BIOCHEMICAL ANALYSIS OF *LEONURUS SIBIRICUS* AND PREDICTION OF ITS BIOACTIVE COMPOUND-PROTEIN INTERACTIONS IN *HOMO SAPIENS*

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ABSTRACT

This study was conducted to investigate the proximate composition of *L. sibiricus*, an herbaceous medicinal plant, and explore the potential effects of its bioactive compounds, rutin and quercetin. Samples were collected from Dinajpur and Nilphamari to assess proximate composition. Proximate composition analysis revealed variations of ash, starch, fat, protein, non-reducing sugar (sucrose), invert (total) sugar, and reducing sugar contents of *L. sibiricus*. These findings indicate that proximate compositions and mineral contents varied depending on the growing place, soil and environmental factors. Furthermore, bioinformatics tools were utilized to investigate the effects of bioactive compounds rutin and quercetin of *L. sibiricus* in *Homo sapiens*. In this study, both rutin and quercetin interacted with 10 proteins, separately. Each individual protein interacted with another 10 proteins and found 100 proteins for rutin and 99 proteins for quercetin. However, 199 proteins were used to blast search in NCBI database and blasted results found only 177 proteins. Venn diagram analysis showed only four (4) common proteins interacted with rutin and quercetin. Functional annotation analysis revealed both positive and negative regulations of rutin and quercetin for biological process, molecular functions and cellular component in human health. Interestingly, KEGG pathway, compound and drug analysis exhibited that some potential drugs have already been discovered by rutin and quercetin that are mostly used for vascular protectant, ophthalmic, liver function improving agent and antimicrobial activity. A number of genes or enzymes involved in rutin and quercetin biosynthesis pathway retrieved in KEGG database. This study revealed that genetic engineering might enhances the biosynthesis of rutin and quercetin in honeyweed that could be utilized for production of valuable drug in industry for controlling different human and animal diseases in Bangladesh.

KEYWORDS: Functional annotation, honeyweed, proximate composition, quercetin, rutin

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Introduction

Medicinal plants have been utilized for treatment to various diseases in human and domestic animals in Bangladesh by folk practitioner since ancient time. In recent times, advancements in science, technology, and medical science have revolutionized the primary healthcare system. Medicinal plants have been extensively documented for their therapeutic uses, including the treatment of diabetes, cardiovascular diseases (CVD), menstrual disorder and other non-communicable diseases (Rahman *et al.*, 2015). These are characterized by possessing substances in their organs that have healing properties or serve as precursors for the synthesis of beneficial drugs. As natural substances are often more effective in specific applications, synthesizing them has gained significant interest in the pharmaceutical industry. The holistic nature of herbal medicine, which offers comprehensive solutions by addressing underlying systemic imbalances, has contributed to its growing popularity. Accordingly, the discovery and development of new chemical substances for controlling diabetes, CVD, cancer are greatly needed and it has been promoted studies of traditionally used medicinal plants, which are generally considered to be very important sources of bioactive substances (Saha *et al.*, 2012). Traditional medicinal plants demonstrate the beneficial outcomes from a long time without having recognized molecular basis of mode of actions. *Leonurus sibiricus* is one of the potential medicinal plants belongs to the family of Lamiaceae used by folk practitioner in Bangladesh (Sayed *et al.*, 2016). It is an omnipresent herbaceous plant that has been

used as a traditional and medicinal herb for many years in Asia, South America and Europe (Sayed *et al.,* 2016; Zhang *et al.,* 2014). This species is known to have antibacterial, anti-inflammatory, and antioxidant activity and has demonstrated a reduction of intracellular reactive oxygen species (Sitarek *et al.,* 2016). *L. sibiricus* has been used to treat in menoxenia, dysmenorrhea, amenorrhea, lochia, edema of the body, oliguresis, sores, ulcerations of many years (Shang *et al.,* 2014). Herbal medicine of *L. sibiricus* are likely to be contaminated with some metals (Saeed, *et al.,* 2010). Despite the presence flavone, flavonoids and phenolic compounds in *L. sibiricus* herbal plants, there are some potential macro and micro minerals that could be provide health beneficiary effects. Among the bioactive compounds, the flavonoid quercetin and its glycoside rutin, which exert antidiabetic, vascular protectant and many other functional activities lead healthy life in human (Kittl, *et al.,* 2016). The presence of bioactive compounds such as diterpenes, triterpenes, flavonoids, and phenolic acids in *L. sibiricus* highlights its therapeutic potential against various diseases, although their molecular mechanisms are yet to be fully explored (Sayed *et al.,* 2016). Therefore, this study aims to comprehensively investigate the composition, properties, and potential applications of *L. sibiricus*, focusing on determining its proximate composition, assessing mineral contents and exploring the interactions as well as molecular mechanisms of its bioactive compounds rutin and quercetin for the development of safe and effective herbal-based treatments.

Materials and Methods

Honeyweed (*L. sibiricus*) samples were collected from Nilphamari and Dinajpur districts of Bangladesh in January 2018. The samples were washed, dried, and ground for proximate composition analysis, macronutrient assessment, and investigating the interaction of rutin and quercetin in *Homo sapiens*. Our previous study enlisted some bioactive compounds in *L. siribicus* (Sayed *et al.,* 2016) and we selected two potential compounds rutin and quercetin for bioinformatics analysis to find the potential effects on human health.

Network retrieval

The interaction of rutin and quercetin with proteins of *Homo sapiens* was explored using the STITCH4.0 (http://stitch.embl.de/) web server (Kuhn *et al.,* 2014), which consolidates protein-chemical interactions from various data sources. Rutin and quercetin interaction with human proteins was examined at a high confidence level of 0.7 to focus on robust protein-ligand interactions. To identify protein-protein interactions within the same species, the list of proteins interacting with rutin and quercetin was further analyzed to identify the protein-protein interaction (PPI) using the STRING9.1 (http://string-db.org/) web server, which integrates known and predicted protein interactions based on both physical and functional associations.

Protein accession, amino acid sequence retrieval and functional annotation analysis

The protein sequences for PPI of rutin and quercetin were used to search in protein database UniProtKB (https://www.uniprot.org/uniprotkb) to find the accession number, gene name and number amino acid. We have found accession number of 199 out of 200 proteins for rutin and quercetin. It was not found any information about this "ENSG0000029209" protein for quercetin in UniProtKB and NCBI data base (https://www.ncbi.nlm.nih.gov/protein) as well. Again, we searched using the "ENSG0000029209" protein in (https://www.ensembl.org) using whole genome of *Homo sapiens* and found in the class of Long noncoding RNA (LncRNA) which is not translated into protein. That is why, it was not found any information in UniProtKB and NCBI data base. However, we searched and blasted the 199-protein accession in NCBI and found 177-protein accession, where 22-protein accession was duplicated and removed automatically by NCBI database. Amino acids sequences of 177 proteins were downloaded as FASTA file for functional annotation analysis. Functional annotation analysis was done using the software OmicBox (Version 3.0.29) as described previously (Götz, *et al.,* 2008). Briefly, fasta file of 177 amino sequences was uploaded in OMICS box to blast. Using blasted data GO mapping and GO annotation were done. Finally, functional annotation analysis was done for finding the biological process, molecular function and cellular components of different proteins in human.

Venn diagram

Protein ID for PPI of rutin and quercetin interaction was taken in the (.txt) file separately. These text file was uploaded in the OMICS box software for the analysis of Venn diagram (Götz, *et al.,* 2008). For Venn diagram, we exclude the protein ID: A0A0U1RQF0, because this protein sequence is found as un-reviewed in the UniProtKB protein database. We also did not find the sequence in NCBI database during the blast search.

KEGG Pathway

In KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis (https://www.genome.jp/kegg/pathway.html), we searched the rutin and quercetin in KEGG compound database and we found rutin (Compound ID: C04194) and quercetin (Compound ID: C00389) and their derivatives compound. Subsequently, rutin and quercetin used to DB search for SIMCOMP to compute the drug using global search and we found a numbers of drug has already been discovered by these two parent compounds. We retrieved drug with structure from each that have similar score at least 0.90. Finally, rutin and quercetin used to search in default to find their biosynthesis pathway from KEGG database with enzymes and subsequently retrieved the flavone and flavonol biosynthesis pathway (Pathway: map00944).

Data Analysis

The experiments on *L. sibiricus* involved triplicate analyses for proximate composition and macronutrient assessment. Results were presented as means with standard deviations and analyzed using MSTATC Program with t-tests.

Results and Discussion

Proximate analysis

The proximate analysis of *L. siribicus* presented in Table 1 where, the total sugar content in *L. sibiricus* collected from Dinajpur and Nilphamari was determined to be 3.39 and 4.20 %, respectively, with no significant difference between the two locations. The reducing sugar content was found to be

1.25 and 2.37% in Dinajpur and Nilphamari samples, respectively. Similarly, the non-reducing sugar (sucrose) content in Dinajpur and Nilphamari samples was 2.15 and 1.83%, respectively, which are similar as described previously (Ullah *et al.,* 2012). The ash content in *L. sibiricus* collected from Dinajpur was 12.83%, while 15.92% at Nilphamari. The crude fat content in *L. sibiricus* from Dinajpur and Nilphamari was 4.95% and 4.79%, respectively; this finding is consistent with previous observations (Pasha *et al.,* 2011). The crude protein content was found higher in Nilphamari (26.77%) than Dinajpur (19.73%). These values are within the range recommended for dietary protein intake (Erukainure *et al.,* 2011). Starch content was 1.12 and 2.13% found that is reported by Bhatty (2000). The crude fat and protein contents revealed the similar results as found previously (Ali *et al.,* 2012; Shakil *et al.,* 2019).

Table 1. Proximate analysis of *L. sibiricus* sample from Dinajpur and Nilphamari

Name of the parameter	Dinajpur (%)	Nilphamari (%)
Reducing sugar	1.25±0.050	2.37±0.048
Non-reducing sugar (Sucrose)	2.15±0.224	1.83±0.153
Invert (Total) sugar	3.39±0.258	4.20±0.136
Protein	19.73±1.616	26.77±0.631
Fat	4.95±0.062	4.79±0.474
Starch	1.12±0.045	2.13±0.043
Ash	12.83±0.942	15.92±0.286
LSD	1.306	0.522
CV %	8.90	2.94

Data expressed as (Mean ± SD).

The mineral contents showed in (Table 2) and found the magnesium (Mg) and calcium (Ca) little bit higher that was the dissimilar with previous results in bean and rice (Paul *et al.,* 2011; Islam *et al.,* 2020). The magnesium content of Dinajpur and Nilphamari was 1555.0 mg/100g and 1418.0 mg/100g, respectively and probably environmental and soil factors influences for synthesis of higher amount of Ca and Mg (Mubarak, 2005). Sulfur and potassium content was found larger than values reported by (Olaofe *et al.,* 2009). The sodium content in samples from Dinajpur (175.0 mg/100g) and Nilphamari (212.5 mg/100g) also did not significantly differ. These mineral levels were within the recommended daily allowances. Phosphorus levels in Dinajpur and Nilphamari samples were 244.1 mg/100g and 244.0 mg/100g, respectively, meeting the recommended dietary allowance (NRC, 1989). However, these mineral contents of *L. siribicus* has many health beneficial effects, if it can be used as traditional medicine. These can be provided the micro and macro nutrients to the daily diet with the main dishes, if it can be used as herbal tea and medicine as well (Sayed *et al.,* 2016). The calcium content in Dinajpur (1523.0 mg/100g) and Nilphamari (1483.0 mg/100g) was consistent with previous findings and contributes to bone health, blood clotting, and normal bodily functions (Francis and Selby, 1997; Holick, 2006; Michael, 1997, 2006). The chemical compositions of *L. siribicus* might be varied due to different location, soil types and climate conditions. According to Agro Ecological Zone (AEZ) of Bangladesh, it has been divided into 30 AEZ based on the soil types, weather and climatic condition, geography, tidal activity and cropping pattern etc. Dinajpur and Nilphamri is situated in different AEZ, it might be one of the reason for differing the chemical composition of *L. siribicus.* However, further extensive studies will be needed for more depth explanation of chemical composition.

Table 2. Mineral composite of *L. sibiricus* sample from Dinajpur and Nilphamari

Minerals	Dinajpur (mg/100g)	Nilphamari (mg/100g)
Calcium	1523.0 ±80.16	1483.0 ±40.08
Magnesium	1555.0 ±48.61	1418.0 ±37.13
Sulfur	447.1 ±9.46	345.6 ±16.27
Phosphorus	244.1 ±7.58	244.0 ±3.96
Sodium	175.0 ±12.50	212.5 ±12.50
Potassium	691.7 ±38.19	745.8 ±19.09
LSD	73.35	46.65
CV %	5.22	3.46

Data expressed as (Mean ± SD).

Rutin and quercetin interaction network

Rutin, also known as rutoside or quercetin-3-O-rutinoside, is a glycoside compound formed between the flavonol quercetin and the disaccharide rutinose. Quercetin is also an important flavonoid that has health beneficial effects in *Homo sapiens*. Our present study is aimed to investigate the effects of rutin and quercetin of *L. siribicus* in human (*Homo sapiens*) through bioinformatics tools. Using STITCH 4.0, an interaction network was established to identify potential targets proteins and protein-protein interactions associated with rutin and quercetin, respectively. Ten potential target proteins was identified, and their interaction network (Figure 1 & Figure 3), functions and interaction scores are listed in Table 3 and Table 5 for rutin and quercetin, respectively.

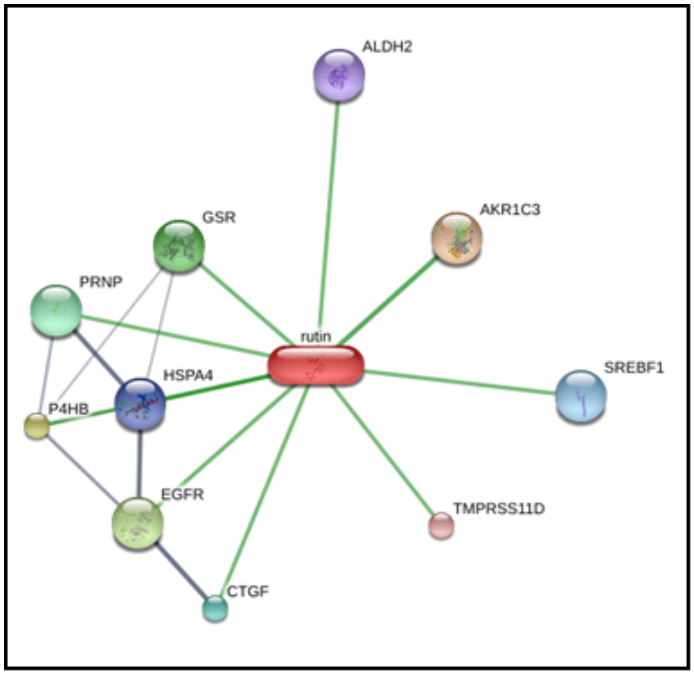


Figure 1. Interactions of rutin ($C_{27}H_{30}O_{16}$) with human protein using STITCH 4.0.

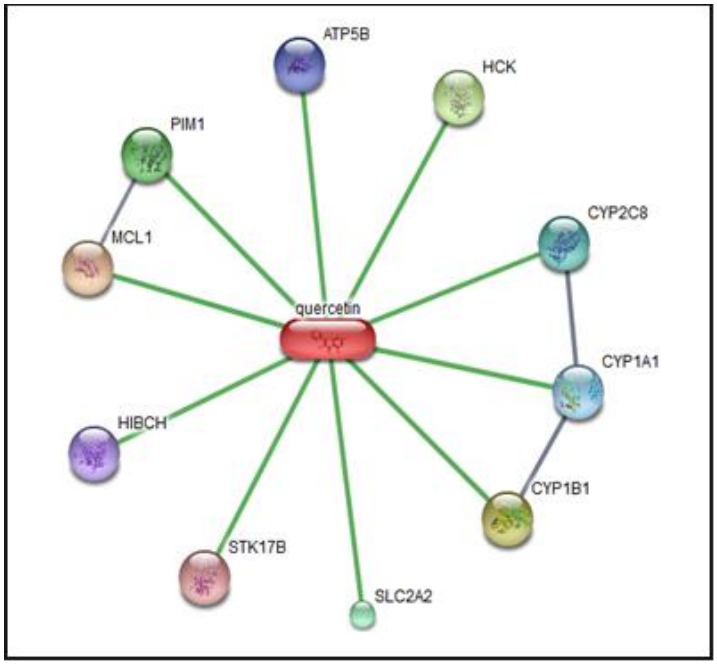


Figure 3. Interactions of quercetin ($C_{15}H_{10}O_7$) with human protein using STITCH 4.0.

Table 3. List of interacting proteins with rutin ($C_{27}H_{30}O_{16}$) detected through STITCH 4.0 along with their brief description

Interacting proteins	Short descriptions	Interaction Score
AKR1C3	Aldo-ketoreductase family 1, member C3 (3-alpha hydroxysteroid dehydrogenase, type II)	0.962
P4HB	Prolyl 4-hydroxylase, beta polypeptide; This multifunctional protein catalyzes the formation, breakage and rearrangement of disulfide bonds. At the cell surface, seems to act as a reductase that cleaves disulfide bonds of proteins attached to the cell.	0.830
EGFR	Epidermal growth factor receptor.	0.817
GSR	Glutathione reductase; Maintains high levels of reduced glutathione in the cytosol	0.809
PRNP	Prion protein	0.800
CTGF	Connective tissue growth factor; Major connective tissue mitoattractant secreted by vascular endothelial cells. Promotes proliferation and differentiation of chondrocytes. Mediates heparin- and divalent cation-dependent cell adhesion in many cell types including fibroblasts, myofibroblasts, endothelial and epithelial cells.	0.800
SREBF1	Sterol regulatory element binding transcription factor 1; Transcriptional activator required for lipid homeostasis. Regulates transcription of the LDL receptor gene as well as the fatty acid and to a lesser degree the cholesterol synthesis pathway	0.800
HSPA4	Heat shock 70kDa protein 4.	0.800
ALDH2	Aldehyde dehydrogenase 2 family	0.800
TMPRSS11D	Transmembrane protease, serine 11D; May play some biological role in the host defense system on the mucous membrane independently of or in cooperation with other substances in airway mucous or bronchial secretions.	0.786

Table 5. List of interacting proteins with quercetin $C_{15}H_{10}O_7$ detected through STITCH 4.0 along with their brief description

Interacting Proteins	Short descriptions	Interaction Score
MCL1	Myeloid cell leukemia sequence 1 (BCL2-related) (350 aa)	0.987
CYP1B1	Cytochrome P450, family 1, subfamily B, polypeptide 1; Cytochromes P450 are a group of heme-thiolatemonooxygenases.	0.975
HCK	Hemopoietic cell kinase (526 aa)	0.969
PIM1	Pim-1 oncogene; Proto-oncogene with serine/threonine kinase activity involved in cell survival and cell proliferation and thus providing a selective	0.969
SLC2A2	Solute carrier family 2 (facilitated glucose transporter), member 2; Facilitative glucose transporter. This isoform likely mediates the bidirectional tran	0.965
CYP2C8	Cytochrome P450, family 2, subfamily C, polypeptide 8; Cytochromes P450 are a group of heme-thiolatemonooxygenases.	0.964
CYP1A1	Cytochrome P450, family 1, subfamily A, polypeptide 1; Cytochromes P450 are a group of heme-thiolatemonooxygenases.	0.963
ATP5B	ATP synthase, H+ transporting, mitochondrial F1 complex, beta polypeptide; Mitochondrial membrane ATP synthase (F(1)F(0) ATP synthase or Com	0.961
HIBCH	3-hydroxyisobutyryl-CoA hydrolase; Hydrolyzes 3-hydroxyisobutyryl-CoA (HIBYL-CoA).	0.958
STK17B	Serine/threonine kinase 17b;	0.958

Each of 10 proteins for rutin and quercetin were interacted to another 10 proteins and made together 100 proteins for rutin and another 100 proteins for quercetin through the bioinformatics tools STRING 9.1. In this analysis, interaction network of each of 10 protein for rutin and quercetin were presented graphically (Figure 2 & 4) and their respective protein-protein interaction (PPI) in Table 4 and Table 6, respectively.

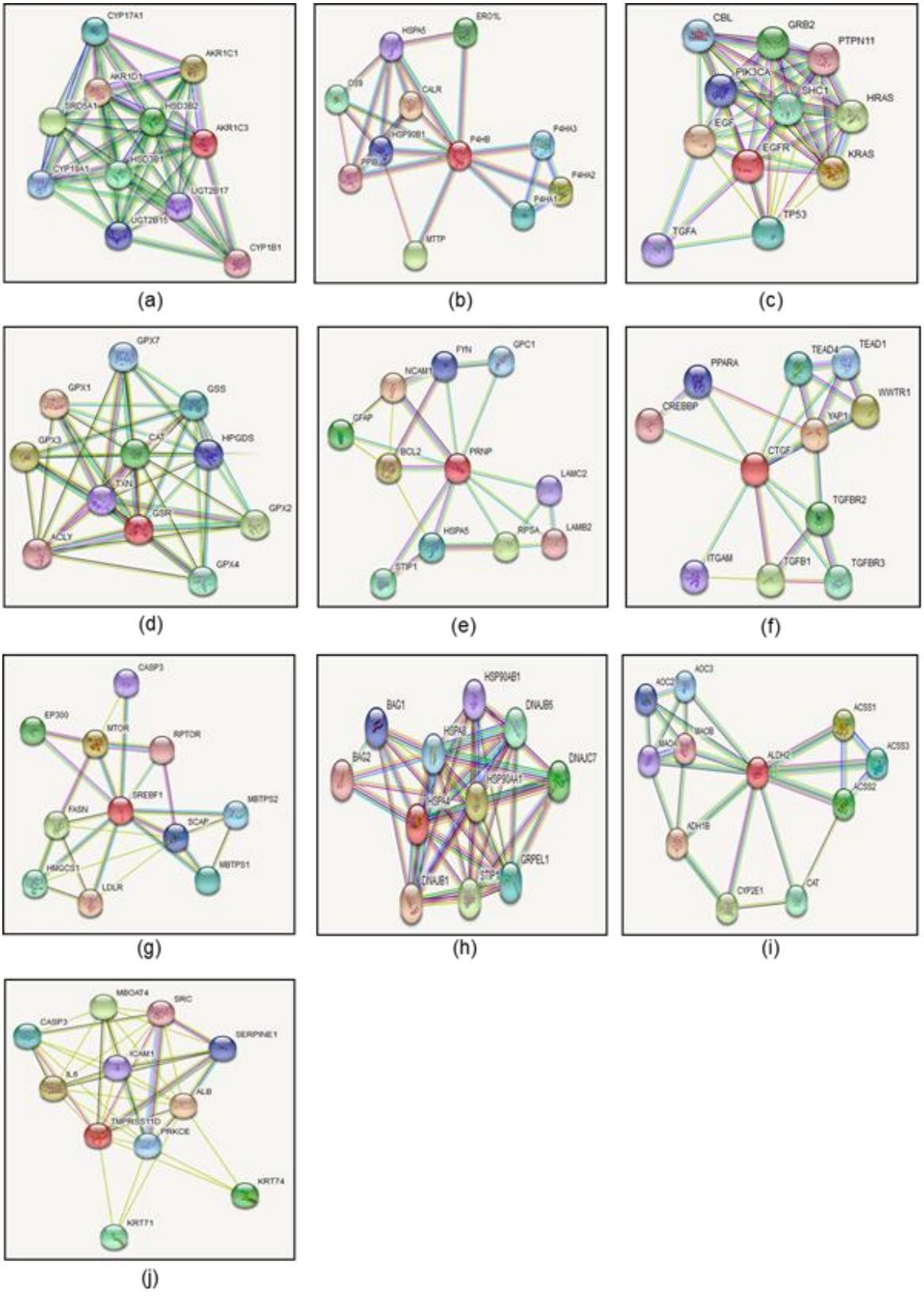


Figure 2. Protein-protein interaction (PPI) of rutin in *Homo sapiens* using STRING 9.1. (a): AKR1C3, (b):P4HB, (c): EGFR, (d): GSR, (e):PRNP, (f): CTGF, (g): SREBF1, (h):HSPA4, (i): ALDH2, (j):TMPRSS11D.

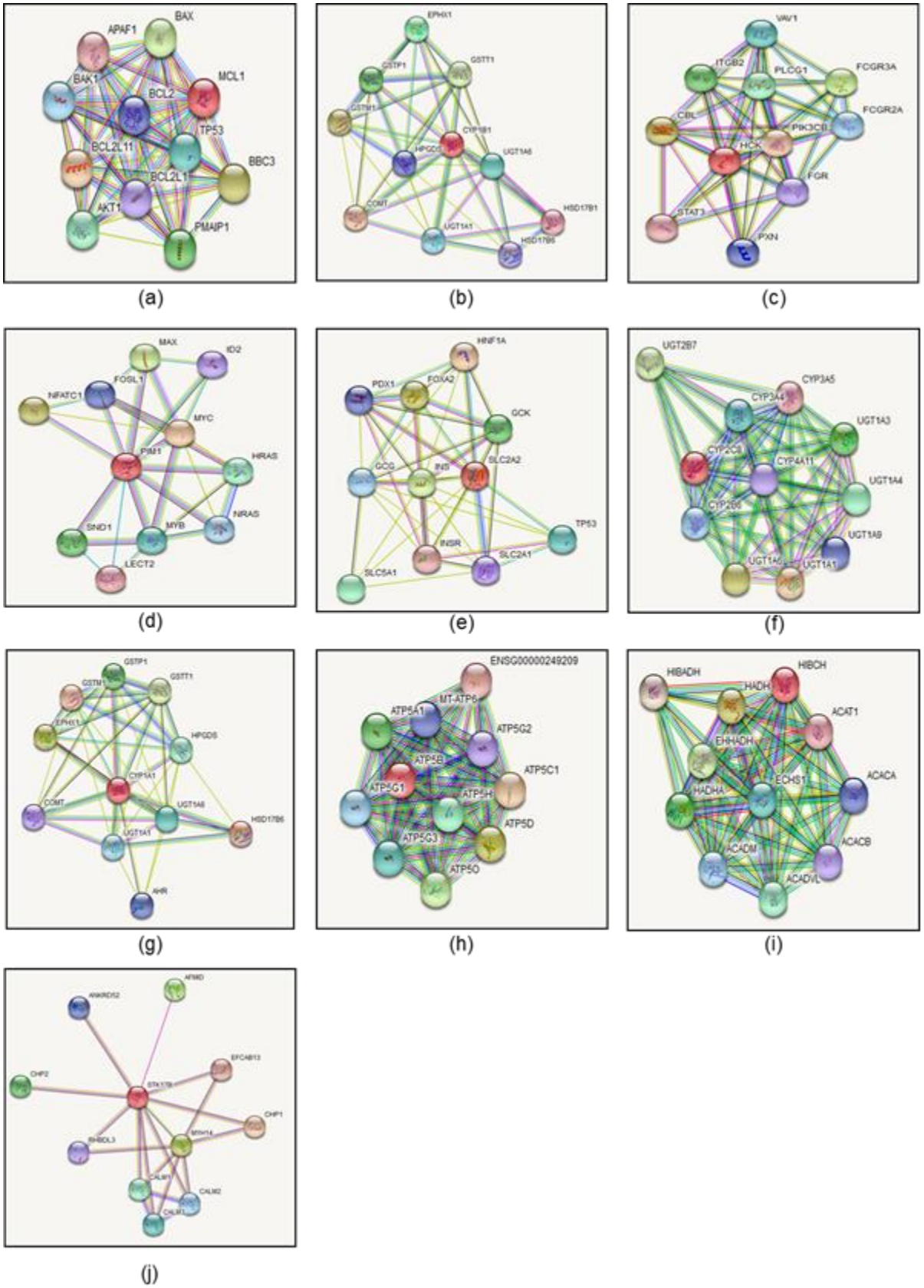


Figure 4. Protein-protein interaction (PPI) of quercetin in *Homo sapiens* using STRING 9.1. (a): MCL1, (b):CYP1B1, (c): HCK, (d): PIM1, (e): SLC2A2, (f): CYP2C8, (g): CYP1A1, (h): ATP5B, (i): HIBCH, (j):STK17B.

Table 4. Protein-Protein Interaction (PPI) of rutin using STRING 9**.1**

No.	AKR1C3	P4HB	EGFR	GSR	PRNP	CTGF	SREBF1	HSPA4	ALDH2	TMPRSS11D
1	AKR1D1	CALR	EGF	GPX1	NCAM1	YAP1	LDLR	DNAJB1	ADH1B	ALB
2	AKR1C1	P4HA2	KRAS	GPX3	BCL2	WWTR1	MTOR	HSP90AA1	ACSS1	IL6
3	SRD5A1	MTTP	HRAS	GPX2	RPSA	TGFB1	FASN	STIP1	CYP2E1	MBOAT4
4	HSD3B2	ERO1L	GRB2	CAT	GFAP	TGFBR2	EP300	DNAJC7	ACSS2	KRT74
5	HSD3B1	OS9	SHC1	GPX4	STIP1	TGFBR3	HMGCS1	DNAJB6	CAT	KRT71
6	CYP17A1	P4HA1	TP53	GSS	HSPA5	TEAD4	MBTPS1	GRPEL1	ACSS3	CASP3
7	CYP19A1	P4HA3	CBL	GPX7	GPC1	TEAD1	MBTPS2	HSPA8	AOC3	PRKCE
8	UGT2B15	HSP90B1	PIK3CA	HPGDS	FYN	PPARA	SCAP	BAG1	AOC2	SERPINE1
9	UGT2B17	HSPA5	TGFA	TXN	LAMC2	ITGAM	CASP3	HSP90AB1	MAOA	ICAM1
10	CYP1B1	PPIB	PTPN11	ACLY	LAMB2	CREBBP	RPTOR	BAG2	MAOB	SRC

Table 6. Protein-Protein Interaction (PPI) of quercetin using STRING 9.1

No.	MCL1	CYP1B1	HCK	PIM1	SLC2A2	CYP2C8	CYP1A1	ATP5B	HIBCH	STK17B
1	BCL2L11	COMT	PIK3CB	MYC	HNF1A	UGT1A1	GSTM1	ATP5C1	HIBADH	CHP1
2	BBC3	GSTM1	CBL	NFATC1	FOXA2	UGT1A6	EPHX1	ATP5D	HADH	MYH14
3	BAX	GSTT1	FCGR3A	MAX	INS	UGT2B7	GSTT1	ATP5O	EHHADH	AFMID
4	PMAIP1	GSTP1	ITGB2	SND1	GCK	UGT1A3	GSTP1	ATP5A1	HADHA	CHP2
5	AKT1	EPHX1	PLCG1	HRAS	SLC5A1	UGT1A4	HPGDS	ATP5H	ACADVL	CALM1
6	TP53	UGT1A6	VAV1	MYB	TP53	CYP3A4	UGT1A6	ATP5G3	ECHS1	CALM3
7	BAK1	UGT1A1	FCGR2A	NRAS	GCG	CYP2B6	UGT1A1	ATP5G1	ACADM	CALM2
8	BCL2	HPGDS	PXN	FOSL1	PDX1	UGT1A9	AHR	MT-ATP6	ACACA	ANKRD52
9	BCL2L1	HSD17B6	FGR	ID2	SLC2A1	CYP4A11	COMT	ATP5G2	ACACB	RHBDL3
10	APAF1	HSD17B1	STAT3	LECT2	INSR	CYP3A5	HSD17B6	ENSG0000029209	ACAT1	EFCAB13

Rutin and quercetin are two bioactive compounds found in buckwheat, tea, honeyweed and many other medicinal plants that can be used as therapeutic and functional foods for maintain the healthy life in animal. These molecules could be the binding ability to some important proteins and predicted their important functions in human body (Szklarczyk *et al.*, 2016). Likewise, rutin and quercetin about 430000 chemicals could be interacted to different proteins in humans (Szklarczyk *et al.*, 2016). Functions of various proteins and cellular locations can be identified in yeast (Schwikowski *et al.*, 2000) through the analysis of different protein-protein interactions (Phizicky and Fields, 1995). Besides that it can be predicted to uncover the functions of proteins, discover the drug (Barabási *et al.*, 2011) and identify the genes related to diseases (Oti *et al.*, 2006). Our study suggested that rutin and quercetin bound with some important proteins and genes that would provide necessary information for drug discovery with these two bioactive compounds and have some advantageous health benefits.

Protein accession, amino acid sequence retrieval and functional annotation analysis

We retrived total 200 proteins (100 for rutin and 100 for quercetin) through the analysis of PPI by suing the STRING9.1. (Ali *et al.*, 2017a & b). Accession number and amino acid length of these proteins were retrieved in protein database UniProtKB and NCBI simultaneously. Using these protein accession numbers, we searched protein- protein blast to remove duplication and collected fasta file for functional annotation analysis. After blast search, we retrieved 177 protein sequence (details in materials and methods) and subsequently functional annotation and Venn diagram analysis were performed. Functional annotation analysis including biological process, cellular component and molecular functions (Figure 5) showed very significant possible effects of rutin and quercetin in *Homo sapiens*.

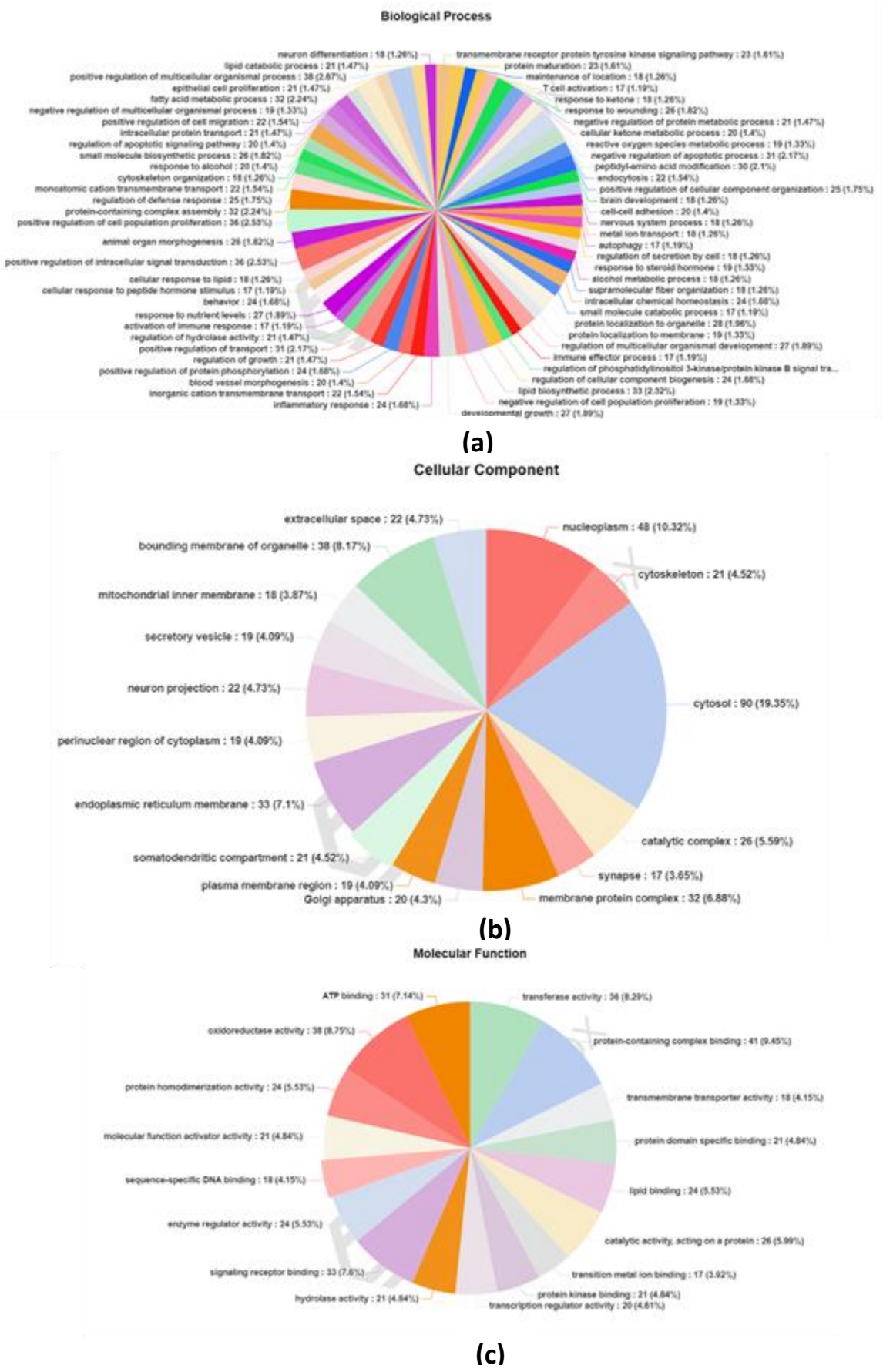


Figure 5. Functional annotation analysis showed the various biological processes (a), localization of cellular components (b) and molecular functions (c) of different proteins in *Homo sapiens*.

In case of biological process showed that rutin and quercetin played a role to regulate the metabolism carbohydrates, lipids, proteins, small molecules and other nutrients. Honeyweed powder and crude extract significantly improved lipid profile and liver function test (Abedin *at el.*, 2020 & 2021) indicating the role of metabolisms of biomolecules by rutin and quercetin. They might have possible effects on development of neuron, nervous system and protein maturation. In addition, these two bioactive compounds can be controlled reactive oxygen species and apoptosis. They have effects on cell and cell membrane development, protein localization, steroid hormone synthesis, vascular morphogenesis, autophagy and regulation of ketone bodies (Figure 5a). These compounds are well known flavonoid due to their inflammation reduction, minimizing the oxidative damage and anticancer activities (Rosiak *et al.*, 2023; Başaran *et al.*, 2022). Interestingly, it was observed that remarkable biological activities has been increased in cell culture system (Yang *et al*, 2019). Cellular component reveals that proteins are localized in cell membrane, cytoplasm, mitochondria and neuron projection due to the effects of rutin and quercetin (Figure 5b). These proteins are involved in different enzyme activities, receptor binding and transmembrane activities (Figure 5c). The molecular mechanism by which rutin and quercetin execute their biochemical attributes by direct interaction with DNA, enzymes and membrane receptors (Malešev and Kuntić, 2007) in cell membrane, cytoplasm and mitochondria.

Venn diagram

Rutin and quercetin is flavonoids compound found in buckwheat, wheat, tea, honeyweed and many other plants. They have significant medicinal values to ameliorate positive effects on human health by interacting at gene and protein level. Our bioinformatics analysis of PPI found 100 proteins and genes for rutin and quercetin separately in *Homo sapiens*. Interestingly, only four (4) proteins are found common among these active 177 proteins (Table 8) that indicated rutin and quercetin have different mode of action to regulate various activities and control disease in animal body (Ali *et al.*, 2017).

Table 7: List of interacting proteins of STRING 9.1 after blast searching

SL. No.	Accession number	Protein name	Amino acid length	SL. No.	Accession number	Protein name	Amino acid length
1.	A0A8I5KQE6	RPSA2	295	46.	P08684	CP3A4	503
2.	O00763	ACACB	2458	47.	P09211	GSTP1	210
3.	O14727	APAF	1248	48.	P09488	GSTM1	218
4.	O14756	H17B6	317	49.	P09769	FGR	529
5.	O14960	LECT2	151	50.	P0DP23	CALM1	149
6.	O15460	P4HA2	535	51.	P0DP24	CALM2	149
7.	O43462	MBTP2	519	52.	P0DP25	CALM3	149
8.	O43521	B2L11	198	53.	P10242	MYB	640
9.	O43745	CHP2	196	54.	P10415	BCL2	239
10.	O60656	UD19	530	55.	P10599	THIO	105
11.	O60760	HPGDS	199	56.	P11021	BIP	654
12.	O75106	AOC2	756	57.	P11142	HSP7C	646
13.	O75190	DNJB6	326	58.	P11166	GTR1	492
14.	O75795	UDB17	530	59.	P11215	ITAM	1152
15.	O75947	ATP5H	161	60.	P11310	ACADM	421
16.	O95644	NFAC1	943	61.	P11511	CP19A	503
17.	O95816	BAG2	211	62.	P12318	FCG2A	317
18.	P00325	ADH1B	375	63.	P12931	SRC	536
19.	P00846	ATP6	226	64.	P13591	NCAM1	858
20.	P01106	MYC	454	65.	P13674	P4HA1	534
21.	P01111	RASN	189	66.	P13866	SC5A1	664
22.	P01112	RASH	189	67.	P14060	3BHS1	373
23.	P01116	RASK	189	68.	P14061	DHB1	328
24.	P01130	LDLR	860	69.	P14136	GFAP	432
25.	P01133	EGF	1207	70.	P14625	ENPL	803
26.	P01135	TGFA	160	71.	P15407	FOSL1	271
27.	P01137	TGFB1	390	72.	P15498	VAV	845
28.	P01275	GLUC	180	73.	P16662	UD2B7	529
29.	P01308	INS	110	74.	P18283	GPX2	190
30.	P02768	ALBU	609	75.	P18405	S5A1	259
31.	P04040	CATA	527	76.	P19174	PLCG1	1290
32.	P05093	CP17A	508	77.	P19224	UD16	532
33.	P05107	ITB2	769	78.	P20813	CP2B6	491
34.	P05121	PAI1	402	79.	P20815	CP3A5	502
35.	P05181	CP2E1	493	80.	P20823	HNF1A	631
36.	P05231	IL6	212	81.	P21397	AOFA	527
37.	P05362	ICAM1	532	82.	P21964	COMT	271
38.	P05496	AT5G1	136	83.	P22309	UD11	533
39.	P06213	INSR	1382	84.	P22310	UD14	534
40.	P06241	FYN	537	85.	P22352	GPX3	226
41.	P07099	HYEP	455	86.	P22681	CBL	906
42.	P07203	GPX1	203	87.	P23284	PPIB	216
43.	P07900	HS90A	732	88.	P24752	THIL	427
44.	P08238	HS90B	724	89.	P25685	DNJB1	340
45.	P08637	FCG3A	254	90.	P25705	ATPA	553

Table 7: List of interacting proteins of STRING 9.1 after blast searching (Continued)

SL. No.	Accession number	Protein name	Amino acid length	SL. No.	Accession number	Protein name	Amino acid length
91.	P26439	3BHS2	372	135.	Q04828	AK1C1	323
92.	P27338	AOFB	520	136.	Q06055	AT5G2	141
93.	P27797	CALR	417	137.	Q06124	PTN11	593
94.	P28347	TEAD1	426	138.	Q07812	BAX	192
95.	P29353	SHC1	583	139.	Q07817	B2CL1	233
96.	P30049	ATPD	168	140.	Q07869	PPARA	468
97.	P30084	ECHM	290	141.	Q08426	ECHP	723
98.	P30711	GSTT1	240	142.	Q09472	EP300	2414
99.	P31749	AKT1	480	143.	Q12770	SCAP	1279
100.	P31937	3HIDH	336	144.	Q12888	TP53B	1972
101.	P31948	STIP1	543	145.	Q13085	ACACA	2346
102.	P35052	GPC1	558	146.	Q13438	OS9	667
103.	P35503	UD13	534	147.	Q13753	LAMC2	1193
104.	P35557	HXK4	465	148.	Q13794	APR	54
105.	P35869	AHR	848	149.	Q14703	MBTP1	1052
106.	P36542	ATPG	298	150.	Q15561	TEAD4	434
107.	P36969	GPX4	197	151.	Q16611	BAK	211
108.	P37173	TGFR2	567	152.	Q16836	HCDH	314
109.	P40763	STAT3	770	153.	Q16853	AOC3	763
110.	P40939	ECHA	763	154.	Q3SY84	K2C71	523
111.	P42336	PK3CA	1068	155.	Q63HM1	KFA	303
112.	P42338	PK3CB	1070	156.	Q7KZF4	SND1	910
113.	P42345	MTOR	2549	157.	Q7RTS7	K2C74	529
114.	P42574	CASP3	277	158.	Q7Z406	MYH14	1995
115.	P46937	YAP1	504	159.	Q7Z4N8	P4HA3	544
116.	P48047	ATPO	213	160.	Q8IY85	EFC13	973
117.	P48201	AT5G3	142	161.	Q8N122	RPTOR	1335
118.	P48637	GSHB	474	162.	Q8NB46	ANR52	1076
119.	P49023	PAXI	591	163.	Q92793	CBP	2442
120.	P49748	ACADV	655	164.	Q96HE7	ERO1A	468
121.	P51857	AK1D1	326	165.	Q96SL4	GPX7	187
122.	P52945	PDX1	283	166.	Q96T53	MBOA4	435
123.	P53396	ACLY	1101	167.	Q99615	DNJC7	494
124.	P54855	UDB15	530	168.	Q99653	CHP1	195
125.	P55157	MTP	894	169.	Q99933	BAG1	345
126.	P55268	LAMB2	1798	170.	Q9BXH1	BBC3	193
127.	P58872	RHBL3	404	171.	Q9GZV5	WWTR1	400
128.	P61244	MAX	160	172.	Q9H6R3	ACSS3	686
129.	P62993	GRB2	217	173.	Q9HAV7	GRPE1	217
130.	Q01581	HMCS1	520	174.	Q9HDD0	PLAT1	168
131.	Q02156	KPCE	737	175.	Q9NR19	ACSA	701
132.	Q02363	ID2	134	176.	Q9NUB1	ACS2L	689
133.	Q02928	CP4AB	519	177.	Q9Y261	FOXA2	457
134.	Q03167	TGBR3	851				

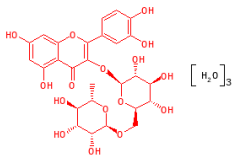
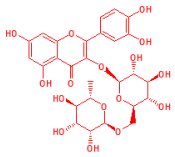
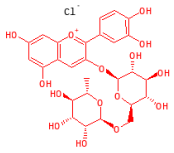
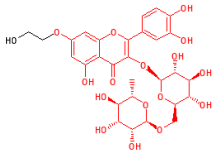
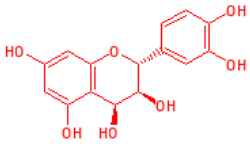
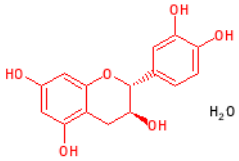
Table 8. Common protein and their respective genes for Rutin and Quercetin in Venn diagram

Accession No.	Protein Name	Full Name of protein	Gene name	Amino acid length
O60760	HPGDS	Hematopoietic prostaglandin D synthase (H-PGDS) (EC 5.3.99.2) (GST class-sigma) (Glutathione S-transferase) (EC 2.5.1.18) (Glutathione-dependent PGD synthase) (Glutathione-requiring prostaglandin D synthase) (Prostaglandin-H2 D-isomerase)	HPGDS GSTS PGDS PTGDS2	199
P10415	BCL2	Apoptosis regulator Bcl-2	BCL2	239
Q12888	TP53B	TP53-binding protein 1 (53BP1) (p53-binding protein 1) (p53BP1)	TP53BP1	1972
P22681	CBL	E3 ubiquitin-protein ligase CBL (EC 2.3.2.27) (Casitas B-lineage lymphoma proto-oncogene) (Proto-oncogene c-Cbl) (RING finger protein 55) (RING-type E3 ubiquitin transferase CBL) (Signal transduction protein CBL)	CBL CBL2 RNF55	906

KEGG Pathway

As we found notable biological, cellular and molecular interactions of rutin and quercetin in *Homo sapiens*, it was investigated to search the potential drug using two compounds through KEGG compound database. Intriguingly, KEGG compound database showed some important drug has already been discovered by these two parent compounds. It was retrieved only four drugs prepared by rutin and two drugs prepared by quercetin considering the similarity score 0.90 with their functions (Table 9).

Table 9. Drugs that discovered from rutin (1-4) and quercetin (5-6) with their similarity score, name and function retrieved from KEGG pathway

SL No.	ID No.	Structure	Similarity Score	Name	Function
1	D00190		1.0	Rutin hydrate (JAN), Rutin trihydrate	Vascular protectant
2	D08499		1.0	Rutoside (INN), Rutin, Venoruton (TN)	Vascular protectant
3	D08097		**0.97**	Keracyanin chloride (INN), Meralop (TN)	Rhodopsin resynthesis promoter (ophthalmic)
4	D07179		**0.94**	Monoxerutin (INN)	Vascular protectant
5	D08112		**0.94**	Leucocianidol (INN), Flavan (TN)	Vascular protectant
6	D00200		**0.91**	Cianidanol(JAN/INN)	Liver function improving agent

Note: International Nonproprietary Names (INN), Japanese Accepted Name (JAN), Trigeminal Neuralgia (TN)

We did not consider some other drug from the database that similarity score less than 0.90, even though they are produced from rutin and quercetin mentioning their functions like antibacterial activity (Soberon *et al.*, 2007), anticancer (Nagasawa *et al.*, 1990), antidiabetic (Schmidt *et al.*, 2013) and neuroprotectant (Sayed *et al.*, 2016). It was studied that honeyweed extract could be used as suppressing the tumor growth, antimicrobial and antidiabetic activities (Sayed *et al.*, 2016). Considering the potentiality of this plant, rutin and quercetin biosynthesis pathway was retrieved in KEGG pathway (Figure 9) and found the apigenin (Sayed *et al.*, 2016) is the substrate for biosynthesis of rutin and quercetin and some important enzymes were involved for this flavone and flavonol biosynthesis pathway (Table 10). It is notable, honeyweed is herbaceous medicinal plants and grown as weed, this study could be useful for production the bioactive compounds for drug design through the up and downregulation of genes by the application of genetic engineering tools (Yan *et al.*, 2021).

Figure 6. Identification of common targeted proteins through Venn diagram in *Homo sapiens.*

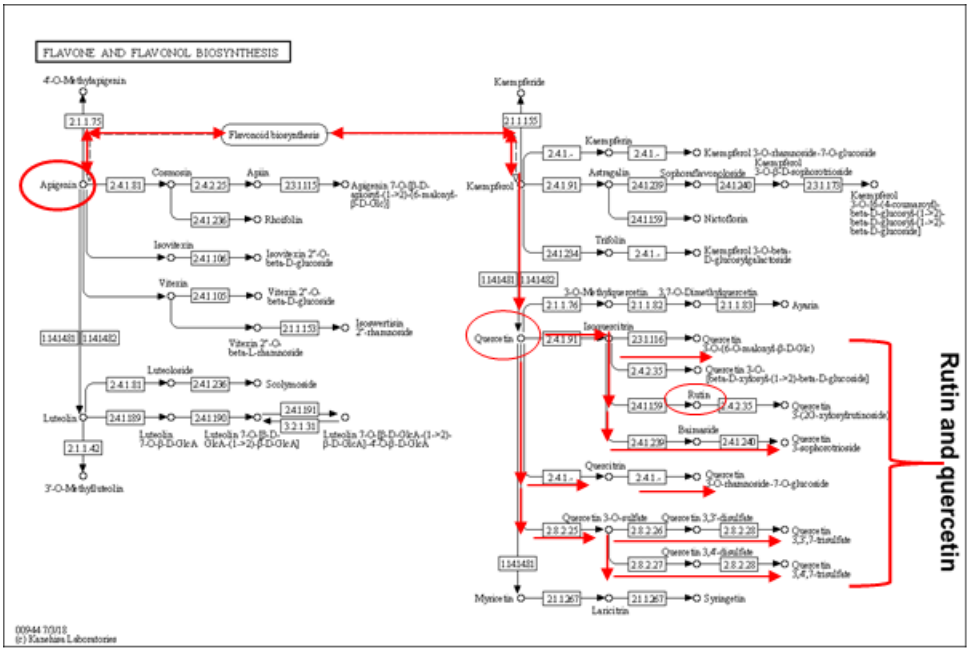


Figure 7. Flavone and flavonol (map00944) biosynthesis pathway retrieved from KEGG pathway database (https://www.genome.jp/pathway/map00944).

Table 10. List of enzymes functioning for flavone and flavonol biosynthesis pathway

Enzyme ID	Enzyme Name
EC 1.14.14.81	Flavanoid 3',5'-hydroxylase; flavonoid 3',5'-hydroxylase
EC 1.14.14.82	flavonoid 3'-monooxygenase; flavonoid 3'-hydroxylase; flavonoid 3-hydroxylase; NADPH:flavonoid-3'-hydroxylase; flavonoid 3-monooxygenase
EC 2.1.1.153	vitexin 2"-O-rhamnoside 7-O-methyltransferase
EC 2.1.1.155	kaempferol 4'-O-methyltransferase; S-adenosyl-L-methionine:flavonoid 4'-O-methyltransferase; F 4'-OMT
EC 2.1.1.267	flavonoid 3',5'-methyltransferase; AOMT; CrOMT2
EC 2.1.1.42	flavone 3'-O-methyltransferase; o-dihydric phenol methyltransferase; luteolin methyltransferase; luteolin 3'-O-methyltransferase; o-diphenol m-O-methyltransferase; o-dihydric phenol meta-O-methyltransferase; S-adenosylmethionine:flavone/flavonol 3'-O-methyltransferase; quercetin 3'-O-methyltransferase
EC 2.1.1.75	apigenin 4'-O-methyltransferase; flavonoid O-methyltransferase; flavonoid methyltransferase; S-adenosyl-L-methionine:5,7,4'-trihydroxyflavone 4'-O-methyltransferase
EC 2.1.1.76	quercetin 3-O-methyltransferase; flavonol 3-O-methyltransferase; flavonoid 3-methyltransferase
EC 2.1.1.82	3-methylquercetin 7-O-methyltransferase; flavonol 7-O methyltransferase; flavonol 7-methyltransferase; 7-OMT; S-adenosyl-L-methionine:3',4',5,7-tetrahydroxy-3-methoxyflavone 7-O-methyltransferase; 3-methylquercitin 7-O-methyltransferase
EC 2.1.1.83	3,7-dimethylquercetin 4'-O-methyltransferase; flavonol 4'-O-methyltransferase; flavonol 4'-methyltransferase; 4'-OMT; S-adenosyl-L-methionine:3',4',5-trihydroxy-3,7-dimethoxyflavone 4'-O-methyltransferase; 3,7-dimethylquercitin 4'-O-methyltransferase
EC 2.3.1.115	isoflavone-7-O-beta-glucoside 6"-O-malonyltransferase; flavone/flavonol 7-O-beta-D-glucoside malonyltransferase; flavone (flavonol) 7-O-glycoside malonyltransferase; malonyl-CoA:flavone/flavonol 7-O-glucoside malonyltransferase; MAT-7; malonyl-coenzyme A:isoflavone 7-O-glucoside-6"-malonyltransferase; malonyl-coenzyme A:flavone/flavonol-7-O-glycoside malonyltransferase
EC 2.3.1.116	flavonol-3-O-beta-glucoside O-malonyltransferase; flavonol 3-O-glucoside malonyltransferase; MAT-3; malonyl-coenzyme A:flavonol-3-O-glucoside malonyltransferase
EC 2.3.1.173	flavonol-3-O-triglucoside O-coumaroyltransferase; 4-coumaroyl-CoA:flavonol-3-O-[beta-D-glucosyl-(1->2)-beta-D-glucoside] 6"'-O-4-coumaroyltransferase
EC 2.4.1.105	vitexin beta-glucosyltransferase; uridine diphosphoglucose-vitexin 2"-glucosyltransferase
EC 2.4.1.106	isovitexin beta-glucosyltransferase; uridine diphosphoglucose-isovitexin 2"-glucosyltransferase

EC 2.4.1.159	flavonol-3-O-glucoside L-rhamnosyltransferase; uridine diphosphorhamnose-flavonol 3-O-glucoside rhamnosyltransferase; UDP-rhamnose:flavonol 3-O-glucoside rhamnosyltransferase; UDP-L rhamnose:flavonol-3-O-D-glucoside 6"-O-L rhamnosyltransferase
EC 2.4.1.189	luteolin 7-O-glucuronosyltransferase; uridine diphosphoglucuronate-luteolin 7-O-glucuronosyltransferase; LGT; UDP-glucuronate:luteolin 7-O-glucuronosyltransferase
EC 2.4.1.190	luteolin-7-O-glucuronide 2"-O-glucuronosyltransferase; uridine diphosphoglucuronate-luteolin 7-O-glucuronide glucuronosyltransferase; LMT; UDP-glucuronate:luteolin 7-O-glucuronide glucuronosyltransferase; UDP-glucuronate:luteolin-7-O-beta-D-glucuronide 2"-O-glucuronosyltransferase
EC 2.4.1.191	luteolin-7-O-diglucuronide 4'-O-glucuronosyltransferase; uridine diphosphoglucuronate-luteolin 7-O-diglucuronide glucuronosyltransferase; UDP-glucuronate:luteolin 7-O-diglucuronide glucuronosyltransferase; UDPglucuronate:luteolin 7-O-diglucuronide-4'-O-glucuronosyl-transferase; LDT; UDP-glucuronate:luteolin-7-O-beta-D-diglucuronide 4'-O-glucuronosyltransferase
EC 2.4.1.234	kaempferol 3-O-galactosyltransferase; F3GalTase; UDP-galactose:kaempferol 3-O-beta-D-galactosyltransferase
EC 2.4.1.236	flavanone 7-O-glucoside 2"-O-beta-L-rhamnosyltransferase; UDP-rhamnose:flavanone-7-O-glucoside-2"-O-rhamnosyltransferase; 1->2 UDP-rhamnosyltransferase; UDP-L-rhamnose:flavanone-7-O-glucoside 2"-O-beta-L-rhamnosyltransferase
EC 2.4.1.239	flavonol-3-O-glucoside glucosyltransferase; UDP-glucose:flavonol-3-O-glucoside 2"-O-beta-D-glucosyltransferase
EC 2.4.1.240	Transferases; Glycosyltransferases; Hexosyltransferases
EC 2.4.1.81	flavone 7-O-beta-glucosyltransferase; UDP-glucose-apigenin beta-glucosyltransferase; UDP-glucose-luteolin beta-D-glucosyltransferase; uridine diphosphoglucose-luteolin glucosyltransferase; uridine diphosphoglucose-apigenin 7-O-glucosyltransferase; UDP-glucosyltransferase (ambiguous)
EC 2.4.1.91	flavonol 3-O-glucosyltransferase; GTI; uridine diphosphoglucose-flavonol 3-O-glucosyltransferase; UDP-glucose:flavonol 3-O-glucosyltransferase; UDPG:flavonoid-3-O-glucosyltransferase
EC 2.4.2.25	flavone apiosyltransferase; uridine diphosphoapiose-flavone apiosyltransferase; UDP-apiose:7-O-(beta-D-glucosyl)-flavone apiosyltransferase
EC 2.4.2.35	flavonol-3-O-glycoside xylosyltransferase; UDP-D-xylose:flavonol-3-O-glycoside 2"-O-beta-D-xylosyltransferase
EC 2.8.2.25	flavonol 3-sulfotransferase; 3'-phosphoadenylyl-sulfate:quercetin 3-sulfotransferase
EC 2.8.2.26	quercetin-3-sulfate 3'-sulfotransferase; flavonol 3'-sulfotransferase; 3'-Sulfotransferase; PAPS:flavonol 3-sulfate 3'-sulfotransferase; 3'-phosphoadenylyl-sulfate:quercetin-3-sulfate 3'-sulfotransferase
EC 2.8.2.27	quercetin-3-sulfate 4'-sulfotransferase; flavonol 4'-sulfotransferase; PAPS:flavonol 3-sulfate 4'-sulfotransferase; 3'-phosphoadenylyl-sulfate:quercetin-3-sulfate 4'-sulfotransferase
EC 2.8.2.28	quercetin-3,3'-bissulfate 7-sulfotransferase; flavonol 7-sulfotransferase; 7-sulfotransferase; PAPS:flavonol 3,3'/3,4'-disulfate 7-sulfotransferase; 3'-phosphoadenylyl-sulfate:quercetin-3,3'-bissulfate 7-sulfotransferase
EC 3.2.1.31	beta-glucuronidase; beta-glucuronide glucuronohydrolase glucuronidase; exo-beta-D-glucuronidase; ketodase

Conclusion

Honeyweed is herbaceous medicinal plants, grown in roadside, crop field as weed in Bangladesh. It could be used as therapeutic drug for controlling the various life threatening diseases. It can be used for isolation, purification and characterization some other new bioactive compounds and thereby used for manufacturing important drug. Production of rutin and quercetin might be increased *in vitro* through tissue culture and genetic engineering tools that can be further used for raw materials for discovering the drug in industry. In addition, honeyweed may be used as traditional medicine that can be pave the way for new direction for manufacturing the herbal drug.

Conflict of interest

Authors declare that there is no conflict of interest.

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