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Potential bioactive phytochemicals, antioxidant properties and anticancer pathways of *Nymphaea nouchali*

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ABSTRACT

Objective: To investigate bioactive phytochemicals and antioxidant activities of *Nymphaea nouchali* and to explore its anticancer pathways by a network pharmacology approach.

Methods: Using a spectrophotometer and high-performance liquid chromatography-diode array detector (HPLC-DAD), we quantified bioactive phytochemicals in methanolic extract of *Nymphaea nouchali* tuber. The extracts were investigated for *in vitro* antioxidant properties. Targets of these bioactive phytochemicals were predicted and anticancer-associated pathways were analyzed by a network pharmacology approach. Moreover, we identified the predicted genes associated with cancer pathways and the hub genes in the protein-protein interaction network of predicted genes.

Results: Quantitative results indicated the total phenolics, total flavonoids, and total proanthocyanidins in the methanolic extract of *Nymphaea nouchali* tuber. HPLC-DAD analysis showed rutin (39.44 mg), catechin (39.20 mg), myricetin (30.77 mg), ellagic acid (11.05 mg), gallic acid (3.67 mg), vanillic acid (0.75 mg), rosmarinic acid (4.81 mg), *p*-coumaric acid (3.35 mg), and quercetin (0.90 mg) in 1 g of dry extract. The extract showed the radical scavenging activities of 2, 2-diphenyl-1-picrylhydrazyl, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) and N,N-dimethyl-*p*-phenylenediamine. By using network pharmacology, we predicted 130 target genes associated with cancer pathways. The top hub genes (*IL6*, *AKT1*, *EGFR*, *JUN*, *PTGS2*, *MAPK3*, *CASP3*, and *CXCL8*) were also identified, which were associated with cancer pathways and interacted with bioactive phytochemicals of the methanolic extract of *Nymphaea nouchali* tuber.

Conclusions: Our study provides insights into the mechanism of anticancer activities of the methanolic extract of *Nymphaea nouchali* tuber.

KEYWORDS: Bioactive phytochemicals; Anticancer pathways; Malignancy; Network pharmacology; *Nymphaea nouchali*

1. Introduction

Nymphaea nouchali (*N. nouchali*) (Burm. f) is a flowering plant belonging to the family Nymphaeceae. It is a national flower of Bangladesh and Srilanka, commonly known as "Shapla" in Bangladesh. *N. nouchali* grows abundantly as a mixed population in nearly all shoals and natural water bodies[1]. This plant has various bioactivities including anti-inflammatory and diuretic activities[2]. In the Ayurveda and Siddha systems of medicines, it is used for the treatment of diabetes, inflammation, liver disorders, urinary disorders, menorrhagia, blennorrhagia, menstruation problem, and used as an aphrodisiac, and a bitter tonic[3,4]. Rhizomes and flowers have been widely used for the treatment of kidney problems[3]. Flower extract can attenuate melanogenesis by regulation of cAMP/CREB/MAPKs/MITF pathway and proteasomal degradation of tyrosinase[1]. Alam *et al.* revealed that *N. nouchali* flower extracts

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had DNA-protecting activities by phosphorylating mitogen-activated protein kinase (MAP kinase) followed by enhancing the nuclear translocation of the nuclear factor erythroid 2-related factor 2 (Nrf2). These cellular signaling activities attenuate cellular ROS generation and are associated with the protection from cell death[5]. The presences of gallic acid, catechin, epicatechin, epigallocatechin, epicatechin gallate, caffeic acid, quercetin, and apigenin were identified in the flower of this plant[5]. *N. nouchali* flower also has antimicrobial activity on human and plant pathogenic bacteria and endophytic fungi[6]. Seed extract promotes adipocyte differentiation and glucose consumption by inducing the PPARγ activation, which in turn increases mRNA GLUT-4 expression[7]. These previous studies proved the medicinal value of *N. nouchali* at molecular levels. Plant-derived natural dietary bioactive phytochemicals have potent antioxidants and cancer chemopreventive agents[8]. Dietary phytochemicals are often used as anticancer agents against breast cancer, skin cancer, diverse types of thyroid cancers, prostate cancer, and gastroenterological cancer, as well as play a role in modulating coding and non-coding genes in cancers[9]. *N. nouchali* tubers are eaten usually boiled or roasted. A novel Ca^{2+}-dependent lectin was isolated from the *N. nouchali* tuber and exhibited antiproliferative properties[10]. Additionally, the tuber and root of *N. nouchali* were used by the folk medicine practitioners in three districts of Bangladesh for the prevention and management of malignancy in cancer[11]. However, to the best of our knowledge, there have been no investigations to date that identify the subset of anticancer pathways associated with the bioactive phytochemicals of *N. nouchali* tuber extract. Therefore, we designed the present study to identify the potential bioactive phytochemicals, to evaluate *in vitro* antioxidant properties, and to investigate the predicted genes interacting with bioactive phytochemicals and the association of these predicted genes with cancer pathways. In addition, we investigated the hub genes, which are regulated by the bioactive phytochemicals of *N. nouchali*.

2. Materials and methods

2.1. Collection and preparation of methanolic crude extract of N. nouchali

The *N. nouchali* tubers were collected from different canals and ponds in Bangladesh. Collected tubers were thoroughly washed with fresh water, dried under shade at room temperature for 2 d. Then the skin of the tubers was removed and the tubers were cut into thin pieces. The pieces were sun-dried and powdered in a mechanical blender. About 100 g of dry powders were macerated in 500 mL methanol for 3 d in a flat bottomed container with occasional shaking, stirring, homogenization, and sonication[12]. Then it was filtered through a filter paper and the supernatant was collected. The extraction was repeated three times. The methanol was evaporated under reduced pressure below 50 ℃ through the rotatory evaporator (RE 200 Sterling, UK). The concentrated *N. nouchali* tuber extract was stored at 4 ℃ until further use.

2.2. Quantification of bioactive phytochemicals in methanolic extract of N. nouchali tuber

The amount of total phenolic and total tannin content was determined following the established method with some modifications by using Folin-Ciocalteu reagent[13]. The content of total flavonoids and proanthocyanidins was determined spectrophotometrically using a standard curve of catechin[14]. Ascorbic acid was assessed based on the established procedure[15]. Methanolic extract of *N. nouchali* tuber was then subjected to the high-performance liquid chromatography-diode array detector (HPLC-DAD) analysis. The composition of bioactive phytochemicals was determined by HPLC[14,16]. Chromatographic analyses were carried out on a Thermo Scientific Dionex ultimate 3000 rapid separation LC (RSLC) systems (Thermo Fisher Scientific Inc., MA, USA), coupled to a quaternary rapid separation pump (LPG-3400RS), Ultimate 3000RS autosampler (WPS-3000) and rapid separation diode array detector (DAD-3000RS). Bioactive phytochemicals were separated on an Acclaim® C18 (4.6 mm × 250 mm × 5 μm) column (Dionix, USA), which was controlled at 30 ℃ using a temperature-controlled column compartment (TCC-3000). For the preparation of calibration curve, a standard stock solution was prepared in methanol containing all bioactive phytochemicals. Data acquisition, peak integration, and calibrations were performed with Dionix Chromeleon software (Version 6.80 RS 10). At least three determinations were conducted for every analysis.

2.3. In vitro antioxidant activity

Total antioxidant activity was determined by using the phosphomolybdenum blue and FRAP reagent[14]. 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and N,N-dimethyl-*p*-phenylenediamine (DMPD) were used to evaluate the *in vitro* radical scavenging activity[14]. Standard ascorbic acid was used in DPPH scavenging assay and gallic acid was used as a standard in ABTS and DMPD scavenging assays. The reducing power was determined by using ascorbic acid as a standard. The chelation of ferrous ions and superoxide radical scavenging capacity were also assessed according to our previous work[14,17]. Based on the screening results of the triplicate measurement of the extract, the inhibition concentration (IC_{50}) value was determined from extrapolating the graph of scavenging activity *versus* the concentration of extract (using linear regression analysis), which is defined as the amount of antioxidant necessary to reduce the initial radical concentration by 50%. EC_{50} value was determined from extrapolating the graph of the concentration of extract or standard *versus* absorbance at a specific nanometer (nm) using linear regression analysis. EC_{50} is the concentration of extract or standard to obtain absorbance=0.50[14].

2.4. Bioinformatics analysis

2.4.1. Target gene prediction for bioactive phytochemicals

On the basis of network pharmacology-based prediction, STITCH 5 (http://stitch.embl.de/) was used to identify target genes related

to bioactive phytochemicals[18]. It calculates a score for each pair of protein-chemical interactions. Chemical names of bioactive compounds (rutin, catechin, myricetin, ellagic acid, gallic acid, vanillic acid, rosmarinic acid, *p*-coumaric acid, quercetin, and ascorbic acid) were input into STITCH 5 singly to match their potential targets, with the organism selected as "*Homo sapiens*" and minimum required interaction score being ≥ 0.4. We predicted 20 genes with top confidence score for each bioactive compound by chromatographic and spectrophotometric analysis. The compound targets without a relationship with the compound-proteins interactions were not considered for further analysis. According to the results, only rosmarinic acid was not detected for any target genes. Usually, interactions with score ≥ 0.4 are considered as medium confidence.

2.4.2. Identification of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway by enrichment analyses of the predicted genes

We performed a gene-set enrichment analysis of the predicted genes[19]. The KEGG[20] pathways significantly associated with the predicted genes were identified. The false discovery rate (FDR) < 0.05, calculated by the Benjamini and Hochberg method[21], was considered as significant.

2.4.3. Construction of protein-protein interaction (PPI) network of the predicted genes

We constructed a PPI network of the predicted genes by search tool for the retrieval of interacting genes (STRING) database (https://string-db.org/cgi/input.pl; STRING-DB v11.0)[22]. The hub genes (with no less than ten edges connected to other nodes) in the PPI network were identified using the node explorer module of NetworkAnalyst software[23].

3. Results

3.1. Bioactive phytochemicals in the methanolic extract of N. nouchali tuber

The yield of methanolic extract of *N. nouchali* tuber was 5.49% (w/w). The content of total phenolics [(353.66±2.98) mg/g of the extract] was higher than total flavonoids [(102.86±14.13) mg/g of the extract], total tannins [(78.14±21.28) mg/g of the extract], and total proanthocyanidins [(4.42±2.67) mg/g of the extract]. The extract contained a moderate amount of ascorbic acid [(27.57±0.12) mg/100 g sun-dried tuber powder]. Furthermore, we identified bioactive phytochemicals using a HPLC system (Figure 1) and the results showed the presence of rutin (39.44 mg), catechin (39.20 mg), myricetin (30.77 mg), ellagic acid (11.05 mg), gallic acid (3.67 mg), vanillic acid (0.75 mg), rosmarinic acid (4.81 mg), *p*-coumaric acid (3.35 mg), and quercetin (0.90 mg) in 1 g of dry methanolic extract of *N. nouchali* tuber.

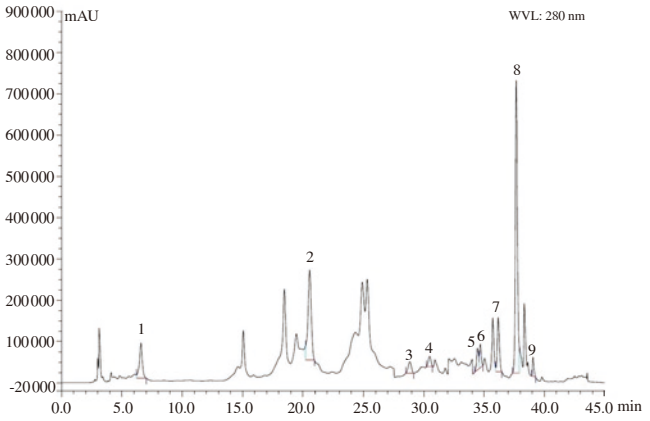

Figure 1. HPLC-DAD chromatogram of the methanolic extract of *Nymphaea nouchali* tuber. 1: gallic acid, 2: (+)-catechin, 3: vanillic acid, 4: *p*-coumaric acid, 5: ellagic acid, 6: rutin, 7: rosmarinic acid, 8: myricetin, and 9: quercetin.

3.2. In-vitro antioxidant activities of methanolic extract of N. nouchali tuber

By using phosphomolybdenum blue, the total antioxidant capacity in the methanolic extract of *N. nouchali* tuber was determined using the following linear regression equation: y=0.010x–0.090, R^2=0.975, where y is absorbance and x is the ascorbic acid concentration in microgram. The total antioxidant capacity was (159.78±23.11) mg ascorbic acid equivalent in 1 g of the methanolic extract. By using FRAP reagents, the total antioxidant capacity was (763.58±36.88) mg ascorbic acid equivalent in 1 g of the methanolic extract (Linear regression equation of standard ascorbic acid: y=0.041x–0.072, R^2=0.998).

We used DPPH, ABTS, and DMPD assays to screen free radical scavenging capacity of the extract. The IC_{50} value of standard ascorbic acid and the *N. nouchali* tuber extract was 13.77 μg/mL and 26.44 μg/mL, respectively in DPPH assay. For the extract and the standard gallic acid in ABTS assay, the IC_{50} value was 25.14 μg/mL and 8.86 μg/mL, respectively. In DMPD assay, the value was 49.32 μg/mL and 22.86 μg/mL, respectively.

Moreover, reducing power, superoxide radical scavenging capacity, and chelation of ferrous ions of the methanolic extract were determined. Ascorbic acid and the extract showed the EC_{50} of 44.42 μg/mL and 164.50 μg/mL, respectively in reducing power assay, indicating that bioactive phytochemicals of the extract are election donors and can reduce the oxidized intermediates. Superoxide was generated by alkaline DMSO method and the IC_{50} value of standard gallic acid and the methanol extract of the tuber was 75.90 μg/mL and 677.50 μg/mL, respectively. The assay for chelation capability of ferrous ion showed the IC_{50} value of standard ethylene diamine tetraacetic acid and the extract was 30.65 μg/mL and 1 949.69 μg/mL, respectively, which indicates that the extract has extremely poor chelation capacity of ferrous ion. These results clearly indicated that the bioactive phytochemicals responsible for the antioxidant

potentials might be present in the methanolic extract of *N. nouchali* tuber.

3.3. Network-based target prediction for bioactive phytochemicals

A drug-target network was constructed to further examine the potential mechanisms of action of the bioactive phytochemicals. The freely available STITCH software (http://stitch.embl.de/) was used to construct the drug-target network and to provide genetic identification in humans. We selected 20 genes/protein for each bioactive phytochemical identified by chromatographic analysis. Altogether, 130 unique target genes were predicted by all the compounds (ascorbic acid, catechin, myricetin, ellagic acid, gallic acid, vanillic acid, *p*-coumaric acid, quercetin, and rutin). The network-based target predicted genes are summarised in Supplementary Table 1.

3.4. Enrichment of cancer pathways of the predicted genes

Enriched KEGG pathways of the predicted 130 unique genes were analyzed. These pathways were mainly involved in cellular development, immune regulation, metabolism, and cancer (Supplementary Table 2). -Log_{10}(FDR) values of some significantly (FDR<0.05) enriched cancer-associated pathways are shown in Figure 2. Some of significant cancer-associated pathways (FDR<0.05) were as follows: pathways in cancer (MAPK3, PIK3CG, AKT1, PRKCA, JUN, CHUK, CXCL8, IL6, CASP3, PTGS2, SLC2A1, ARNT, EGFR, BMP2, MMP2, MMP9, NOS2, and FGF2), renal cell carcinoma (MAPK3, PIK3CG, AKT1, JUN, SLC2A1, and ARNT), bladder cancer (MAPK3, MMP2, EGFR, MMP9, and CXCL8), non-small cell lung cancer (MAPK3, PIK3CG, AKT1, PRKCA, and EGFR), acute myeloid leukemia (MAPK3, PIK3CG, AKT1, CHUK, and PIM1), colorectal cancer (MAPK3, PIK3CG, AKT1, CASP3, and JUN), glioma (MAPK3, PIK3CG, AKT1, PRKCA, and EGFR), pancreatic cancer (MAPK3, PIK3CG, AKT1, CHUK, and EGFR), melanoma (MAPK3, PIK3CG, AKT1, FGF2, and EGFR), small cell lung cancer (PIK3CG, AKT1, NOS2, CHUK, and PTGS2), prostate cancer (MAPK3, PIK3CG, AKT1, CHUK, and EGFR), endometrial cancer (MAPK3, PIK3CG, AKT1, and EGFR), and chronic myeloid leukemia (MAPK3, PIK3CG, AKT1, and CHUK).

3.5. Identification of hub genes associated with pathways in cancer

Using STRING database and NetworkAnalyst software, a total of 130 predicted genes were mapped into the PPI network, including 128 nodes and 841 edges with the PPI enrichment *P*-value less than 1.0×10^{-16}. Interestingly, we found that 117 genes were involved in the PPI network and 65 genes (hub genes) had PPI connectivity degree not less than 10 (Figure 3 and Supplementary Table 3). In addition, we identified top ten hub genes (*IL6*, *AKT1*, *EGFR*, *CAT*, *JUN*, *PTGS2*, *MAPK3*, *CASP3*, *CXCL8*, and *NOS3*) with a higher degree of connectivity, which are associated with pathways in cancer in this study, and eight of them interacted with bioactive phytochemicals of the methanolic extract of *N. nouchali* tuber (Figure 4 and Table 1). These hub genes interacted with one or more bioactive phytochemicals. For example, *AKT1* interacted with gallic acid, myricetin, and quercetin; *CASP3* with gallic acid, rutin, quercetin, and myricetin. Based on the hub genes and the bioactive phytochemicals, we established a gene-bioactive phytochemicals regulatory network that may contribute to the treatment of cancer. This network included the interactions of *IL6*-catechin, *AKT1*-gallic acid/myricetin/quercetin, *EGFR*/*MAPK3*/*CXCL8*-rutin, *JUN*-gallic acid, *PTGS2*-catechin/*p*-coumaric acid/quercetin, and *CASP3*-gallic acid/rutin/quercetin/myricetin.

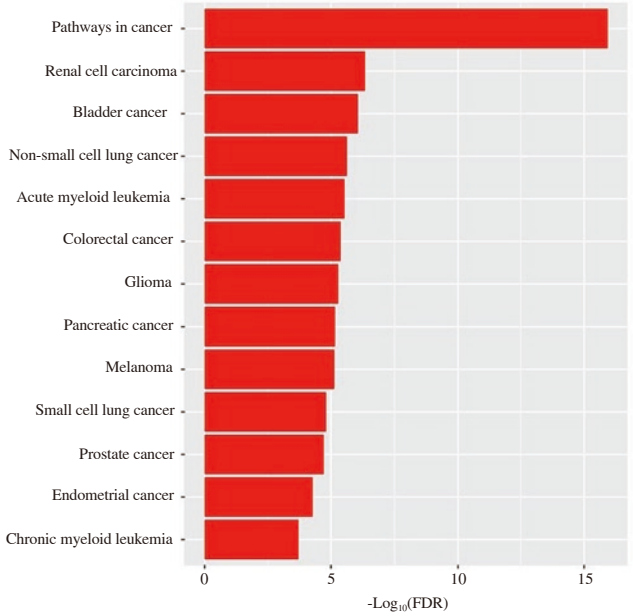

Figure 2. Some of significantly enriched cancer-associated KEGG pathways in human. FDR: false discovery rate.

Table 1. The top ten hub genes with a higher degree of connectivity which are targeted by bioactive phytochemicals from the methanolic extract of *Nymphaea nouchali* tuber.

Gene	Interacting degree	Betweenness	Targeting bioactive phytochemicals
IL6	55	246.11	Catechin
AKT1	52	43.13	Gallic acid, myricetin, quercetin
EGFR	46	343.36	Rutin
CAT	46	219.48	Gallic acid, ascorbic acid
JUN	45	255.77	Gallic acid
PTGS2	44	255.46	Catechin, *p*-coumaric acid, quercetin
MAPK3	44	215.66	Rutin
CASP3	42	220.62	Gallic acid, rutin, quercetin, myricetin
CXCL8	37	116.81	Rutin
NOS3	35	177.58	Catechin, ellagic acid

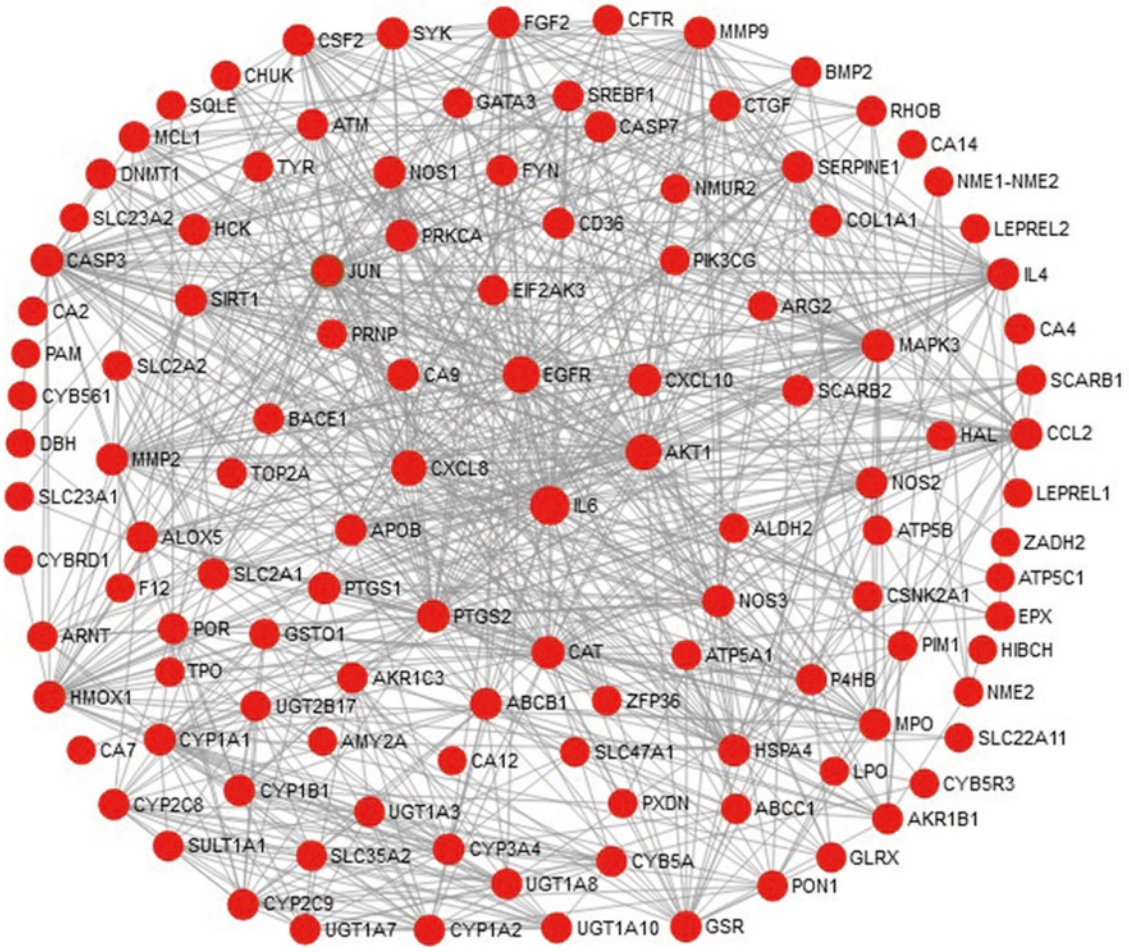

Figure 3. Protein-protein interaction network of the 130 predicted genes. 117 genes were involved in the protein-protein interaction network.

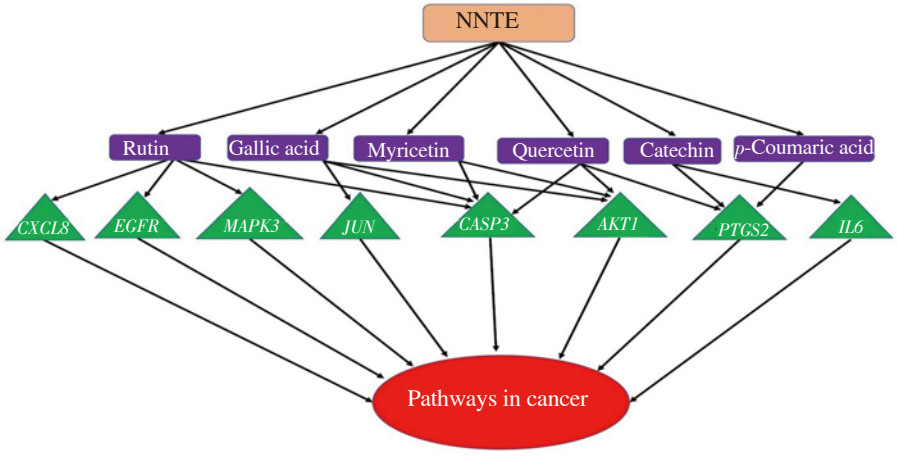

Figure 4. Hub genes (*CXCL8*, *EGFR*, *MAPK3*, *JUN*, *CASP3*, *AKT1*, *PTGS2*, and *IL6*) interacted with bioactive phytochemicals of *Nymphaea nouchali* tuber extract (NNTE) and associated with cancer pathways.

4. Discussion

Phenolics have remarkable bioactivities including anticancer, anti-inflammatory, antibacterial, anti-diabetes, anti-cardiovascular, anti-neurodegenerative, anti-analgesic, anti-allergic, and anti-Alzheimer's properties[24]. Phenolics also stimulate the expression of tumor-suppressing proteins such as p53, phosphatase and tensin homolog (PTEN), p21, and p27[25]. The biological and pharmacological effects of flavonoids include antioxidant, anti-inflammatory, cardioprotective, hepatoprotective, antimicrobial, and anticancer activities[26]. Proanthocyanidins and tannins have various pharmacological effects, including antioxidant and free radical

scavenging activity, antimicrobial, anti-cancer, anti-nutritional, and cardioprotective properties[27]. Our study showed the presence of bioactive phytochemicals in methanolic extract of *N. nouchali* tuber. Natural antioxidants exhibit a wide range of biological effects, such as anti-inflammatory, antibacterial, antiviral, anti-aging, and anticancer[28]. Therefore, our results suggested that the extract of *N. nouchali* tuber might be used as an effective and safe source of natural antioxidants against various diseases, including cancer.

Network medicine offers a platform to systematically explore the molecular complexity of a particular disease, which helps identify modules and pathways of diseases as well as the molecular relationships among apparently distinct phenotypes[29]. Integrating network biology and pharmacology is a paradigm for drug discovery[30]. Phytochemicals activate pathways and thereby enhance the ability of cells to resist injury and disease[31]. Dietary phytochemicals demonstrate strong anticancer effects and affect several cancer-related pathways[32]. Dietary bioactive phytochemicals have gained tremendous attention because of their ability to inhibit multiple signaling pathways as a promising approach to prevent and treat cancers[33]. Our findings indicate that bioactive phytochemicals in the methanolic extract of *N. nouchali* tuber are associated with anticancer pathways, and these bioactive phytochemicals may be better therapeutic agents for treating cancers. PPI network plays an important role in molecular processes, and abnormal PPI is the basis of many pathological conditions, including tumors[34]. The IL-6/JAK/STAT3 pathway is aberrantly hyperactivated in many types of cancer[35]. Another hub gene *PTGS2* is upregulated during both inflammation and cancer[36]. *IL6* and *PTGS2* both interact with catechin, and it was stated that catechin intake may ensure a protective epigenetic status against the adverse effects through the interaction with *IL6*[37]. An important antioxidant enzyme catalase (*CAT*) is another hub gene with 46 connectivity degree and is associated with genetic, epigenetic, and posttranscriptional processes. Abnormal expression levels of CAT have been reported in cancer tissues[38]. Gallic acid significantly increased the activity of CAT in rat model. A recent study demonstrated the link between aberrant cell cycle progression and AKT hyperactivation in cancer[39]. Gallic acid inhibited the activities of AKT as well as migration and invasion in human osteosarcoma[40]. Quercetin facilitates cell death and chemosensitivity through RAGE/PI3K/AKT/mTOR axis in human pancreatic cancer cells[41]. A proto-oncogene *JUN* is a molecular target for improving cancer therapy[42]. *CASP3* is used as a marker for efficacy of cancer therapy and this gene may not only increase the sensitivity of cancer cells to chemotherapy and radiotherapy, but also inhibit cancer cell invasion and metastases[43]. Gallic acid, rutin, tannic acid, and quercetin significantly inhibited cell proliferation of human prostate cancer by increasing the caspase-3 activity[44]. Gallic acid also upregulated the expression of *CASP3* that induced apoptosis[45]. Aberrant expression of *MAPK3* is associated with invasion, metastasis, and drug resistance of multiple tumor cells[46]. *EGFR* is involved in the pathogenesis and progression of different carcinoma types[47]. *CXCL8* is the most studied chemokine in human malignancies and displays multifaceted pro-tumorigenic effects, including tumor cell growth, metastatization, and angiogenesis, contributing to the progression of several cancers[48]. Polyphenols downregulated gene expression of pro-inflammatory cytokines/enzymes and differentially modulated the inflammatory response of human keratinocytes through distinct signal transduction pathways, including EGFR-MAPK pathways[49]. Our results reveal that three hub genes (*MAPK3*, *EGFR*, and *CXCL8*) interacted with rutin. The above findings suggested that these hub genes might play a crucial role in various types of cancers and interact with bioactive compounds of the *N. nouchali* tuber extract.

5. Conclusion

The discovery of bioactive phytochemicals and their predicted target genes will be of great importance in various treatments against chronic diseases including various cancers. The results strongly indicate that this medicinal plant can serve as a very useful source of antioxidant and anticancer components. The network pharmacology contributes to understanding the complex interactions between bioactive phytochemicals and anticancer pathways from a network perspective. Consequently, network pharmacology provides a novel approach to promote drug discovery in a precise manner from this plant. Furthermore, clinical experiments are required to characterize the potent molecule from the pharmaceutical point of view in future studies.

Conflict of interest statement

The authors declare that they have no conflicts of interest.

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Authors' contributions

MNU performed data analyses, conceived the research, designed analysis strategies, and wrote the manuscript. MZH and MAZ conceived the research and designed analysis strategies. MAS and TAK performed data analyses. MAH, AS, and AA drew the figures and helped write the manuscript. KM helped to revise the

manuscript. All the authors read and approved the final manuscript.

References

[1] Alam MB, Ahmed A, Motin MA, Kim S, Lee SH. Attenuation of melanogenesis by *Nymphaea nouchali* (Burm. f) flower extract through the regulation of cAMP/CREB/MAPKs/MITF and proteasomal degradation of tyrosinase. *Sci Rep* 2018; **8**(1): 13928. Doi: 10.1038/s41598-018-32303-7.

[2] Siddhanta AK, Mody KH, Ramavat BK, Chauhan VD, Garg HS, Goel AK, et al. Bioactivity of marine organisms: Part Ⅷ-Screening of some marine flora of western coast of India. *Indian J Exp Biol* 1997; **35**(6): 638-643.

[3] Bhandarkar MR, Khan A. Antihepatotoxic effect of *Nymphaea stellata* willd., against carbon tetrachloride-induced hepatic damage in albino rats. *J Ethnopharmacol* 2004; **91**(1): 61-64.

[4] Raja MKMM, Sethiya NK, Mishra SH. A comprehensive review on *Nymphaea stellata*: A traditionally used bitter. *J Adv Pharm Technol Res* 2010; **1**(3): 311-319.

[5] Alam MB, Ju MK, Lee SH. DNA protecting activities of *Nymphaea nouchali* (Burm. f) flower extract attenuate t-BHP-induced oxidative stress cell death through Nrf2-mediated induction of heme oxygenase-1 expression by activating MAP-kinases. *Int J Mol Sci* 2017; **18**(10). Doi: 10.3390/ijms18102069.

[6] Dissanayake RK, Ratnaweera PB, Williams DE, Wijayarathne CD, Wijesundera RLC, Andersen RJ, et al. Antimicrobial activities of endophytic fungi of the Sri Lankan aquatic plant *Nymphaea nouchali* and chaetoglobosin A and C, produced by the endophytic fungus *Chaetomium globosum*. *Mycology* 2016; **7**(1): 1-8.

[7] Parimala M, Debjani M, Vasanthi HR, Shoba FG. *Nymphaea nouchali* Burm. f. hydroalcoholic seed extract increases glucose consumption in 3T3-L1 adipocytes through activation of peroxisome proliferator-activated receptor gamma and insulin sensitization. *J Adv Pharm Technol Res* 2015; **6**(4): 183-189.

[8] Shankar E, Kanwal R, Candamo M, Gupta S. Dietary phytochemicals as epigenetic modifiers in cancer: Promise and challenges. *Semin Cancer Biol* 2016; **40–41**: 82-99.

[9] Budisan L, Gulei D, Zanoaga OM, Irimie AI, Chira S, Braicu C, et al. Dietary intervention by phytochemicals and their role in modulating coding and non-coding genes in cancer. *Int J Mol Sci* 2017; **18**(6). Doi: 10.3390/ijms18061178.

[10]Kabir SR, Zubair MA, Nurujjaman M, Haque MA, Hasan I, Islam MF, et al. Purification and characterization of a Ca(2+)-dependent novel lectin from *Nymphaea nouchali* tuber with antiproliferative activities. *Biosci Rep* 2011; **31**(6): 465-475.

[11]Kabidul Azam MdN, Rahman MdM, Biswas S, Ahmed MdN. Appraisals of Bangladeshi medicinal plants used by folk medicine practitioners in the prevention and management of malignant neoplastic diseases. *Int Sch Res Not* 2016; **2016**: 7832120. Doi: 10.1155/2016/7832120.

[12]Pisoschi AM, Pop A, Cimpeanu C, Predoi G. Antioxidant capacity determination in plants and plant-derived products: A review. *Oxid Med Cell Longev* 2016; **2016**: 9130976. Doi: 10.1155/2016/9130976.

[13]Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nat Protoc* 2007; **2**(4): 875-877.

[14]Uddin MN, Mitra K, Haque MZ. Comparative bio-active compounds determination and *in vitro* antioxidant properties of newly developed soy mixed wheat flour and traditional wheat flour. *Int J Food Prop* 2016; **19**(9): 2113-2126.

[15]Khalil I, Moniruzzaman M, Boukraâ L, Benhanifia M, Islam A, Islam N, et al. Physicochemical and antioxidant properties of Algerian honey. *Mol Basel Switz* 2012; **17**(9): 11199-11215.

[16]Chuanphongpanich S, Phanichphant S. Method development and determination of phenolic compounds in broccoli seeds samples. *J Sci* 2006; **33**(1): 103-107.

[17]Uddin MN, Rahman MA, Hossain H, Khan TA, Akter R. High-performance liquid chromatographic analysis explores the potential antioxidative agents of *Argyreia argentea* ARN. EX CHOISY extract. *J Pharm Bioallied Sci* 2019; **11**(1): 16.

[18]Szklarczyk D, Santos A, von Mering C, Jensen LJ, Bork P, Kuhn M. STITCH 5: Augmenting protein-chemical interaction networks with tissue and affinity data. *Nucleic Acids Res* 2016; **44**(D1): D380-D384.

[19]Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A* 2005; **102**(43): 15545-15550.

[20]Kanehisa M, Furumichi M, Tanabe M, Sato Y, Morishima K. KEGG: New perspectives on genomes, pathways, diseases and drugs. *Nucleic Acids Res* 2017; **45**(D1): D353-D361.

[21]Benjamini Y, Hochberg Y. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J R Stat Soc Ser B Methodol* 1995; **57**(1): 289-300.

[22]Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, et al. STRING v11: Protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res* 2019; **47**(Database issue): D607-D613.

[23]Xia J, Benner MJ, Hancock REW. NetworkAnalyst - integrative approaches for protein–protein interaction network analysis and visual exploration. *Nucleic Acids Res* 2014; **42**(Web Server issue): W167-W174.

[24]Shahidi F, Yeo J. Bioactivities of phenolics by focusing on suppression of chronic diseases: A review. *Int J Mol Sci* 2018; **19**(6). Doi: 10.3390/ijms19061573.

[25]Anantharaju PG, Gowda PC, Vimalambike MG, Madhunapantula SV. An overview on the role of dietary phenolics for the treatment of cancers. *Nutr J* 2016; **15**(1): 99.

[26]Gontijo VS, Dos Santos MH, Viegas C. Biological and chemical aspects of natural biflavonoids from plants: A brief review. *Mini Rev Med Chem* 2017; **17**(10): 834-862.

[27]Smeriglio A, Barreca D, Bellocco E, Trombetta D. Proanthocyanidins and

hydrolysable tannins: Occurrence, dietary intake and pharmacological effects. *Br J Pharmacol* 2017; 1**74**(11): 1244-1262.

[28]Zheng J, Zhou Y, Li Y, Xu DP, Li S, Li HB. Spices for prevention and treatment of cancers. *Nutrients* 2016; **8**(8): 495. Doi: 10.3390/nu8080495.

[29]Barabási AL, Gulbahce N, Loscalzo J. Network medicine: A network-based approach to human disease. *Nat Rev Genet* 2011; **12**(1): 56-68.

[30]Hopkins AL. Network pharmacology: The next paradigm in drug discovery. *Nat Chem Biol* 2008; **4**(11): 682-690.

[31]Lee J, Jo DG, Park D, Chung HY, Mattson MP. Adaptive cellular stress pathways as therapeutic targets of dietary phytochemicals: Focus on the nervous system. *Pharmacol Rev* 2014; **66**(3): 815-868.

[32]Kapinova A, Kubatka P, Golubnitschaja O, Kello M, Zubor P, Solar P, et al. Dietary phytochemicals in breast cancer research: Anticancer effects and potential utility for effective chemoprevention. *Environ Health Prev Med* 2018; **23**(1): 36. Doi: 10.1186/s12199-018-0724-1.

[33]Dandawate PR, Subramaniam D, Jensen RA, Anant S. Targeting cancer stem cells and signaling pathways by phytochemicals: Novel approach for breast cancer therapy. *Semin Cancer Biol* 2016; **40–41**: 192-208.

[34]Wong FH, Huang CYF, Su LJ, Wu YC, Lin YS, Hsia JY, et al. Combination of microarray profiling and protein-protein interaction databases delineates the minimal discriminators as a metastasis network for esophageal squamous cell carcinoma. *Int J Oncol* 2009; **34**(1): 117-128.

[35]Johnson DE, O'Keefe RA, Grandis JR. Targeting the IL-6/JAK/STAT3 signalling axis in cancer. *Nat Rev Clin Oncol* 2018; **15**(4): 234-248.

[36]Desai SJ, Prickril B, Rasooly A. Mechanisms of phytonutrient modulation of cyclooxygenase-2 (COX-2) and inflammation related to cancer. *Nutr Cancer* 2018; **70**(3): 350-375.

[37]Zhong J, Colicino E, Lin X, Mehta A, Kloog I, Zanobetti A, et al. Cardiac autonomic dysfunction: Particulate air pollution effects are modulated by epigenetic immunoregulation of toll-like receptor 2 and dietary flavonoid intake. *J Am Heart Assoc* 2015; **4**(1): e001423. Doi: 10.1161/JAHA.114.001423.

[38]Glorieux C, Zamocky M, Sandoval JM, Verrax J, Calderon PB. Regulation of catalase expression in healthy and cancerous cells. *Free Radic Biol Med* 2015; **87**: 84-97.

[39]Liu P, Begley M, Michowski W, Inuzuka H, Ginzberg M, Gao D, et al. Cell-cycle-regulated activation of Akt kinase by phosphorylation at its carboxyl terminus. *Nature* 2014; **508**(7497): 541-545.

[40]Liao CL, Lai KC, Huang AC, Yang JS, Lin JJ, Wu SH, et al. Gallic acid inhibits migration and invasion in human osteosarcoma U-2 OS cells through suppressing the matrix metalloproteinase-2/-9, protein kinase B (PKB) and PKC signaling pathways. *Food Chem Toxicol Int J Publ Br Ind Biol Res Assoc* 2012; **50**(5): 1734-1740.

[41]Lan CY, Chen SY, Kuo CW, Lu CC, Yen GC. Quercetin facilitates cell death and chemosensitivity through RAGE/PI3K/AKT/mTOR axis in human pancreatic cancer cells. *J Food Drug Anal* 2019; **27**(4): 887-896. Doi: 10.1016/j.jfda.2019.07.001.

[42]Xia Y, Yang W, Bu W, Ji H, Zhao X, Zheng Y, et al. Differential regulation of c-Jun protein plays an instrumental role in chemoresistance of cancer cells. *J Biol Chem* 2013; **288**(27): 19321-19329.

[43]Zhou M, Liu X, Li Z, Huang Q, Li F, Li CY. Caspase-3 regulates the migration, invasion, and metastasis of colon cancer cells. *Int J Cancer* 2018; 1**43**(4): 921-930.

[44]Ferruelo A, Romero I, Cabrera PM, Arance I, Andrés G, Angulo JC. Effects of resveratrol and other wine polyphenols on the proliferation, apoptosis and androgen receptor expression in LNCaP cells. *Actas Urol Esp* 2014; **38**(6): 397-404.

[45]Chandramohan Reddy T, Bharat Reddy D, Aparna A, Arunasree KM, Gupta G, Achari C, et al. Anti-leukemic effects of gallic acid on human leukemia K562 cells: Downregulation of COX-2, inhibition of BCR/ABL kinase and NF-κB inactivation. *Toxicol Vitro Int J Publ Assoc BIBRA* 2012; **26**(3): 396-405.

[46]Cao HY, Xiao CH, Lu HJ, Yu HZ, Hong H, Guo CY, et al. MiR-129 reduces CDDP resistance in gastric cancer cells by inhibiting MAPK3. *Eur Rev Med Pharmacol Sci* 2019; **23**(15): 6478-6485.

[47]Normanno N, De Luca A, Bianco C, Strizzi L, Mancino M, Maiello MR, et al. Epidermal growth factor receptor (EGFR) signaling in cancer. *Gene* 2006; **366**(1): 2-16.

[48]Rotondi M, Coperchini F, Latrofa F, Chiovato L. Role of chemokines in thyroid cancer microenvironment: Is CXCL8 the main player? *Front Endocrinol* 2018; **9**: 314. Doi:10.3389/fendo.2018.00314.

[49]Potapovich AI, Lulli D, Fidanza P, Kostyuk VA, De Luca C, Pastore S, et al. Plant polyphenols differentially modulate inflammatory responses of human keratinocytes by interfering with activation of transcription factors NFκB and AhR and EGFR-ERK pathway. *Toxicol Appl Pharmacol* 2011; **255**(2): 138-149.

Supplementary Table 1. The predicted genes for the bioactive compounds of *Nymphaea nouchali*

Ascorbic acid	Gallic acid	Catechin	Rutin	Myricetin	Ellagic acid	Vanillic acid	p-coumaric acid	Quercetin
SLC23A2	*MMP2*	*DNMT1*	*AKR1C3*	*SIRT1*	*F12*	*UGT1A3*	*TGR1*	*MCL1*
DBH	*TYR*	*IL6*	*P4HB*	*PIK3CG*	*CSNK2A1*	*UGT1A8*	*TYR*	*CYP1B1*
GSTO1	*ATM*	*PTGS2*	*EGFR*	*CYP1B1*	*TYR*	*UGT1A7*	*LPO*	*HCK*
CYBRD1	*ABCB1*	*NOS2*	*GSR*	*SLC2A2*	*IL4*	*UGT1A10*	*HAL*	*PIM1*
SLC23A1	*JUN*	*NOS1*	*PRNP*	*AKT1*	*NOS3*		*CYP1A1*	*SLC2A2*
CAT	*UGT2B17*	*HMOX1*	*CTGF*	*ALOX5*	*SQLE*		*CYP1A2*	*CYP2C8*
HMOX1	*GATA3*	*NOS3*	*SREBF1*	*TOP2A*	*NME2*		*EPX*	*CYP1A1*
NOS3	*EIF2AK3*	*SLC47A1*	*HSPA4*	*AMY2A*	*NME1-NME2*		*MPO*	*ATP5B*
CFTR	*SERPINE1*	*APOB*	*ALDH2*	*BMP2*	*CA2*		*TPO*	*HIBCH*
CYBASC3	*CASP3*	*PON1*	*TMPRSS11D*	*PIM1*	*SYK*		*SULT1A1*	*STK17B*
CYB5R3	*SULT1A1*	*SLC35A2*	*FGF2*	*FYN*	*CA1*		*ENSG00000258653*	*ATP5C1*
CYB5A	*AKT1*	*BACE1*	*CASP3*	*CYP3A4*	*CA9*		*PTGR2*	*ATP5A1*
PAM	*MMP9*	*SLC22A11*	*NOS2*	*CD36*	*CA6*		*PXDNL*	*AKT1*
GLRX	*APOB*	*CSF2*	*CXCL10*	*SCARB2*	*CA14*		*PXDN*	*MMP9*
NOS2	*PRKCA*	*KIAA1149*	*IL8*	*SCARB1*	*CA7*		*PTGS2*	*PTGS2*
P4HB	*CHUK*	*ABCB1*	*CCL2*	*CYP2C9*	*CA5B*		*ZADH2*	*CYP1A2*
COL1A1	*CASP7*	*ARG2*	*CASP7*	*CYP1A1*	*CA5A*		*PTGS1*	*CASP3*
CYB561	*RHOB*	*ZFP36*	*ARNT*	*ABCC1*	*CA4*		*TMEM37*	*SIRT1*
LEPREL2	*MPO*	*POR*	*MAPK3*	*CASP3*	*CA3*		*AKR1B1*	*CYP3A4*
LEPREL1	*CAT*		*CYP1B1*	*SLC2A1*	*CA12*			*ABCB1*

Supplementary Table 2. KEGG pathways that were significantly associated with the predicted genes for the bioactive compounds of *Nymphaea nouchali*

KEGG pathways	Genes in Overlap (k)	p-value	FDR q-value
Nitrogen metabolism	12	1.53E-25	2.85E-23
Metabolism of xenobiotics by cytochrome P450	13	1.21E-20	1.13E-18
Pathways in cancer	18	1.98E-18	1.23E-16
Retinol metabolism	10	2.42E-15	1.12E-13
Drug metabolism - cytochrome P450	10	8.40E-15	3.12E-13
Steroid hormone biosynthesis	9	4.02E-14	1.25E-12
Fc epsilon RI signaling pathway	8	6.02E-11	1.56E-09
Ascorbate and aldarate metabolism	6	6.71E-11	1.56E-09
Pentose and glucuronate interconversions	6	1.42E-10	2.93E-09
Toll-like receptor signaling pathway	8	4.80E-10	8.93E-09
T cell receptor signaling pathway	8	7.60E-10	1.29E-08
Alzheimer's disease	9	9.82E-10	1.52E-08
Porphyrin and chlorophyll metabolism	6	1.64E-09	2.35E-08
Drug metabolism - other enzymes	6	6.43E-09	8.54E-08
Starch and sucrose metabolism	6	7.25E-09	8.99E-08
Arachidonic acid metabolism	6	1.42E-08	1.65E-07
Renal cell carcinoma	6	4.49E-08	4.91E-07
Cytokine-cytokine receptor interaction	9	6.03E-08	6.10E-07
Chemokine signaling pathway	8	6.23E-08	6.10E-07
B cell receptor signaling pathway	6	6.81E-08	6.33E-07
VEGF signaling pathway	6	7.38E-08	6.53E-07
Tryptophan metabolism	5	9.07E-08	7.49E-07
Focal adhesion	8	9.26E-08	7.49E-07
Bladder cancer	5	1.17E-07	8.85E-07
Natural killer cell mediated cytotoxicity	7	1.19E-07	8.85E-07
Apoptosis	6	1.66E-07	1.15E-06
ErbB signaling pathway	6	1.66E-07	1.15E-06
Jak-STAT signaling pathway	7	2.76E-07	1.83E-06
Fc gamma R-mediated phagocytosis	6	2.99E-07	1.92E-06
Arginine and proline metabolism	5	4.22E-07	2.53E-06
Non-small cell lung cancer	5	4.22E-07	2.53E-06
Acute myeloid leukemia	5	5.56E-07	3.23E-06
Colorectal cancer	5	8.49E-07	4.59E-06
NOD-like receptor signaling pathway	5	8.49E-07	4.59E-06
MAPK signaling pathway	8	8.63E-07	4.59E-06
Glioma	5	1.08E-06	5.56E-06
Linoleic acid metabolism	4	1.25E-06	6.29E-06

Epithelial cell signaling in Helicobacter pylori infection	5	1.35E-06	6.61E-06
Pancreatic cancer	5	1.56E-06	7.44E-06
Melanoma	5	1.67E-06	7.79E-06
Leishmania infection	5	1.80E-06	8.15E-06
Prion diseases	4	2.72E-06	1.21E-05
Small cell lung cancer	5	3.86E-06	1.67E-05
Hematopoietic cell lineage	5	4.59E-06	1.94E-05
Prostate cancer	5	5.13E-06	2.12E-05
GnRH signaling pathway	5	9.54E-06	3.86E-05
Endometrial cancer	4	1.36E-05	5.38E-05
Adipocytokine signaling pathway	4	3.73E-05	1.44E-04
Adherens junction	4	5.22E-05	1.94E-04
Chronic myeloid leukemia	4	5.22E-05	1.94E-04
Calcium signaling pathway	5	1.39E-04	5.08E-04
Aldosterone-regulated sodium reabsorption	3	2.19E-04	7.67E-04
Tyrosine metabolism	3	2.19E-04	7.67E-04
ABC transporters	3	2.51E-04	8.65E-04
Type II diabetes mellitus	3	3.06E-04	1.03E-03
Leukocyte transendothelial migration	4	3.14E-04	1.04E-03
mTOR signaling pathway	3	4.13E-04	1.35E-03
Neurotrophin signaling pathway	4	4.29E-04	1.38E-03
Amyotrophic lateral sclerosis (ALS)	3	4.36E-04	1.38E-03
Parkinson's disease	4	4.55E-04	1.41E-03
Cytosolic DNA-sensing pathway	3	4.87E-04	1.48E-03
Insulin signaling pathway	4	5.88E-04	1.76E-03
p53 signaling pathway	3	9.06E-04	2.67E-03
Long-term depression	3	9.86E-04	2.86E-03
RIG-I-like receptor signaling pathway	3	1.03E-03	2.94E-03
Huntington's disease	4	1.62E-03	4.56E-03
beta-Alanine metabolism	2	1.69E-03	4.69E-03
Progesterone-mediated oocyte maturation	3	1.73E-03	4.72E-03
Proximal tubule bicarbonate reclamation	2	1.85E-03	4.97E-03
Dorso-ventral axis formation	2	2.01E-03	5.32E-03
Gap junction	3	2.03E-03	5.32E-03
Melanogenesis	3	2.82E-03	7.28E-03
Histidine metabolism	2	2.93E-03	7.46E-03
Regulation of actin cytoskeleton	4	2.97E-03	7.47E-03
Asthma	2	3.13E-03	7.77E-03
Propanoate metabolism	2	3.78E-03	9.26E-03
Pyruvate metabolism	2	5.52E-03	1.33E-02
Oxidative phosphorylation	3	5.83E-03	1.39E-02

Tight junction	3	5.96E-03	1.40E-02
Valine, leucine and isoleucine degradation	2	6.64E-03	1.54E-02
Intestinal immune network for IgA production	2	7.87E-03	1.81E-02
Glycerolipid metabolism	2	8.19E-03	1.86E-02
Wnt signaling pathway	3	8.46E-03	1.88E-02
Glutathione metabolism	2	8.51E-03	1.88E-02
Autoimmune thyroid disease	2	9.18E-03	2.01E-02
Vibrio cholerae infection	2	9.87E-03	2.13E-02
Pathogenic Escherichia coli infection	2	1.06E-02	2.26E-02
Complement and coagulation cascades	2	1.58E-02	3.33E-02
Long-term potentiation	2	1.62E-02	3.35E-02
Viral myocarditis	2	1.62E-02	3.35E-02
Phosphatidylinositol signaling system	2	1.89E-02	3.86E-02
Peroxisome	2	1.98E-02	4.01E-02
ECM-receptor interaction	2	2.28E-02	4.56E-02
TGF-beta signaling pathway	2	2.33E-02	4.61E-02

Supplementary Table 3. Hub genes in the protein-protein interaction network of the predicted genes		
Gene symbol	**Connectivity degree**	**Betweenness**
IL6	55	246.11
AKT1	52	43.13
EGFR	46	343.36
CAT	46	219.48
JUN	45	255.77
PTGS2	44	255.46
MAPK3	44	215.66
CASP3	42	220.62
CXCL8	37	116.81
NOS3	35	177.58
HSPA4	33	135.49
CCL2	32	32.65
HMOX1	32	10.52
IL4	31	47.85
MMP9	30	41.12
MMP2	30	4.88
FGF2	28	79.35
CYP1A1	27	158.68
MPO	26	23.78
SERPINE1	26	18.34
SIRT1	26	0
CYP3A4	25	185.69
CSF2	25	38.42
ABCB1	25	18.7
GSR	22	13.07
CYP1B1	21	0
CTGF	20	25.68
SLC2A1	20	17.54
CYP2C9	20	16.99
NOS2	20	16.14
APOB	19	67.65
CYP1A2	19	22.18
CYP2C8	18	89.89
MCL1	18	30.5
CXCL10	18	16.17
ATM	18	10.4
UGT1A8	17	41.66

ALOX5	17	31.97
P4HB	16	150.77
AKR1B1	16	72.39
SREBF1	16	60.2
SYK	16	15.55
NOS1	16	0
PTGS1	15	30
COL1A1	15	14.43
CYB5A	14	50.02
FYN	14	33.11
PON1	14	20.31
UGT1A3	14	2.17
PRKCA	13	29.12
AKR1C3	13	23.2
BACE1	13	7.54
POR	13	2.55
UGT1A7	13	2.23
SLC35A2	13	1.64
UGT1A10	13	1.59
DNMT1	12	26.22
SULT1A1	12	0.35
GSTO1	11	72.51
TYR	11	61.61
CA9	11	1.35
CFTR	11	0
BMP2	11	0
ABCC1	10	11.18
ARNT	10	8.74
CSNK2A1	9	1.09
EIF2AK3	9	0.76
UGT2B17	9	0.18
SLC2A2	8	68.67
RHOB	8	7.82
GLRX	8	1.75
SCARB1	8	1.11
CASP7	8	0
TOP2A	7	2.74
CHUK	7	1.12
GATA3	7	0
ATP5A1	6	19.55
PIM1	6	0.65

HCK	6	0
PRNP	6	0
CD36	6	0
ZFP36	6	0
SCARB2	5	20
SLC47A1	5	5.33
PIK3CG	5	0.44
ATP5B	5	0
NME2	5	0
EPX	5	0
TPO	4	18.5
ALDH2	4	0.78
ARG2	4	0.29
NME1-NME2	3	7.93
HAL	3	0.61
CYB5R3	3	0.33
CA2	3	0.2
PXDN	3	0.11
LEPREL1	3	0
LEPREL2	3	0
SQLE	3	0
DBH	3	0
PAM	2	18
CYB561	2	1
ATP5C1	2	0
NMUR2	2	0
SLC22A11	2	0
SLC23A1	2	0
F12	2	0
LPO	1	0
CA7	1	0
SLC23A2	1	0
ZADH2	1	0
CYBRD1	1	0
CA14	1	0
AMY2A	1	0
HIBCH	1	0
CA4	1	0
CA12	1	0