
Malic acid	-1.57	94.83	9	134.09	5	3	0	3	108.28
Malonic acid	-0.93	74.60	7	104.06	4	2	0	2	83.44
Mandelic acid	0.37	57.53	11	152.15	3	2	0	2	135.89
(2R)-2-Methoxybutanedioic acid	-0.95	83.83	10	148.11	5	2	0	4	125.81
Methylmalonic acid	-0.45	74.60	8	118.09	4	2	0	2	100.03
Oxalic acid	-1.20	74.60	6	90.03	4	2	0	1	66.64
2-oxopentanoic acid	0.17	54.37	8	116.12	3	1	0	3	108.78
Phenylacetic acid	1.36	37.30	10	136.15	2	1	0	2	127.85
Picolinic acid	0.34	50.19	9	123.11	3	1	0	1	106.89
Propionic acid	0.44	37.30	5	74.08	2	1	0	1	73.00
DL-Pyroglutamic acid	-2.40	66.40	9	129.12	4	2	0	1	110.61
Pyruvic acid	-0.90	54.37	6	88.06	3	1	0	1	75.18
Quinic acid	-2.33	118.21	13	192.17	6	5	0	1	161.46
Shikimic acid	-1.57	97.98	12	174.15	5	4	0	1	147.55
Succinate	-3.22	80.26	8	116.07	4	0	0	3	94.76
Succinic acid	-0.66	74.60	8	118.09	4	2	0	3	100.24
Tartaric acid	-2.49	115.05	10	150.09	6	4	0	3	116.33
Valeric acid	1.50	37.30	7	102.13	2	1	0	3	106.60

Note:: [1]Octanol-Water partition coefficient, [2]Polar surface area, [3]Number of non-hydrogen atoms, [4]Molecular weight, [5]Number of hydrogen bond acceptors [O and N atoms], [6]Number of hydrogen bond donors [OH and NH groups], [7]Number of Rule of 5 violations, [8]Number of rotatable bonds, [9]Molecular volume.

The bioactivity score is analyzed in order to evaluate the drug-likeness nature of the ligands (**Narayanaswamy *et al.*, 2015**). With reference to bioactivity score analysis of fifty-three selected (honey) ligands, only one ligand (lactobionic acid) has shown active (>0) bioactivity score against all the descriptors except kinase inhibitor (**Table 4**).

Table 4 Bioactivity score analysis of fifty-three selected (honey) ligands using Molinspiration online server

Ligands	G-protein coupled receptor ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Acetic acid	-3.71	-3.69	-3.88	-3.52	-3.62	-3.62
Alpha ketoglutaric acid	-0.92	-0.28	-1.74	-0.73	-0.59	0.20
Anthranilic acid	-0.97	-0.31	-1.06	-1.19	-1.11	-0.34
Ascorbic acid	-0.53	-0.24	-1.09	-1.01	-0.81	0.20
Benzoic acid	-2.21	-1.57	-2.49	-2.05	-2.31	-1.60
Butyric acid	-3.34	-3.52	-3.77	-3.13	-3.32	-3.18
Cis Aconitic acid	-0.52	0.09	-0.99	-0.12	-0.55	0.21
Citramalic acid	-0.50	-0.28	-1.23	-0.07	-0.69	0.27
Citric acid	-0.26	-0.14	-0.79	-0.12	-0.47	0.37
2,4-Dihydroxybenzoic acid	-0.81	-0.33	-0.99	-0.50	-1.02	-0.28
2,6-Dihydroxybenzoic acid	-0.91	-0.48	-1.20	-0.77	-1.07	-0.28
3,4-Dihydroxybenzoic acid	-0.88	-0.35	-1.10	-0.58	-1.09	-0.34
Erythorbic acid	-0.53	-0.24	-1.09	-1.01	-0.81	0.20
Folic acid	0.31	-0.09	0.23	-0.54	0.10	0.55
Formic acid	-3.77	-3.76	-3.84	-3.73	-3.73	-3.33
Fumarate	-3.14	-2.64	-3.29	-3.21	-3.21	-2.81
Fumaric acid	-2.87	-2.60	-3.46	-2.61	-2.83	-2.48
D-Galacturonic acid	-0.29	0.03	-1.03	-0.36	-0.38	0.46
Gluconic acid	-0.47	-0.15	-0.96	-0.51	-0.43	0.23
Glutamic acid	-0.29	0.25	-1.07	-0.96	-0.16	0.23
Glutaric acid	-1.84	-1.35	-2.48	-1.89	-1.93	-1.31
Glyoxylic acid	-3.66	-3.59	-3.81	-3.25	-2.96	-3.46
Hexanoic acid	-2.65	-2.46	-3.49	-2.56	-2.67	-2.31
3-Hydroxyanthranilic acid	-0.76	-0.54	-1.04	-0.89	-1.15	-0.28
2-Hydroxybutyric acid	-3.18	-3.25	-3.74	-2.71	-2.99	-2.94
2-Hydroxyglutaric acid	-0.55	-0.21	-1.31	-0.45	-0.60	-0.01
4-Hydroxyphenylacetic acid	-0.53	-0.07	-1.02	-0.18	-0.69	-0.09
Isobutyric acid	-3.61	-3.56	-3.83	-3.21	-3.40	-3.54
Isocitric acid	-0.23	-0.02	-0.94	-0.19	-0.18	0.04
Kynurenic acid	-0.43	-0.07	-0.77	-0.44	-0.79	-0.12
Lactic acid	-3.52	-3.58	-3.79	-2.99	-3.36	-3.29
Lactobionic acid	0.19	0.07	-0.13	0.05	0.24	0.52
Malic acid	-1.66	-1.19	-2.39	-1.47	-1.58	-0.89
Malonic acid	-3.31	-3.19	-3.66	-3.15	-3.26	-2.94
Mandelic acid	-0.50	-0.34	-0.99	-0.41	-0.77	-0.25
(2R)-2-Methoxybutanedioic acid	-0.45	-0.17	-1.21	-0.40	-0.33	0.21
Methylmalonic acid	-2.94	-2.53	-3.55	-2.70	-2.75	-2.60
Oxalic acid	-3.58	-3.54	-3.73	-3.46	-3.38	-3.31
2-oxopentanoic acid	-3.07	-2.51	-3.69	-2.66	-2.52	-1.95
Phenylacetic acid	-0.71	-0.21	-1.27	-0.54	-0.78	-0.24
Picolinic acid	-1.84	-0.99	-2.15	-2.05	-2.12	-1.19
Propionic acid	-3.56	-3.74	-3.91	-3.23	-3.54	-3.53
DL-Pyroglutamic acid	-1.70	-1.23	-3.02	-2.82	-1.50	-1.34
Pyruvic acid	-3.69	-3.52	-3.88	-3.48	-3.23	-3.16
Quinic acid	-0.24	0.10	-0.77	0.16	-0.26	0.60
Shikimic acid	-0.38	0.22	-1.13	0.01	-0.37	0.65
Succinate	-3.13	-2.62	-3.25	-3.25	-3.17	-2.81
Succinic acid	-2.74	-2.45	-3.37	-2.68	-2.76	-2.38
Tartaric acid	-0.87	-0.41	-1.43	-0.74	-0.75	-0.20
Valeric acid	-3.06	-3.15	-3.68	-2.91	-3.05	-2.84

Swiss ADME is an important online tool used in the drug discovery process to predict the pharmacokinetics attributes of ligands (or) small molecules (**Daina *et al*., 2017**). **Table 5** represents the Absorption, Distribution, Metabolism, and Excretion (ADME) property of fifty-three selected (honey) ligands where two ligands (D-galacturonic acid and quinic acid) have predicated to possess P-glycoprotein (P-gp) inhibition effect.

Table 5 Absorption, Distribution, Metabolism, Excretion (ADME) analysis of fifty-three selected (honey) ligands using Swiss ADME online server

Ligands	GI[1]	BBB[2]	P-gp[3]	CYP1A2[4]	CYP2C19[4]	CYP2C9[4]	CYP2D6[4]	CYP3A4[4]	Log Kp[5]
Acetic acid	High	No	No	No	No	No	No	No	-6.82
Alpha ketoglutaric acid	High	No	No	No	No	No	No	No	-7.83
Anthranilic acid	High	Yes	No	No	No	No	No	No	-6.28
Ascorbic acid	High	No	No	No	No	No	No	No	-8.54
Benzoic acid	High	Yes	No	No	No	No	No	No	-5.72
Butyric acid	High	Yes	No	No	No	No	No	No	-6.28
Cis Aconitic acid	High	No	No	No	No	No	No	No	-8.05
Citramalic acid	High	No	No	No	No	No	No	No	-7.81
Citric acid	Low	No	No	No	No	No	No	No	-8.69
2,4-Dihydroxybenzoic acid	High	No	No	No	No	No	No	Yes	-6.08
2,6-Dihydroxybenzoic acid	High	No	No	No	No	No	No	Yes	-5.68
3,4-Dihydroxybenzoic acid	High	No	No	No	No	No	No	Yes	-6.42
Erythorbic acid	High	No	No	No	No	No	No	No	-8.54
Folic acid	Low	No	No	No	No	No	No	No	-9.76
Formic acid	High	No	No	No	No	No	No	No	-6.72
Fumarate	Low	No	No	No	No	No	No	No	-7.24

Fumaric acid	High	No	No	No	No	No	No	No	-7.25
D-Galacturonic acid	Low	No	Yes	No	No	No	No	No	-9.15
Gluconic acid	Low	No	No	No	No	No	No	Yes	-9.89
Glutamic acid	High	No	No	No	No	No	No	No	-9.82
Glutaric acid	High	No	No	No	No	No	No	No	-7.31
Glyoxylic acid	High	No	No	No	No	No	No	No	-6.98
Hexanoic acid	High	Yes	No	No	No	No	No	No	-5.65
3-Hydroxyanthranilic acid	High	No	No	No	No	No	No	No	-6.62
2-Hydroxybutyric acid	High	No	No	No	No	No	No	No	-6.89
2-Hydroxyglutaric acid	High	No	No	No	No	No	No	No	-7.89
4-Hydroxyphenylacetic acid	High	Yes	No	No	No	No	No	No	-6.70
Isobutyric acid	High	Yes	No	No	No	No	No	No	-6.17
Isocitric acid	Low	No	No	No	No	No	No	No	-8.75
Kynurenic acid	High	No	No	No	No	No	No	No	-6.54
Lactic acid	High	No	No	No	No	No	No	No	-7.36
Lactobionic acid	Low	No	No	No	No	No	No	No	-12.01
Malic acid	High	No	No	No	No	No	No	No	-8.01
Malonic acid	High	No	No	No	No	No	No	No	-7.51
Mandelic acid	High	No	No	No	No	No	No	No	-6.79
(2R)-2-Methoxybutanedioic acid	High	No	No	No	No	No	No	No	-7.76
Methylmalonic acid	High	No	No	No	No	No	No	No	-6.98
Oxalic acid	High	No	No	No	No	No	No	No	-7.03
2-oxopentanoic acid	High	No	No	No	No	No	No	No	-6.65
Phenylacetic acid	High	Yes	No	No	No	No	No	No	-6.13
Picolinic acid	High	Yes	No	No	No	No	No	No	-6.54
Propionic acid	High	Yes	No	No	No	No	No	No	-6.52
DL-Pyroglutamic acid	High	No	No	No	No	No	No	No	-7.63
Pyruvic acid	High	No	No	No	No	No	No	No	-7.07
Quinic acid	Low	No	Yes	No	No	No	No	No	-9.15
Shikimic acid	High	No	No	No	No	No	No	No	-8.58
Succinate	Low	No	No	No	No	No	No	No	-7.43
Succinic acid	High	No	No	No	No	No	No	No	-7.44
Tartaric acid	Low	No	No	No	No	No	No	No	-8.55
Valeric acid	High	Yes	No	No	No	No	No	No	-5.94

Note: [1]Gastrointerstinal absorption, [2]Blood-brain barrier permeant, [3]P-gp-P-glycoprotein substrate, [4]CYP- Cytochrome P450 Inhibitors,[5]Log Kp-Skin Permeation (cm/s)

Pro Tox II is an important online tool used in the drug discovery process to predict the toxicity nature of ligands (or) small molecules (**Banerjee *et al.*, 2018**). Regarding toxicity analysis of fifty-three selected (honey) ligands where four ligands (anthranilic acid, 3-hydroxy anthranilic acid, phenylacetic acid, and picolinic acid) have predicated to possess hepato-toxicity effect. Similarly, one ligand (glyoxylic acid) has been predicted to possess a mutagenicity effect (**Table 6**). Molecular Physico-chemical, bioactivity score, ADME, and toxicity analysis results for three ligands, namely 3-phenyl lactic acid, 4-hydroxybenzoic acid, and kojic acid, have not been shown in the current study as reported by us in the previous study (**Narayanaswamy *et al.*, 2015).**

Table 6 Toxicity analysis of fifty-three selected (honey) ligands using Pro Tox II online server

Ligands	**HT[1]**	**CG[2]**	**IT[3]**	**MG[4]**	**CT[5]**	**AhR[6]**	**AR[7]**	**AR-LBD[8]**	**Aromatase**	**ER[9]**	**ER-LBD[10]**	**PPAR-γ[11]**	**nrf2/ARE[12]**	**HSE[14]**	**MMP[13]**	**p53[15]**	**ATAD5[16]**	**LD50[17]**	**Toxicity class**
Acetic acid	IA[18]	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	333	1
Alpha ketoglutaric acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	7100	6
Anthranilic acid	A[19]	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	1400	4
Ascorbic acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	3367	5
Benzoic acid	A	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	290	3
Butyric acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	91	3
Cis Aconitic acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	1320	4
Citramalic acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	2497	5
Citric acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	80	3
2,4-Dihydroxybenzoic acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	1250	4
2,6-Dihydroxybenzoic acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	1250	4
3,4-Dihydroxybenzoic acid	IA	A	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	2000	4
Erythorbic acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	3367	5
Folic acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	A	IA	IA	IA	IA	IA	IA	IA	135	3
Formic acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	162	3
Fumarate	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	1350	4
Fumaric acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	1350	4
D-Galacturonic acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	10000	6
Gluconic acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	1900	6
Glutamic acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	4500	5
Glutaric acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	2750	5
Glyoxylic acid	IA	IA	IA	A	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	3620	4
Hexanoic acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	94	3
3Hydroxyanthranilic acid	A	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	2000	4
2-Hydroxybutyric acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	1037	3
2-Hydroxyglutaric acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	1000	3
4-Hydroxyphenylacetic acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	1550	4
Isobutyric acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	300	3
Isocitric acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	80	3
Kynurenic acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	1550	4
Lactic acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	A	IA	IA	IA	IA	IA	IA	IA	75	3
Lactobionic acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	10000	6

Malic acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	2497	5
Malonic acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	19	2
Mandelic acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	2000	4
(2R)-2-Methoxybutanedioic acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	3030	5
Methylmalonic acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	19	2
Oxalic acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	660	4
2-oxopentanoic acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	600	3
Phenylacetic acid	A	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	2250	5
Picolinic acid	A	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	1550	4
Propionic acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	300	3
DL-Pyroglutamic acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	1000	4
Pyruvic acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	200	3
Quinic acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	9800	6
Shikimic acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	9000	6
Succinate	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	2260	5
Succinic acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	2260	5
Tartaric acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	2497	5
Valeric acid	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	134	3

Note: HT[1]- Hepatotoxicity, CG[2]- Carcinogenicity, IT[3]- Immunotoxicity, MG[4]- Mutagenicity, CT[5]- Cytotoxicity. [6]Aryl Hydrocarbon Receptor, [7]Androgen Receptor, [8]Androgen Receptor Ligand Binding Domain, [9]Estrogen Receptor Alpha, [10]Estrogen Receptor Ligand Binding Domain, [11]Peroxisome Proliferator-Activated Receptor Gamma, [12]Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element, [13]Heat shock factor response element, [14]Mitochondrial Membrane Potential, [15]Phosphoprotein (Tumor suppressor), [16]ATPase family AAA domain-containing protein 5, [17]Lethal Dose, [18]IA- Inactive,[19]A- Active.

Salovaara and coworkers (2002) had reported that nine organic acids (acetic acid, citric acid, fumaric acid, lactic acid, malic acid, oxalic acid, propionic acid, succinic acid, and tartaric acid) influence iron [both ferrous (Fe^{2+}) and ferric (Fe^{3+})] absorption in the human epithelial (Caco-2) cells. **Bu and coworkers (2012)** had reported that arachidonate binds to Ferritin and stimulates iron absorption. **Zanzoni and coworkers (2017)** had reported that oleate (fatty acid) binds to human Ferritin and enhances iron biomineralization. **Shamsi and coworkers (2021)** had reported that quercetin and naringenin (flavonoid) bind to human Ferritin by using molecular docking study.

The docking studies showed that folic acid (vitamin) and kynurenic acid (organic acid) had exhibited the maximum atomic contact energy (-227.41 and -133.39 kcal/mol) with human Ferritin as tabulated in **Table 7**. The present finding was in good agreement with the previous report, where kynurenic acid has shown a good effect on ferritin level (**Wenninger *et al*., 2019**). .In contrast, formic acid (organic acid) has shown the mininum atomic contact energy (+26.09 kcal/mol) with human Ferritin, which may be due to a very weak binding effect (**Akdoğan *et al.*, 2011**). Interestingly, 39 ligands showed interaction with Tyr 29, Asp 89, Cys 102, His 105, and Asn 109 amino acid residues of human Ferritin as shown in **Table 7**. The present finding was in good agreement with the previous report, where quercetin and naringenin have been shown to interact with Tyr 29 and Asp 89 of human Ferritin (**Shamsi *et al.*, 2021**). However, fourteen ligands (acetic acid, butyric acid, 2,6-dihydroxybenzoic acid, formic acid, glutamic acid, hexanoic acid, 4-hydroxyphenyl acetic acid, kynurenic acid (**Figure 1**), lactobionic acid, methylmalonic acid, oxalic acid, 3-phenyl lactic acid, pyruvic acid, and valeric acid) did not interact with amino acid residues of human Ferritin.

Table 7 The interaction energy analysis of fifty-three selected (honey) ligands with that of human Ferritin using PatchDock method

Ligands	**ACE[1]** (kcal/mol)	**Amino acid interaction**	**Bond distance** (Å)[2]
Acetic acid	-40.13	No interaction	-
Alpha ketoglutaric acid	-65.41	Tyr 29 Asn 109	3.5 2.4
Anthranilic acid	-108.95	Tyr 29	2.3
Ascorbic acid	-80.88	Tyr 29 Asn 109	2.8 and 3.3 3.0
Benzoic acid	-83.64	Asn 109	2.3
Butyric acid	-59.93	No interaction	-
Cis aconitic acid	-90.36	Tyr 29 Asn 109	2.9 and 3.3 2.9
Citramalic acid	-72.91	Tyr 29	3.3
Citric acid	-80.45	Tyr 29	3.2
2,4-Dihydroxybenzoic acid	-101.20	Tyr 29 Asn 109	2.6 2.8
2,6-Dihydroxybenzoic acid	-97.66	No interaction	-
3,4-Dihydroxybenzoic acid	-90.49	Tyr 29 Asn 109	3.5 2.3
Erythrobic acid	-80.88	Tyr 29 Asn109	2.8 and 3.3 3.0
Folic acid	-227.41	Cys102	3.1
Formic acid	+26.09	No interaction	-
Fumarate	-58.36	Asn 109	2.9 and 3.2
Fumaric acid	-48.51	His 105 Asn 109	3.2 2.2
D-Galacturonic acid	-94.51	Tyr 29 Asp 89 Asn 109	3.3 and 3.4 3.5 3.0
Gluconic acid	-70.53	Asn 109	2.3
Glutamic acid	-82.28	No interaction	-
Glutaric acid	-61.89	Asn 109	3.3
Glyoxalic acid	-47.21	Asp 89	3.4
Hexanoic acid	-81.49	No interaction	-
3-Hydroxyanthranilic acid	-116.09	Asn 109	2.5
4-Hydroxybenzoic acid	-89.92	Asn 109	3.5
2-Hydroxybutyric acid	-61.47	Tyr 29	2.8
2-Hydroxyglutaric acid	-77.67	Tyr 29 Asn 109 Cys 102 Asp 89	3.3 3.3 2.4 3.3

4-Hydroxyphenylacetic acid	-99.46	No interaction	-
Isobutyric acid	-68.35	Asp 89	1.8
Isocitric acid	-79.03	Tyr 29 Tyr 29	3.1 3.3
Kojic acid	-85.90	Cys 102	1.9
Kynurenic acid	-133.39	No interaction	-
Lactic acid	-56.08	Tyr 29	3.3
Lactobionic acid	-125.04	No interaction	-
Malic acid	-60.47	Tyr 29 Asp 89 Asn 109	2.4 3.1 3.6
Malonic acid	-48.15	Tyr 29 Tyr 29 Asn 109	3.4 3.2 3.1
Mandelic acid	-85.41	His 105 Asn 109 Asn 109	3.2 2.9 3.4
(2R)-2-Methoxybutanedioic acid	-60.11	Tyr 29 Tyr 29 Asp 89	3.1 2.0 3.4
Methylmalonic acid	-65.07	No interaction	-
Oxalic acid	-60.18	No interaction	-
2-oxopentanoic acid	-68.75	Tyr 29	3.4
Phenylacetic acid	-93.11	Asn 109	2.4
3-Phenyllactic acid	-99.06	No interaction	-
Picolinic acid	-80.50	Tyr 29 Asn 109	2.6 3.3
Propionic acid	-46.86	Tyr 29	3.0
DL-Pyroglutamic acid	-93.18	Asn 109	3.1
Pyruvic acid	-48.84	No interaction	-
Quinic acid	-110.74	Tyr 29 Asp 89	2.4 3.0
Shikimic acid	-84.84	Tyr 29	2.8
Succinate	-56.69	Asn 109	3.0
Succinic acid	-68.14	Tyr 29	2.5
Tartaric acid	-61.56	Tyr 29 Asp 89 Asn 109 Cys 102	2.6 3.5 2.9 2.0
Valeric acid	-72.28	No interaction	-

Note: ACE[1] – Atomic contact energy, Å[2] – Angstrom

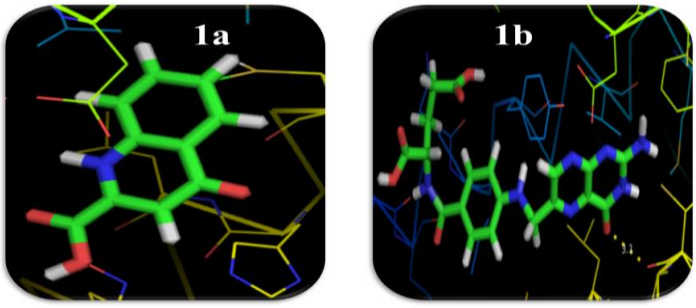

Figure 1 Represents the interaction analysis of human Ferritin with that of a) kynurenic acid and b) folic acid using PyMOL software
Note: Dotted lines represent the hydrogen bond between the ligand (1b-folic acid) and protein (Ferritin).

The docking studies showed that folic acid (vitamin) and kynurenic acid (organic acid) had exhibited the highest atomic contact energy (-334.94 and -102.84 kcal/mol) with human Transferrin as tabulated in **Table 8**. The present finding was in good agreement with the previous study, where kynurenic acid has shown a good correlation with soluble transferrin level (**Wenninger *et al.*, 2019**). In contrast, glyoxylic acid (organic acid) has shown the mininum atomic contact energy (-12.98 kcal/mol) with human Transferrin. Interestingly, 41 ligands showed interaction with Glu 15, Gly 34, Pro 35, Thr 61, Asp 63, Glu 83, Arg 124, His 249, Asp 277, Lys 291, and Asp 292 amino acid residues of human Transferrin as shown in **Table 8** and **Figure 2**. The present finding was in close agreement with the previous report, where the Arg124 plays a critical role in regulating the iron from the N-lobe of human Transferrin (**Adams *et al.*, 2003**). However, 12 ligands (butyric acid, citramalic acid, formic acid, 4-hydroxyphenyl acetic acid, isobutyric acid, isocitric acid, lactic acid, malic acid, malonic acid, phenyl lactic acid, propionic acid, and valeric acid) did not interact with amino acid residues of human Transferrin.

Table 8 The interaction energy analysis of fifty-three selected (honey) ligands with that of human Transferrin using PatchDock method

Ligands	**ACE[1]** (kcal/mol)	**Amino acid interaction**	**Bond distance** (Å)[2]
Acetic acid	-35.40	Pro 35	2.4
Alpha ketoglutaric acid	-61.94	Glu 15 Asp 63	2.5 2.8
Anthranilic acid	-83.90	Glu 15	2.5
Ascorbic acid	-44.36	Thr 61 Asp 63 Arg 124 Asp 292	2.5 3.4 2.4, 3.4 and 3.6 2.0
Benzoic acid	-72.43	Asp 63 Arg 124	2.8 2.0 and 2.5
Butyric acid	-36.85	No interaction	-
Cis aconitic acid	-30.07	Arg 124	3.5
Citramalic acid	-55.55	No interaction	-
Citric acid	-51.47	Arg 124 Asp 63	3.1 3.1

2,4-Dihydroxybenzoic acid	-23.74	Arg 124	2.6 and 3.3
2,6-Dihydroxybenzoic acid	-68.76	Asp 63 Arg 124 His 249	2.9 2.3 2.8
3,4-Dihydroxybenzoic acid	-80.76	Glu 15 Asp 63	2.3 3.2
Erythrobic acid	-44.36	Thr 61 Asp 63 Arg 124 Asp 292	2.5 3.4 2.4, 3.4 and 3.6 2.0
Folic acid	-334.94	Thr 61 Arg 124 Lys 291	2.7 3.5 3.1
Formic acid	-42.57	No interaction	-
Fumarate	-42.34	Asp 63	3.3
Fumaric acid	-30.64	Arg 124	2.3 and 2.6
D-Galacturonic acid	-55.03	Thr 61 Asp 63	3.1 3.3
Gluconic acid	-33.30	Glu 15 Thr 61 Arg 124	2.3 1.7 2.2
Glutamic acid	-57.24	Arg 124 His 249	2.3 and 3.2 3.3
Glutaric acid	-45.94	Arg 124	2.3 and 2.6
Glyoxylic acid	-12.98	Asp 277	2.4
Hexanoic acid	-66.78	Glu 83	2.6
3-Hydroxyanthranilic acid	-53.68	Glu 15	2.0
4-Hydroxybenzoic acid	-57.70	Asp 63	2.9
2-Hydroxybutyric acid	-48.34	Thr 61	2.3
2-Hydroxyglutaric acid	-47.89	Asp 63 Arg 124	2.5 2.3
4-Hydroxyphenylacetic acid	-60.40	No interaction	-
Isobutyric acid	-56.14	No interaction	-
Isocitric acid	-54.36	No interaction	-
Kojic acid	-69.98	Arg 124	2.6
Kynurenic acid	-102.84	Glu 15 Arg 124 Leu 294	2.9 3.6 3.2
Lactic acid	-48.88	No interaction	-
Lactobionic acid	-94.23	Asp 63 Arg 124 Lys 291	3.0 3.5 2.5
Malic acid	-24.01	No interaction	-
Malonic acid	-26.64	No interaction	-
Mandelic acid	-47.14	No interaction	-
(2R)-2-Methoxybutanedioic acid	-23.74	Arg 124	2.6 and 3.3
Methylmalonic acid	-18.77	Arg 124	2.4, 3.0 and 3.2
Oxalic acid	-30.08	Gly 34	2.4
2-oxopentanoic acid	-41.21	Glu 15 Arg 124	2.3 2.6, 2.7 and 3.2
Phenylacetic acid	-56.10	No interaction	-
3-Phenyllactic acid	-48.58	Thr 61	2.7
Picolinic acid	-57.38	Asp 63	2.9
Propionic acid	-23.28	No interaction	-
DL-Pyroglutamic acid	-43.18	Leu 294	2.4
Pyruvic acid	-18.92	Arg 124	2.8, 3.2 and 3.4
Quinic acid	-43.50	Arg 124	3.3
Shikimic acid	-29.12	Arg 124	3.2 and 3.5
Succinate	-49.76	Asp 63	3.3
Succinic acid	-20.57	Arg 124 Leu 294	2.7, 2.9 and 3.5 2.3
Tartaric acid	-50.69	Glu 15 Arg 124	2.1 1.9 and 2.4
Valeric acid	-36.03	No interaction	-

Note: ACE[1] **–** Atomic contact energy, Å[2] – Angstrom

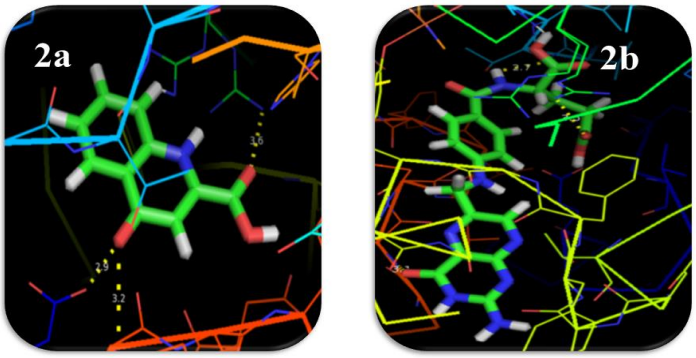

Figure 2 Represents the interaction analysis of human Transferrin with that of a) kynurenic acid and b) folic acid using PyMOL software.

Note: Dotted lines represent the hydrogen bond between the ligand (2a- kynurenic acid and 2b-folic acid) and protein (Transferrin).

The docking studies showed that folic acid (vitamin) and kynurenic acid (organic acid) had exhibited the highest atomic contact energy (-325.69 and -197.64 kcal/mol) with human Hepcidin as tabulated in **Table 9**. In contrast, lactic acid (organic acid) has shown the mininum atomic contact energy (-8.77 kcal/mol) with human Hepcidin. Interestingly, 22 ligands showed interaction with His 3, Phe 4, Ile 8, Cys 14, Arg 16, Cys 19, Met 21, Cys 22, Cys 23, and Lys 24 amino acid residues of human Hepcidin as shown in **Table 9**. The present finding was in close agreement with the previous report, where the Phe 9, Cys 10, Cys 13, Cys 14, His 15, Arg 16, Ser 17, Cys 19, Gly 20, Met 21, and Cys 22 were the most common interaction amino acid residues of human Hepcidin (**Angmo *et al.*, 2017**). However, 31 ligands, including folic acid and kynurenic acid, did not interact with amino acid residues of human Hepcidin (as shown in **Figure 3)**.

Table 9 The interaction energy analysis of fifty-three selected (honey) ligands with that of human Hepcidin using the PatchDock method

Ligands	**ACE**[1] **(**kcal/mol)	**Amino acid interaction**	**Bond distance** (Å)[2]
Acetic acid	-52.58	Cys 23	2.5
Alpha ketoglutaric acid	-134.03	Cys 23	2.7
Anthranilic acid	-144.55	No Interaction	-
Ascorbic acid	-148.46	No Interaction	-
Benzoic acid	-33.83	No Interaction	-
Butyric acid	-9.75	Arg 16	3.5
Cis aconitic acid	-143.89	Cys 14 Lys 24	3.5 2.2
Citramalic acid	-16.01	Met 21	3.1
Citric acid	-166.99	Ile 8 Lys 24	3.2 2.8
2,4-Dihydroxybenzoic acid	-152.89	No Interaction	-
2,6-Dihydroxybenzoic acid	-144.39	No Interaction	-
3,4-Dihydroxybenzoic acid	-162.43	No Interaction	-
Erythrobic acid	-148.46	No Interaction	-
Folic acid	-325.69	No Interaction	-
Formic acid	-34.44	No Interaction	-
Fumarate	-13.45	No Interaction	-
Fumaric acid	-13.16	No Interaction	-
D-Galacturonic acid	-172.31	No Interaction	-
Gluconic acid	-154.85	No Interaction	-
Glutamic acid	-125.36	Lys 24	2.2
Glutaric acid	-118.65	Lys 24	2.6
Glyoxalic acid	-41.60	Lys 24	2.4
Hexanoic acid	-24.53	Met 21	2.8 and 3.5
3-Hydroxyanthranilic acid	-173.46	No Interaction	-
4-Hydroxybenzoic acid	-35.55	Met 21	3.4
2-Hydroxybutyric acid	-19.55	No Interaction	-
2-Hydroxyglutaric acid	-126.98	No Interaction	-
4-Hydroxyphenylacetic acid	-66.92	No Interaction	-
Isobutyric acid	-15.95	No Interaction	-
Isocitric acid	-133.60	Cys 14 Cys 23	3.4 2.7
Kojic acid	-124.08	Cys 23	2.6
Kynurenic acid	-197.64	No Interaction	-
Lactic acid	-8.77	Cys 19 Arg 16	2.0 2.5
Lactobionic acid	-147.47	No Interaction	-
Malic acid	-17.27	Arg 16 Met 21	3.2 3.1
Malonic acid	-10.13	No Interaction	-
Mandelic acid	-145.22	No Interaction	-
(2R)-2-Methoxybutanedioic acid	-9.50	Cys 19 Met 21	2.2 2.8
Methylmalonic acid	-108.71	Cys 22	2.6
Oxalic acid	-41.10	His 3 His 3 Phe 4	2.7 2.7 3.4
2-oxopentanoic acid	-21.54	Met 21 Met 21	3.0 3.4
Phenylacetic acid	-106.68	No Interaction	-
3-Phenyllactic acid	-33.40	No Interaction	-
Picolinic acid	-122.14	No Interaction	-
Propionic acid	-46.35	No Interaction	-
DL-Pyroglutamic acid	-109.64	No Interaction	-
Pyruvic acid	-11.94	Arg 16	3.3
Quinic acid	-157.29	Cys 23	1.7

Shikimic acid	-160.74	No Interaction	-
Succinate	-15.42	Arg 16	3.3
Succinic acid	-56.78	No Interaction	-
Tartaric acid	-122.17	No Interaction	-
Valeric acid	-105.79	No Interaction	-

Note: ACE[1] – Atomic contact energy, Å[2] – Angstrom

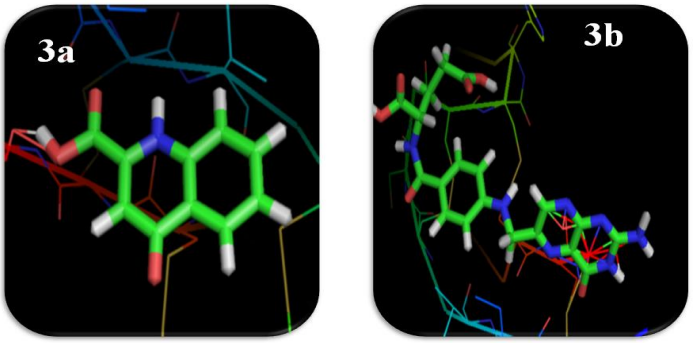

Figure 3 Represents the interaction analysis of human Hepcidin with that of a) kynurenic acid and b) folic acid using PyMOL software.

CONCLUSION

In the present study, all fifty-three selected ligands showed the potential to dock with all the targeted proteins. Interestingly folic acid (vitamin) and kynurenic acid (organic acid) exhibited the highest atomic contact energy for human Ferritin, Transferrin, and Hepcidin. In contrast, formic acid, glyoxylic acid, and lactic acid have shown the least atomic contact energy with Ferritin, Transferrin, and Hepcidin, respectively. Thus the present study has highlighted these forty-six ligands of honey and seven other ligands (such as anthranilic acid, erythrobic acid, fumarate, 3-hydroxy anthranilic acid, lactobionic acid, picolinic acid, and succinate) as modulators of human Ferritin, Transferrin, and Hepcidin via by preventing associated disorders like iron deficiency anemia and neurological disorders.

REFERENCES

Adams, T. E., Mason, A. B., He, Q. Y., Halbrooks, P. J., Briggs, S. K., Smith, V. C., MacGillivray, R. T., & Everse, S. J. (2003). The position of arginine 124 controls the rate of iron release from the N-lobe of human serum transferrin: A structural study. *Journal of Biological Chemistry*, *278*(8), 6027-6033. https://dx.doi.org/10.1074/jbc.m210349200

Akdoğan, E. D., Erman, B., & Yelekçi, K. (2011). *In silico* design of novel and highly selective lysine-specific histone demethylase inhibitors. *Turkish Journal of Chemistry*, *35*(4), 523-542. http://dx.doi.org/10.3906/kim-1102-985

Anand, S., Deighton, M., Livanos, G., Morrison, P. D., Pang, E. C., & Mantri, N. (2019). Antimicrobial activity of Agastache honey and characterization of its bioactive compounds in comparison with important commercial honeys. *Frontiers in Microbiology*, *10*, 263. https://dx.doi.org/10.3389/fmicb.2019.00263

Angmo, S., Tripathi, N., Abbat, S., Sharma, S., Singh, S. S., Halder, A., Yadav, K., Shukla, G., Sandhir, R., Rishi, V., Bharatam, P.V., Yadav, H., & Singhal, N. K. (2017). Identification of guanosine 5′-diphosphate as potential iron mobilizer: Preventing the hepcidin-ferroportin interaction and modulating the interleukin-6/stat-3 pathway. *Scientific reports*, *7*(1), 1-12. https://dx.doi.org/ 10.1038/srep40097

Banerjee, P., Eckert, A. O., Schrey, A. K., & Preissner, R. (2018). ProTox-II: a webserver for the prediction of toxicity of chemicals. *Nucleic acids Research*, *46*(W1), W257-W263. https://dx.doi.org/10.1093/nar/gky318

Beretta, G., Caneva, E., & Facino, R. M. (2007). Kynurenic acid in honey from arboreal plants: MS and NMR evidence. *Planta Medica*, *73*(15), 1592-1595. https://dx.doi.org/10.1055/s-2007-993740

Bermejo, F., & García-López, S. (2009). A guide to diagnosis of iron deficiency and iron deficiency anemia in digestive diseases. *World journal of gastroenterology: WJG*, *15*(37), 4638. https://dx.doi.org/10.3748/wjg.15.4638

Bu, W., Liu, R., Cheung-Lau, J.C., Dmochowski, I.J., Loll, P.J., & Eckenhoff, R.G. (2012) Ferritin couples iron and fatty acid metabolism. *Federation of American Societies for Experimental Biology Journal*, 26(6):2394-2400. https://dx.doi.org/10.1096/fj.11-198853

Chobot, V., Hadacek, F., Weckwerth, W., & Kubicova, L. (2015). Iron chelation and redox chemistry of anthranilic acid and 3-hydroxyanthranilic acid: A comparison of two structurally related kynurenine pathway metabolites to obtain improved insights into their potential role in neurological disease development. *Journal of Organometallic Chemistry*, *782*, 103-110. https://dx.doi.org/10.1016/j.jorganchem.2015.01.005

Christina, I., Radhakrishnan, N., & Vallivitan, K. (2021). Molecular docking analysis of selected *Clitoria ternatea* constituents as matrix metalloproteinases (MMP 2 & MMP 9) inhibitors. *Rasayan Journal of Chemistry*, *14*(1). 659-664. https://dx.doi.org/10.31788/RJC.2021.1416107

Cianciosi, D., Forbes-Hernández, T. Y., Afrin, S., Gasparrini, M., Reboredo-Rodriguez, P., Manna, P. P., Zhang, J., Bravo Lamas, L., Martínez Flórez, S., Agudo Toyos, P., & Quiles, J. L. (2018). Phenolic compounds in honey and their associated health benefits: A review. *Molecules*, *23*(9), 2322. https://dx.doi.org/10.3390/molecules23092322

Ciulu, M., Solinas, S., Floris, I., Panzanelli, A., Pilo, M. I., Piu, P.C., Spano, N., & Sanna, G. (2011). RP-HPLC determination of water-soluble vitamins in honey. *Talanta*, *83*(3), 924-929. http://dx.doi.org/10.1016/j.talanta.2010.10.059

Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports*, *7*(1), 1-13. https://dx.doi.org/10.1038/srep42717

Derewiaka, D., Majewska, E., Kuzak, K., & Szadkowska, D. (2021). Comparison of Volatiles and Chemical Composition of Traditional and Non-Traditional Honey Available on the Polish Market. *Applied Sciences*, *11*(14), 6371. https://dx.doi.org/10.3390/APP11146371

Drakesmith, H., & Prentice, A. M. (2012). Hepcidin and the iron-infection axis. *Science*, *338*(6108), 768-772. https://dx.doi.org/10.1126/science.1224577

Echigo, T., & Takenaka, T. (1974). Production of organic acids in honey by honeybees. Journal of the Agricultural Chemical Society of Japan, *48* (4), 225-230.

El Baaboua, A., El Maadoudi, M., Bouyahya, A., Belmehdi, O., Kounnoun, A., Zahli, R., & Abrini, J. (2018). Evaluation of antimicrobial activity of four organic acids used in chicks feed to control *Salmonella typhimurium*: Suggestion of amendment in the search standard. *International Journal of Microbiology*, *2018*, 7352593. https://dx.doi.org/10.1155/2018/7352593

Escandar, G. M., Olivieri, A. C., González-Sierra, M., & Sala, L. F. (1994). Iron (III) complexes of lactobionic acid: equilibrium and structural studies in aqueous solution. *Journal of the Chemical Society, Dalton Transactions*, (8), 1189-1192. https://dx.doi.org/10.1039/DT9940001189

Fidler, M. C., Davidsson, L., Zeder, C., & Hurrell, R. F. (2004). Erythorbic acid is a potent enhancer of nonheme-iron absorption. *The American Journal of Clinical Nutrition*, *79*(1), 99-102. http://dx.doi.org/10.1093/ajcn/79.1.99

Gillooly, M., Bothwell, T. H., Torrance, J. D., MacPhail, A. P., Derman, D. P., Bezwoda, W. R., Mills, W., Charlton, R. W., & Mayet, F. (1983). The effects of organic acids, phytates and polyphenols on the absorption of iron from vegetables. *British Journal of Nutrition*, *49*(3), 331-342. https://dx.doi.org/10.1079/bjn19830042

Hazell, T., & Johnson, I. T. (1987). *In vitro* estimation of iron availability from a range of plant foods: influence of phytate, ascorbate and citrate. *British Journal of Nutrition*, *57*(2), 223-233. https://dx.doi.org/10.1079/bjn19870028

Horváth, K., & Molnár-Perl, I. (1998). Simultaneous GC-MS quantitation of *o*-phosphoric, aliphatic and aromatic carboxylic acids, proline, hydroxymethylfurfurol and sugars as their TMS derivatives: In honeys. *Chromatographia*, *48*(1-2), 120-126. http://dx.doi.org/10.1007/BF02467527

Koulis, G. A., Tsagkaris, A. S., Aalizadeh, R., Dasenaki, M. E., Panagopoulou, E. I., Drivelos, S., Halagarda, M., Georgiou, C. A., Proestos, C., & Thomaidis, N. S. (2021). Honey Phenolic Compound Profiling and Authenticity Assessment Using HRMS Targeted and Untargeted Metabolomics. *Molecules*, *26*(9), 2769. http://dx.doi.org/10.3390/molecules26092769

Mato, I., Huidobro, J. F., Simal-Lozano, J., & Sancho, M. T. (2006). Analytical methods for the determination of organic acids in honey. *Critical Reviews in Analytical Chemistry*, *36*(1), 3-11. https://dx.doi.org/10.1080/10408340500451957

Mohan, S., Prabhakaran, V. S., & Narayanaswamy, R. (2022). In Silico Analysis of Cissus rotundifolia Constituents as Human Neutrophil Elastase (HNE), Matrix Metalloproteinases (MMP 2 and MMP 9), and Tyrosinase Inhibitors. *Applied Biochemistry and Biotechnology*, **194**, 232–245. https://dx.doi.org/10.1007/s12010-021-03758-8

Nair, K. M., & Iyengar, V. (2009). Iron content, bioavailability & factors affecting iron status of Indians. *Indian Journal of Medical Research*, *130*(5), 634-645.

Nair, K. M. (2019). Contextualizing the principles of iron fortification of foods in India. *Bulletin of the Nutrition Foundation of India*, *40*(2), 1-5.

Narayanaswamy, R., Kok Wai, L., & Ismail, I. S. (2015). *In silico* analysis of selected honey constituents as human neutrophil elastase (HNE) and matrix metalloproteinases (MMP 2 and 9) inhibitors. *International Journal of Food Properties*, *18*(10), 2155-2164. http://dx.doi.org/10.1080/10942912.2014.963870

Nelson, E. K., & Mottern, H. H. (1931). Some organic acids in honey. *Industrial & Engineering Chemistry*, *23*(3), 335-336. https://dx.doi.org/10.1021/ie50255a033

Nozal, M. J., Bernal, J. L., Diego, J. C., Gómez, L. A., & Higes, M. (2003). HPLC determination of low molecular weight organic acids in honey with series-coupled ion-exclusion columns. *Journal of Liquid Chromatography & Related Technologies*, *26*(8), 1231-1253. https://dx.doi.org/10.1081/JLC-120020107

Prica, N., Baloš, M. Ž., Jakšić, S., Mihaljev, Ž., Kartalović, B., Babić, J., & Savić, S. (2014). Moisture and acidity as indicators of the quality of honey originating from Vojvodina region. *Archives of Veterinary Medicine*, *7*(2), 99-109. https://dx.doi.org/10.46784/e-avm.v7i2.135

Puścion-Jakubik, A., Borawska, M. H., & Socha, K. (2020). Modern Methods for Assessing the Quality of Bee Honey and Botanical Origin Identification. *Foods*, *9*(8), 1028. http://dx.doi.org/10.3390/foods9081028

Sabatini, A. G., Marcazzan, G. L., Colombo, R., & Garagnani, M. (1994). Applicazione di un metodo enzimatico per la determinazione dell'acido formico e dell'acido lattico presenti nel miele. *Apicoltura*, *9*, 135-145.

Salovaara, S., Sandberg, A. S., & Andlid, T. (2002). Organic acids influence iron uptake in the human epithelial cell line Caco-2. *Journal of Agricultural and Food Chemistry*, *50*(21), 6233-6238. https://dx.doi.org/10.1021/jf0203040

Seraglio, S. K. T., Silva, B., Bergamo, G., Brugnerotto, P., Gonzaga, L. V., Fett, R. and Costa, A. C. O. (2019). An overview of physicochemical characteristics and health-promoting properties of honeydew honey. *Food Research International*, *119*, 44-66. http://dx.doi.org/10.1016/j.foodres.2019.01.028

Shamsi, A., Shahwan, M., Khan, M. S., Husain, F. M., Alhumaydhi, F. A., Aljohani, A. S., Rehman, M. T., Hassan, M. I., & Islam, A. (2021). Elucidating the interaction of human ferritin with quercetin and naringenin: Implication of natural products in neurodegenerative diseases: Molecular docking and dynamics simulation insight. *ACS Omega*, *6*(11), 7922-7930. https://dx.doi.org/10.1021/acsomega.1c00527

Soldin, O. P., Bierbower, L. H., Choi, J. J., Choi, J. J., Thompson-Hoffman, S., & Soldin, S. J. (2004). Serum iron, ferritin, transferrin, total iron binding capacity, hs-CRP, LDL cholesterol and magnesium in children; new reference intervals using the Dade Dimension Clinical Chemistry System. *Clinica Chimica Acta*, *342*(1-2), 211-217. https://dx.doi.org/10.1016/j.cccn.2004.01.002

Solomon, A., Hailu, M., Manoj, V. R., Chen, Yen-Po., & Radhakrishnan, N. (2021) Molecular docking analysis of *Azadirachta indica* constituents as inhibitors of aflatoxin polyketide synthase (APKS). *Rasayan Journal of Chemistry*, *14*(2), 920-929. https://dx.doi.org/10.31788/RJC.2021.1426063

Speer, V. K., & Montag, A. (1985). Phenylacetic acid and benzoic acid contents in honey. *Lebensmittelchem. Gerichtl. Chem*, *39*, 11-12.

Starowicz, M., Hanus, P., Lamparski, G., & Sawicki, T. (2021). Characterizing the Volatile and Sensory Profiles, and Sugar Content of Beeswax, Beebread, Bee Pollen, and Honey. *Molecules*, *26*(11), 3410. https://dx.doi.org/10.3390/molecules26113410

Steeg, E., & Montag, A. (1988). Quantitative Bestimmung aromatischer Carbonsäuren in Honig. *Zeitschrift für Lebensmittel-Untersuchung und Forschung*, *187*(2), 115-120.

Tan, S. T., Holland, P. T., Wilkins, A. L., & Molan, P. C. (1988). Extractives from New Zealand honeys. 1. White clover, manuka and kanuka unifloral honeys. *Journal of Agricultural and Food Chemistry*, *36*(3), 453-460. https://dx.doi.org/10.1021/jf00081a012

Testa, U., Louache, F., Titeux, M., Thomopoulos, P., & Rochant, H. (1985). The iron-chelating agent picolinic acid enhances transferrin receptors expression in human erythroleukaemic cell lines. *British Journal of Haematology*, *60*(3), 491-502. https://dx.doi.org/10.1111/j.1365-2141.1985.tb07446.x

Tran, V. G., & Zhao, H. (2021). Engineering robust microorganisms for organic acid production. *Journal of Industrial Microbiology and Biotechnology*.

Verzera, A., Campisi, S., Zappalà, M., & Bonaccorsi, I, (2001). SPME-GC-MS analysis of honey volatile components for the characterization of different floral origin. *American Laboratory*, *33*(15), 18-23. https://dx.doi.org/10.1093/jimb/kuab067

Wenninger, J., Meinitzer, A., Holasek, S., Schnedl, W. J., Zelzer, S., Mangge, H., Herrmann, M., & Enko, D. (2019). Associations between tryptophan and iron metabolism observed in individuals with and without iron deficiency. *Scientific Reports*, *9*(1), 1-9. https://dx.doi.org/10.1038/s41598-019-51215-8

White Jr, J. W., 1978. Honey. *Advances in Food Research*, *24*, 287-374. https://dx.doi.org/10.1016/s0065-2628(08)60160-3

Zanzoni, S., Pagano, K., D'Onofrio, M., Assfalg, M., Ciambellotti, S., Bernacchioni, C., Turano, P., Aime, S., Ragona, L., & Molinari, H. (2017). Unsaturated Long-Chain Fatty Acids Are Preferred Ferritin Ligands That Enhance Iron Biomineralization. *Chemistry–A European Journal*, *23*(41), 9879-9887. https://dx.doi.org/10.1002/chem.201701164

Zielińska-Dawidziak, M. (2015). Plant ferritin—a source of iron to prevent its deficiency. *Nutrients*, *7*(2), 1184-1201. https://dx.doi.org/10.3390/nu7021184