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Assessing the Anti-inflammatory Effects of Bacopa-Derived Bioactive Compounds Using Network Pharmacology and *In Vitro* Studies

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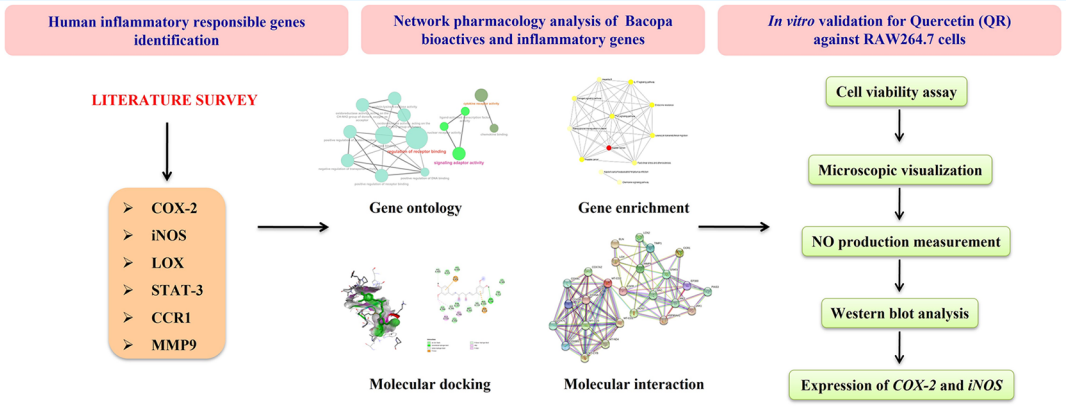

ABSTRACT: *Bacopa monnieri* is reported as a potent Indian medicinal plant that possesses numerous pharmacological activities due to the presence of various bioactive compounds. These pharmacological activities were used in the ancient medicine system to cure inflammatory conditions. Bacopa has the ability to reduce acute pain and inflammation by inhibiting the enzyme cyclo-oxygenase-2 (*COX-2*) and reducing *COX-2*-arbitrated prostanoid mediators. Moreover, the anti-inflammatory property may also be associated with the neuroprotective activity of Bacopa. Considering this importance, the current pilot study focused on the anti-inflammatory potential of various phytocompounds of bacopa and their interaction with inflammation responsible genes such as *COX2*, *iNOS*, *LOX*, *STAT3*, *CCR1*, and *MMP9* through pharmacology analysis of its systems. Docking results revealed that, quercetin (QR) showed significant binding energies with inflammatory genes. Hence, we selected QR as a potential phytocompound for further *in vitro* experiments. This existing study aimed to evaluate the efficacy of QR as a potent anti-inflammatory compound against lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages. The *in vitro* analysis concludes that QR effectively reduces the production of nitric oxide (NO) in LPS-induced RAW264.7 cells and downregulates the expression of *COX-2* and *iNOS* genes due to the inhibitory potential of QR on LPS-stimulated NO production.

INTRODUCTION

Bacopa monnieri (L.) belongs to the family Scrophulariaceae (recently included in Plantaginaceae), also called "Brahmi", which grows in warm and marshy areas. Bacopa has small sessile and fleshy leaves, and flowers are white or light purple in color with more than 70 species.[1] This plant occurs naturally in the Indian subcontinent, Australia, East Asia, and the United States, and it has been used in Ayurvedic medicine in India for more than 3000 years to treat various inflammation-related diseases, such as cancer, asthma, arthritis, and Alzheimer's and Parkinson's diseases. In ancient times, this plant was reputedly used as brain tonic to memorize scriptures and sacred hymns by Ayurvedic scholars.

Bacopa is a notable nootropic herb used to improve memory and cognition.[2−5] The plant contains a number of bioactive compounds which includes several alkaloids, triterpenoid saponins, and sterols.[6] Active constituents including Brahmin, herpestine, D-mannitol, hersaponin, bacosides, bacopasaponins, beta-sitosterol, stigmastanol, and betulic acid[7] have been identified and characterized. Bacoside

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A is the well studied potential triterpenoid saponin of *B. monnieri*, which is composed of bacoside A3, bacopasaponin C, bacopaside II and a jujubogenin isomer of bacosaponin C.[8] To date, bacosides and triterpenoid saponins have been able to repair neuronal damage, promote kinase enzyme activity, restore synaptic activity, and enhance the neural impulse transmission. Bacopa acts pharmacologically as antifungal,[9] antidepressant,[5,10] antiepileptic,[11,12] antioxidant,[11,13] anti-inflammatory,[14,15] antitoxic,[16] antibacterial,[9] anticancer,[17] memory enhancer,[18] analgesic,[19] hepatoprotective,[20] antihyperglycemic,[21] and anticonvulsant.[22]

The anti-inflammatory effect of Bacopa may also be related to neuroprotective effects. Inflammatory responses within the central nervous system (CNS) (brain and spinal cord) are generally referred to as "neuroinflammation". Inflammation is one of the host immune response mechanisms against infection, disease, and tissue injury. The inflammatory response can be caused by different forms of injury and the severity of inflammation associated with multiple organ dysfunction syndrome (MODS), most importantly in sepsis. Moreover, even with best medical treatment the mortality rates in severe sepsis can reach 50%.[23] To overcome this problem, medical scores such as acute physiology, sequential organ dysfunction assessment, and chronic health evaluation II are greatly used to analyze patient inflammation severity and mortality risk.[24,25] In addition, inflammation is a defense mechanism against invading pathogens such as bacteria and virus. During this process, the body's white blood cells release certain chemicals especially chemokines, which enter the bloodstream, reach the site of infection, and protect the body from invaders.[26]

Lipopolysaccharide (LPS) is abundantly found in the outer membrane of Gram-negative bacteria and may activate common myeloid progenitor cells, in particular, monocytes and macrophages to produce proinflammatory cytokines in various cell types.[27] Macrophages predominantly participate in inflammatory reactions through various proinflammatory mediators release, including interleukin-6 (IL-6), nitric oxide (NO), prostaglandins via inducible cyclo-oxygenase (*COX-2*) and tumor necrosis factor-α (TNF-α), which causes tissue damage and pathogenesis of many diseases including cancer, cardiovascular diseases, neurodegenerative disorder, diabetes, and obesity.[28]

At present, various plant-derived products are used to treat inflammation, among which *B. monnieri* possess a significant anti-inflammatory property. Experimentation on edema induced in the hind paws of rats through methanolic extract of *B. monnieri* showed that the edema paw volume was significantly reduced.[29] Various *in vitro* studies demonstrated that *B. monnieri* extract could enhance memory in animal models.[30,31] These experiments revealed that, Bacopa has the ability to hinder or modulate systemic inflammation.

The current study focused on the identification of potential phytocompounds of *B. monnieri* against inflammation-responsible genes through *in silico* analysis such as gene ontology, molecular interactions of inflammation responsive genes, and molecular docking analysis, and the selected phytocompounds was used for further study. Among them, quercetin (QR) is one of the most important phytocompounds present in *B. monnieri*. Further this pilot study was aimed to investigate the anti-inflammatory efficacy of QR against RAW264.7 cells by evaluating the NO production and expression level of *COX-2* and inducible nitric oxide synthase (*iNOS*). Hence, this study could pave the way to recognize the potential phytocompounds to treat inflammation associated diseases and other diseases in the course of time.

MATERIALS AND METHODS

Network Pharmacology Analysis. Network pharmacology was successfully applied in this study to explore the novel drugs and or decipher the mechanism of drugs based on integrated omics, pharmacology, and computational biology.

Retrieval of Phytocompounds. Available literature and our previous research findings reported the presence of about 52 phytocompounds and their respective pharmacological properties in *B. monnieri*.[4,32] Among these phytocompounds, compounds with '0' number of violations (nVio) were considered as highly significant and used for further analyses.

Identification and Mining of Human Targets. Genes responsible for inflammation were identified through a literature survey.[33−38] Furthermore, canonical SMILES of eight selected phytocompounds were employed to find human targets through the SwissTargetPrediction web tool (http://www.swisstargetprediction.ch/)[39] (Table S1).

Molecular Interactome and Gene Enrichment Analysis. The protein−protein interaction (PPI) of inflammation responsible genes was performed by STRING *v*10.5 (https://string-db.org/[40]) with a high confidence filter score of 0.7. This physical interaction network was used to unveil the regulatory functions of inflammation responsible human targets. This PPI network information was collected in TSV format and subjected to ClueGO *v*2.5.9/CluePedia *v*1.5.9 plugin of Cytoscape *v*3.9.1 (http://www.cytoscape.org/) to acquire gene ontology (GO) analysis against *Homo sapiens* and classified as molecular functions and biological processes based on the two-sided hypergeometric statistical test and Bonferroni step-down *p*-value correction method with Kappa threshold level of 0.40.[41−43] Identified inflammatory responsible genes were then imported to Network Analyst database for analyzing the KEGG pathway enrichment (https://www.networkanalyst.ca/[44]). Further, these identified genes were imported to g:Profiler (https://biit.cs.ut.ee/gprofiler/gost accessed on 25 September 2022) against "*Homo sapiens*" and a KEGG, reactome. Wikipathways with their respective term IDs with an adjusted *p*-value < 0.05 were considered as significant (Figure S1).

MOLECULAR DOCKING

Preparation of the receptor proteins. The target proteins with their 3-dimensional (3D) structure were obtained from protein data bank (PDB) and then prepared for molecular docking by Autodock tools *v*1.5.6.[45]

Preparation of the Ligand Molecules. The 3D structures of the phytocompounds from *B. monnieri* were obtained from the PubChem database and prepared for molecular docking via Autodock tools *v*1.5.6.[45]

Molecular Docking. All the selected active phytocompounds were docked with their respective target proteins using Autodock tools, maintained by 'The Scripps Research Institute and Olson Laboratory'.[46] After docking simulation, the stability of docked poses was assessed by determining the binding energies between proteins and ligands.

Cell Culture and Growth. The RAW 264.7 (mouse macrophage cells) was collected from ATCC (American Type Culture Collection). The cells were grown in DMEM

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Table 1. List of Potential Bioactive Compounds and Their Features[a]

Compounds	Identifiers	GPCR lg	Ki	Ncr	Pi	Ei	nVio
3,4-Dimethoxycinnamic acid	3,4DMCA	−0.42	−0.66	−0.15	−0.68	−0.13	0
Ascorbic acid	ASBA	−0.53	−1.09	−0.01	−0.81	0.2	0
Asiatic acid	ASTA	0.2	−0.46	0.91	0.28	0.66	0
Wogonin	WG	−0.14	0.12	0.13	−0.31	0.23	0
Loliolide	LLD	−0.45	−0.91	−0.04	−0.33	0.56	0
Apigenin	AG	−0.07	0.18	0.34	−0.25	0.26	0
Quercetin	QR	−0.06	0.28	0.36	−0.25	0.28	0
Luteolin	LT	−0.02	0.26	0.39	−0.22	0.28	0

[a]Properties: GPCR, G protein-coupled receptors; Ki, Kinase inhibitor activity; Pi, protease inhibitor activity; Ncr, enzymes and nuclear receptors; nVio, number of violations.

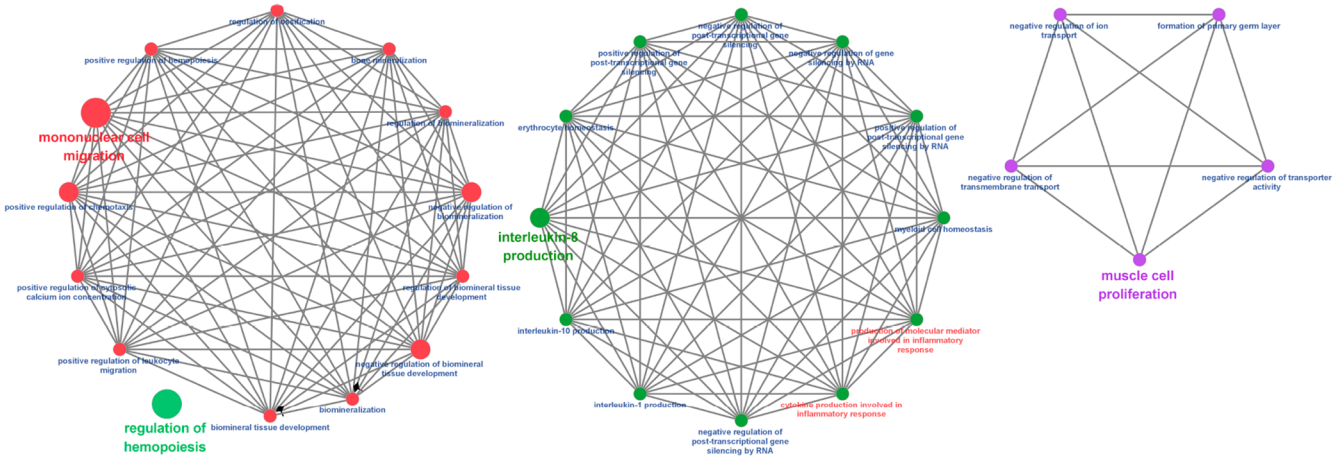

Figure 1. Biological processes inflammatory genes. GO analysis and visualization of inflammatory responsive genes were performed by the ClueGO v2.5.9/CluePedia v1.5.9 plug-in for Cytoscape *v*3.9.1. Node size directly proportional to number of genes belongs to biological processes. The node colors correspond to each GO category of inflammatory genes according to the significance level of GO terms.

(Dulbecco's Modified Eagle Medium) (HyClone; GE Healthcare Life Sciences, Illinois, USA) along with 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin, and 100 g/mL streptomycin supplements and maintained at 37 °C with 5% (v/v) CO_2 in a humidified incubator.[47,48]

MTT/Cell Viability Assay. RAW 264.7 cells were cultured and stimulated in 96 well MTP with 1 μg/mL of lipopolysaccharide (LPS) for 1 h and then treated with the presence of various concentrations (0, 12.5, 25, 50, 75, and 100 μM) of QR (Sigma-Aldrich, Missouri, USA) for 24 h at 37 °C. Further, the same volume of the solvent dimethyl sulfoxide (DMSO) was added to the control and LPS-alone treated cells. After washing the cells with DMSO, in each well 0.05% MTT solution was added and incubated at 37 °C for 3 h. Followed by incubation, absorbance was measured at 570 nm using a multifunctional UV−vis spectrophotometer (Spectra Max 3, Molecular Devices, USA). The cell viability was expressed and determined as a viable cell, and its percentage was compared with that of untreated control cells.

Measurement of Nitric Oxide (NO) Level. To measure NO expression, Griess reagent kit (Promega Corporation, TB229) was used, according to the manufacturer's protocol. RAW 264.7 cells were cultured and seeded (1.5×10^4) in 96-well plates, and then the cells were pretreated with 1 μg/mL LPS for 1 h. After incubation, the cells were treated with QR at a concentration of 12.5 and 25 μM for 24 h at 37 °C. Cell culture supernatant and sulfanilamide solution (1% sulfanilamide in 5% phosphoric acid) mixture was incubated for 10 min at 37 °C, and kept in dark place. After incubation, *N*-(1-naphthyl) ethylene diamine hydrochloride (NEDA·2HCl) was added to the mixture and incubated for 10 min at room temperature, and kept in dark place. The absorbance was measured at a wavelength of 520 nm using multifunctional UV−vis spectrophotometer (Spectra Max 3, Molecular Devices, USA). To measure the NO production, sodium nitrite (0, 1.56, 3.13, 6.25, 12.5, 25, 50, and 100 μM) was used as the nitrate standard.

Protein Estimation by Bradford's Method. The cells treated with LPS and/or QR were collected and washed twice in phosphate-buffered saline (PBS). To acquire whole-cell proteins, cells were homogenized for 30 min in ice-cold lysis buffer. Bradford's method was employed to estimate the amounts of isolated proteins. The 48-well microtiter plate was used for estimation. Bovine Serum Albumin (BSA) was used as standard, and quantification was performed. Concentrations of BSA such as 50, 100, 150, 250, 500, and 1000 μg were prepared from the stock of 1 mg/mL, and their final volume was made up to 50 μL using milli-Q water. Isolated protein samples of 10 μL each were taken, volume is adjusted to 50 μL with milli-Q water, and 100 μL of Bradford's reagent was added. The setup was incubated for 15 min in the dark. After incubation, the sample was quantified using UV−vis spectrophotometer measuring the absorbance at 595 nm. The concentrations of unknown samples were estimated by comparing them with that of the known standard.

Western Blot Analysis. In this analysis, equal concentrations of proteins from control, LPS stimulated, and QR treated cells (12.5 and 25 μM) were employed. To separate

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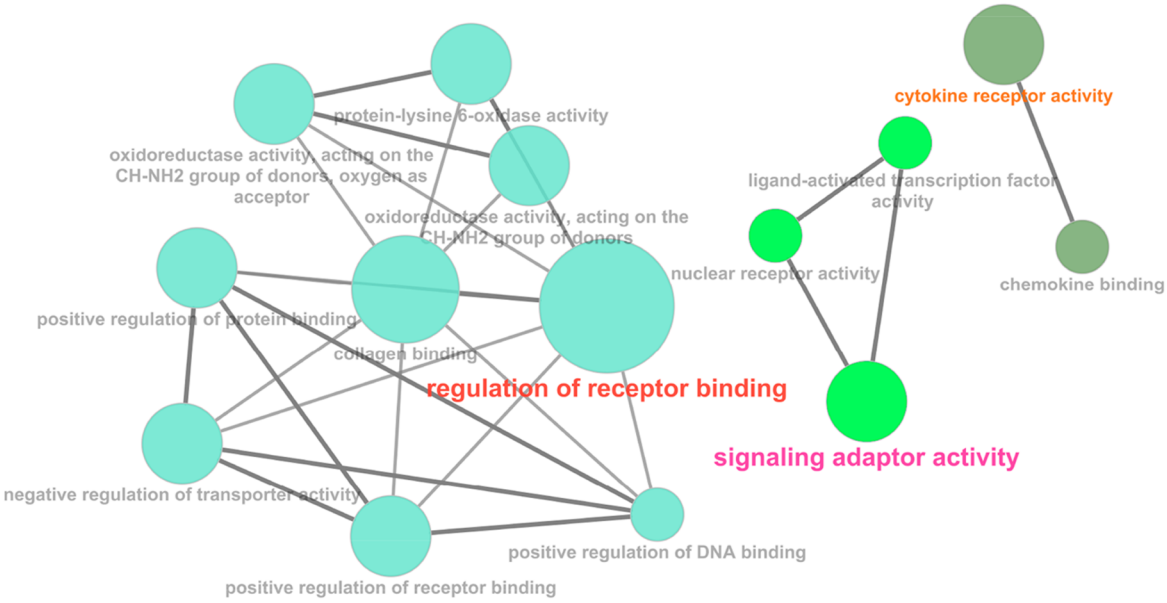

Figure 2. Inflammatory associated genes and their molecular functions. GO molecular function was predicted using ClueGO v2.5.9/CluePedia v1.5.9 plug-in for Cytoscape *v*3.9.1. Node size directly proportional to number of genes belongs to molecular functions. The node colors correspond to each GO category of inflammatory genes according to the significance level of GO terms.

the proteins, 10% SDS-PAGE gels were used, which were then transferred to polyvinylidene fluoride membranes. Tris-buffered saline (TBS) (composed of 0.1% Tween-20 and 5% skimmed milk) was used to block transferred membranes at 4 °C. After blocking, the membranes were probed overnight gently shaking at 4 °C with diluted primary antibodies (1:1000). Followed by incubation, the membranes were then treated with horseradish peroxidase (HRP)-conjugated antirabbit IgG secondary antibodies (1:1000). Afterward, protein bands were observed with enhanced chemiluminescence (ECL) substrate and visualized (Bio-Rad, California, USA).

RESULTS

Retrieval of Phytocompounds and Human Target Prediction. Among the 52 phytocompounds, eight bioactive compounds were selected from Bacopa based on the '0' nVio for further analyses. The details of active compounds and their pharmacological features were given in Table 1. According to the literature survey and SwissTargetPrediction, these phytocompounds target six unique human compounds such as *COX2*, inducible nitric oxide synthase (*iNOS*), C−C chemokine receptor type 1 (*CCR1*), Lysyl oxidase (*LOX*), signal transducer and activator of transcription-3 (*STAT3*) and Matrix metalloproteinase-9 (*MMP9*), and these genes were employed for further analysis.

Gene Ontology and Enrichment Analysis. GO analysis was imputed by ClueGO *v*2.5.9/CluePedia *v*1.5.9 plugin of Cytoscape *v*3.9.1 and showed the involvement of human targets in various biological processes and molecular functions. Biological processes including regulation of hemopoiesis, mononuclear cell migration, types of interleukin production, inflammatory responses, biomineralization, and muscle cell proliferation, etc. (Figure 1). These processes play an important role in inflammatory processes. Further, these genes are involved in diverse molecular functions such as regulation of receptor binding, signaling adaptor activity, and cytokine receptor activity, etc. (Figure 2). As a result of ontology analyses, it is confirmed that inflammation can be treated by targeting these genes.

Gene enrichment network exhibited the activity of inflammatory responsible genes involved in different disorders like bladder cancer, prostate cancer, estrogen signaling pathway, transcriptional misregulation in cancer, IL-17 signaling pathway, Hepatitis B, endocrine resistance (Figure 3). The effect of these phytocompounds and their human targets may reduce the risk of inflammation and its associated diseases.

Interactome Analysis. The molecular interaction of inflammation responsive genes had 26 nodes and 107 edges. The average nodal degrees within the neighbor proteins are 8.23. PPI of inflammation responsive genes *p*-value enrichment score was 1.47×10^{-12} (Figure 4). This signaling network revealed the complexity of inflammation responsive genes and their associated genes.

Molecular Docking. Molecular docking analysis was executed for the phytocompounds against the respective targets. Docking scores revealed that QR showed significant binding energies when compared to other bioactive molecules (Table 2 and Figure 5). Hence, QR was selected for the further analysis of anti-inflammatory activity.

Cell Viability Assay. The cytotoxic effects of QR were assessed by the MTT assay. RAW 264.7 macrophage cells were treated with QR at various concentrations ranging from 12.5 to 100 μM, with or without LPS (1 μg/mL). This analysis revealed that QR did not possess notable toxicity to the cells at concentrations of 12.5 and 25 μM (Figure 6 and 7). Hence, these concentrations of QR were thought suitable for analyzing its role in LPS-evoked inflammatory response.

NO Production Assay. To examine the consequence of QR on NO production, initially, the cells were induced with 1 μg/mL of LPS for 1 h and then treated with QR (12.5 and 25 μM) for 24 h. Cells without the treatment of LPS and QR serve as control. As a result, LPS alone significantly increased NO production in comparison with control. Cells treated with

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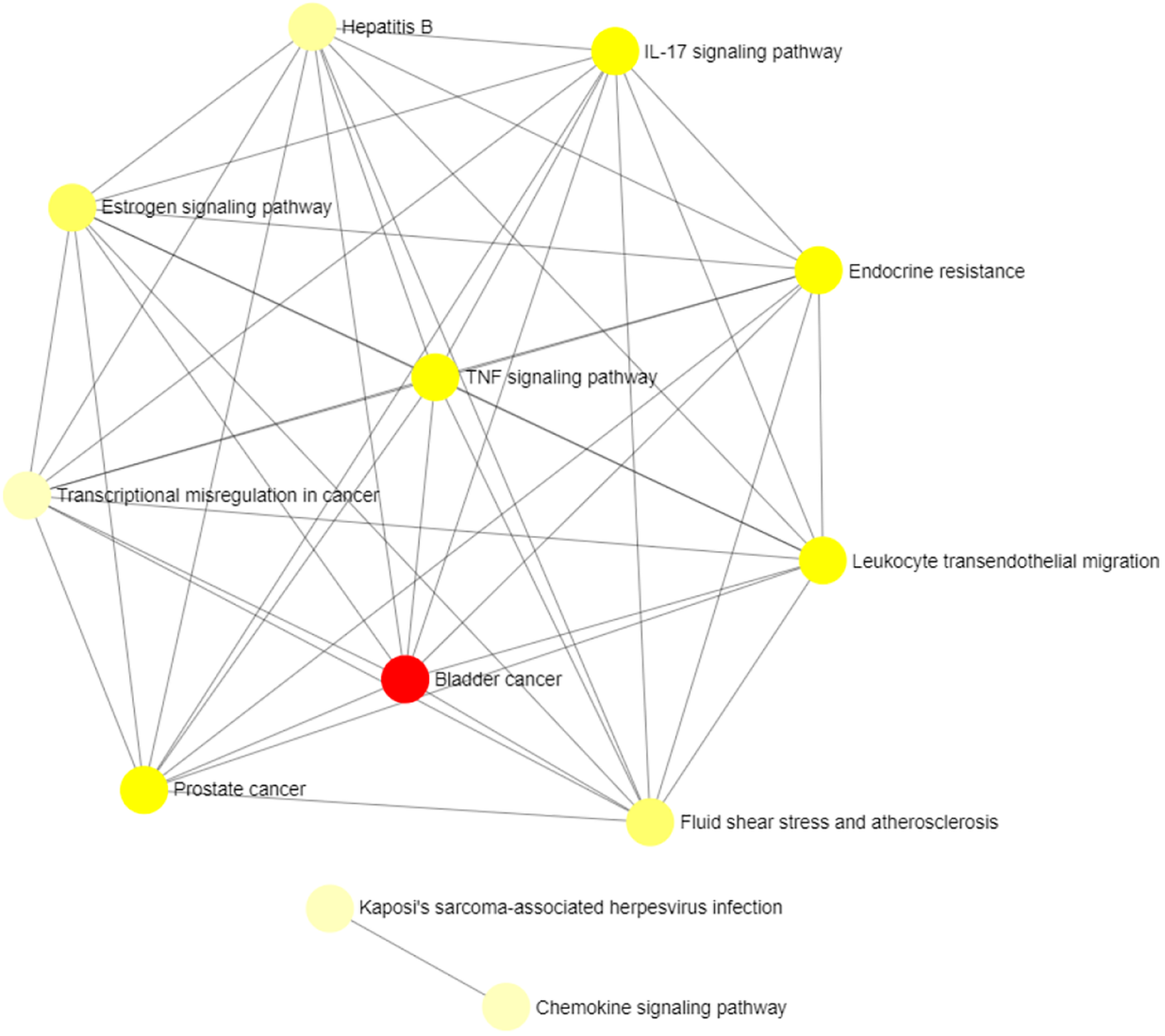

Figure 3. Visualization of network-based pathway enrichment analysis. The nodes are color shaded according to the significance level (adjusted *p*-value < 0.05).

QR exhibited faint changes in NO levels in a concentration-dependent manner (Figure 8).

Western Blot Analysis. To analyze the effect of QR role in modification of protein expression associated with NO production, *COX-2* and *iNOS* expression levels were determined through Western blot analysis. The results revealed that, *COX-2* and *iNOS* expression were significantly increased after 1 h of treatment with 1 μg/mL LPS, whereas their expression was downregulated after treatment with QR (Figure 9). This analysis concludes that the reduced protein expression of *COX-2* and *iNOS* leads to the inhibitory effect of QR on LPS-induced NO production.

DISCUSSION

B. monnieri is a valuable therapeutic plant with a variety of promising pharmacological actions that can be used for the treatment of many diseases and ailments.[4,7,49] This Indian potent plant has pharmacologically active biomolecules and prominently acted as anti-inflammatory, antiamneatic, nootropic, anti-Alzheimer's, memory boosting, neuroprotective/antioxidant, cardio- and hepato-protective, antiaging, antitumor, antiarthritic, cytotoxic, and chemo-preventive.[7] There are 52 bioactive compounds present in *B. monnieri*.[4,32] In this study, among the 52 phytocompounds, we selected eight active compounds from Bacopa dependent on the molecular properties, bioactive scores, and significant nVio values (Table 1). This is the first and foremost study mainly focused on the anti-inflammation property of Bacopa derived bioactive compounds through *in silico* and *in vitro* analysis. There are six inflammation responsible genes identified through a literature survey and SwissTargetPrediction tool: *COX2*, *iNOS*, *LOX*, *STAT3*, *CCR1*, and *MMP9*. These genes are major mediators of pain and inflammation associated diseases, and expression of these genes can be induced by proinflammatory factors like LPS, cytokines (TNF-α, IL-1), and interferons (IFN gamma).[50,51] These factors stimulate inflammation by interaction with IL and TNF receptors[52] and activate inflammatory pathways such as mitogen-activated protein kinase (MAPK), nuclear factor kappa-B (NF-κB), signal transducer, and activator of transcription (STAT) pathways.[53−55] *LOX* actively participates in inflammation initiation and termination processes, and this enzyme modulation is essential for treatment of inflammation. *MMP9* is involved in regulation of inflammatory cytokines

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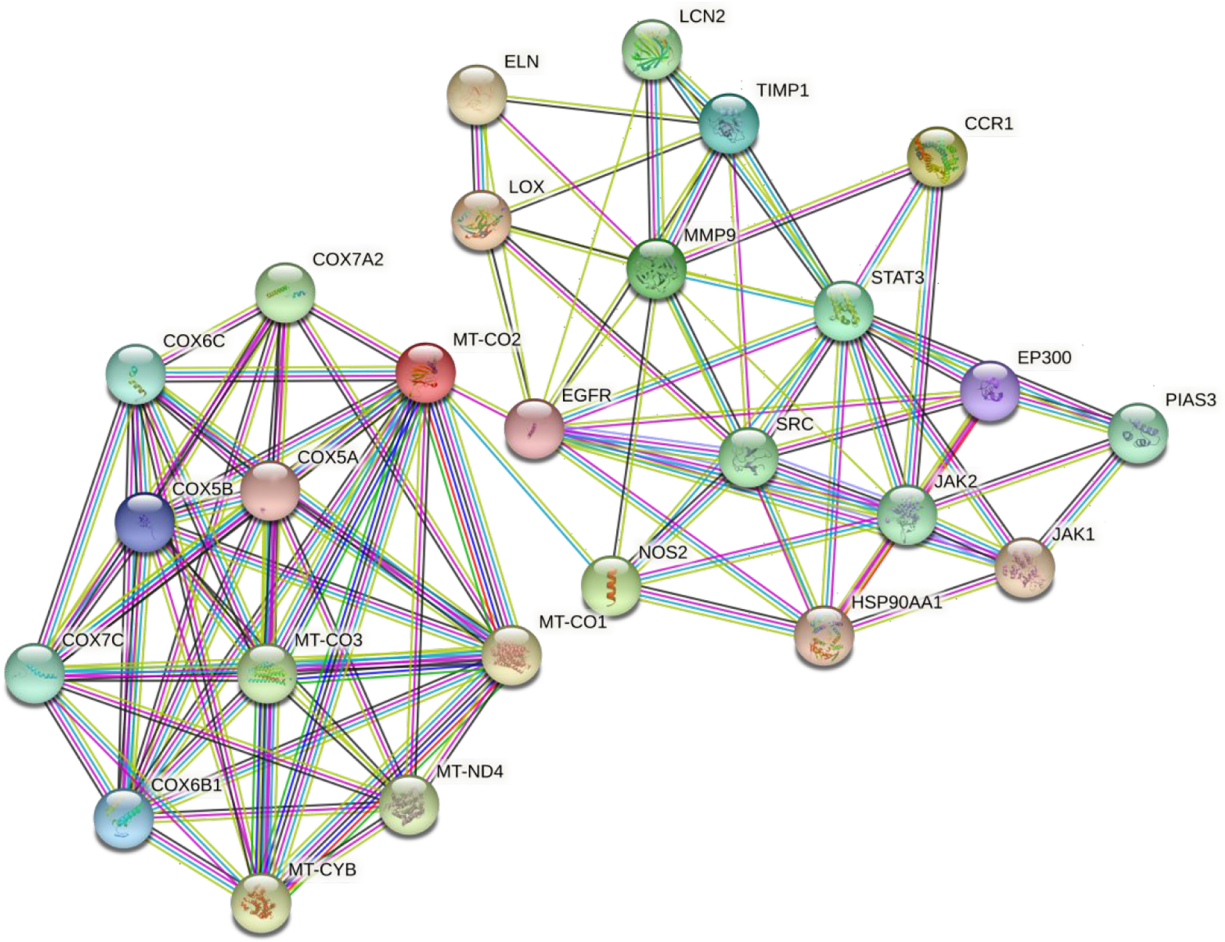

Figure 4. Molecular interaction network of inflammation responsive genes. The line thickness indicates the level of interaction between different proteins and colored lines between the proteins indicate different types of interaction network. Red, gene fusion; violet, protein homology; pink, experimentally determined; blue, gene co-occurrence; green, gene neighborhood.

Table 2. Binding Energies (in kcal/mol) of the Phytocompounds Found Using Docking

Phytocompounds	*COX2*	*iNOS*	*LOX*	*STAT3*	*CCR1*	*MMP9*
3,4-DCMA	−7	−6.9	−6.3	−5.5	−6.8	−6.8
AG	−9.7	−9.5	−8.3	−7.2	−7.9	−6.9
ASBA	−5.4	−6.1	−6.3	−5.3	−6.1	−6
ASTA	−9.8	−9.5	−10.5	−7.2	−7.6	−7.5
LLD	−5.7	−6.5	−6.8	−5.5	−6.5	−6.4
LT	−9.8	−8.7	−8.3	−7.2	−8.2	−7.3
QR[a]	**−9.9**	**−9.6**	**−8.5**	**−7.3**	**−7.5**	**−7.4**
WG	−9.6	−9.2	−8.2	−6.8	−7.5	−6.8

[a]Bold font indicates selected for further analysis of anti-inflammatory activity.

and physical barriers modulation. *CCR1* is a chemokine receptor, playing a critical role in leukocytes trafficking at the site of inflammation.[56] In this study, a series of *in silico* analyses were performed for better understanding of inflammatory pathways and molecular interactions of inflammation responsive genes.

For further confirmation, GO ontology was performed to reveal the involvement of target genes/proteins in various biological processes and molecular functions (Figure 1 and 2). For example, in biological processes these genes actively participate in regulation of inflammatory response, myeloid cell differentiation, cell chemotaxis, and cellular response to chemokine. The pro-inflammatory cytokines drive myeloid cells differentiation and production in response to pneumovirus infection, and inflammatory chemokines control the recruited leucocytes in infection, tissue injury, inflammation, and tumors.[57,58] Hence, our GO analysis results strongly suggest that targeting these genes may help to treat inflammation associated diseases. Gene enrichment was executed to observe the activity of inflammatory-responsible genes involved in the associated signaling pathways of various diseases. From the interactome analysis, we confirmed that these genes actively participate with other inflammation related genes such as *TIMP1*, *SRC*, *JAK1*, and *JAK2*. Molecular docking was conducted for the eight active phytocompounds against the respective human targets. Among the eight phytocompounds, QR showed significant binding energies with *COX-2* and *iNOS* human targets (Table 2 and Figure 5) and these two genes were chosen for our further *in vitro* experiments.

QR is yellow-colored polyphenolic flavonoids that have various pharmaceutical activities, such as antiviral, anticancer, and antimicrobial effects, treatment of allergies, inflammation, and cardiovascular diseases.[59] In this pilot study, the anti-inflammatory effects of QR in LPS-stimulated RAW264.7 mouse macrophage cells were evaluated by *in vitro* analysis. In a host defense mechanism, macrophages play a pivotal role

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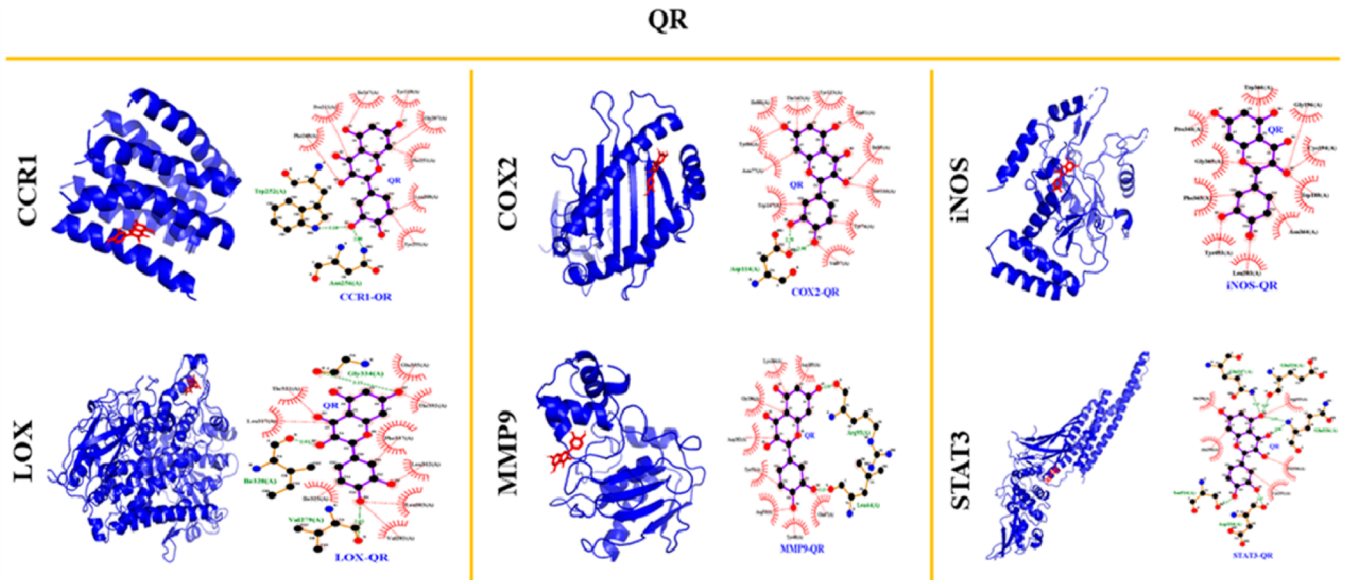

Figure 5. Visualization of molecular docking results.

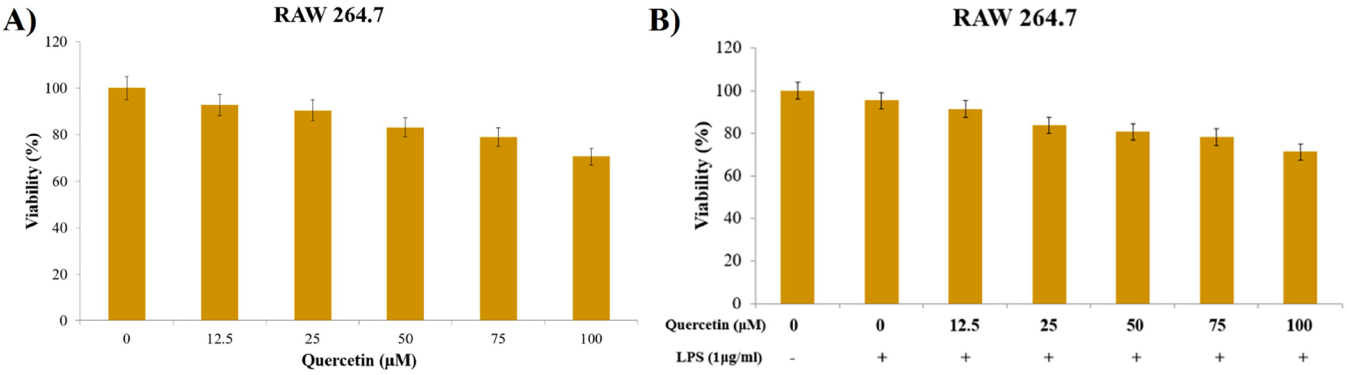

Figure 6. Cytotoxic effect of quercetin (QR) on RAW264.7 cells. These cells were pretreated with or without LPS (1 μg/mL) at 37 °C for 1 h and then treated with QR (0, 12.5, 25, 50, 75, and 100 μM) at 37 °C for 24 h. (A) Effect of QR on non-LPS-induced cell viability in RAW264.7 cells. (B) Effect of QR on LPS-induced cell viability in RAW264.7 cells. Error bars represent the mean ± SD calculated from three independent experiments.

against various infections and cascading inflammatory processes by releasing chemicals such as TNF, IL-6, and NO. Overproduction of these mediators has been associated with a variety of inflammatory and malignant illnesses.[60] The MTT assay was conducted using RAW264.7 macrophage cell lines, and study results revealed that QR had no cytotoxicity up to doses of 25 μM in macrophage cells. In addition, concentrations higher than that were not considered for further *in vitro* experimental analysis.

There are three nitric oxide synthase (NOS) enzymes that generate NO from L-arginine including neuronal (nNOS), endothelial (eNOS), and inducible (iNOS).[61] Constitutively expressed eNOS and nNOS produce low physiological levels of NO, although *iNOS* plays a significant role for the long-term production of high NO levels.[62] In macrophages and other cells, bacterial products and inflammatory cytokines trigger the production of *iNOS*.[63] Furthermore, these results showed QR significantly reduced the LPS-induced production of NO by inhibiting the *iNOS* gene expression in RAW264.7 cells (Figure 6). So, the results suggested that inhibition of *iNOS* gene expression may be one of the QR mechanisms responsible for its anti-inflammatory activity.

COX-2 is another important inducible enzyme that plays a variety of pathophysiological processes such as angiogenesis, inflammation, atherosclerosis, tissue injury, and tumorigenesis. Arachidonic acid is converted to prostaglandin H_2 by *COX-2*, and further the specific enzymes convert the prostaglandin H2 to biologically active prostaglandins and thromboxane A2 (TXA2). Increased prostaglandin levels and *COX-2* activity have been linked to inflammatory pain.[64] As a result, modulating the expression of *iNOS* and *COX-2* are considered a potential method to combat inflammatory diseases. The obtained results showed that for LPS-stimulated RAW264.7 cells, QR suppressed NO generation by downregulating *COX2* and *iNOS* expression. Previous studies reported that QR significantly decreased the expression of *iNOS* but *COX2* expression was not affected.[65] However, our findings showed QR effectively downregulated the expression of both *COX2* and *iNOS* at the concentration of 12.5 and 25 μM (Figure 7). Hence, the variations in obtained results mainly depended on the *COX-2* and *iNOS* specific promoters on the different transcription factors.

So far, there is lack of high throughput *in silico* reports available for the interaction network of inflammation

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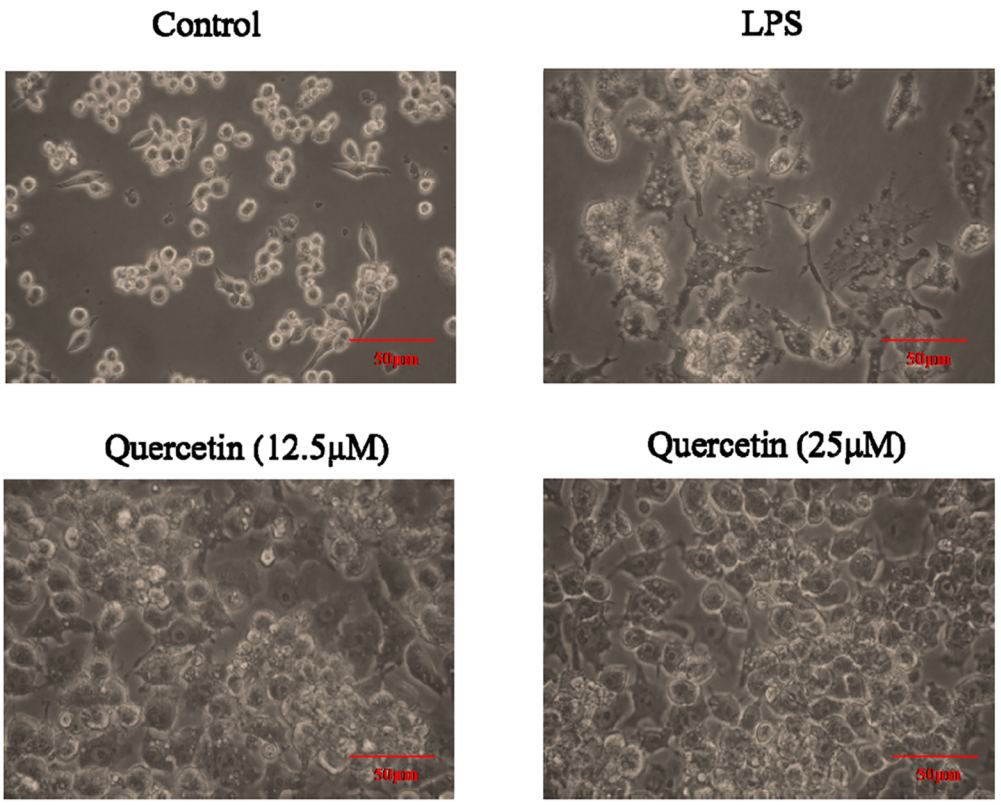

Figure 7. Morphology of RAW 264.7 cells induced with LPS (1 μg/mL) alone and cotreated with quercetin (QR) (12.5 and 25 μM). Scale bar indicates 50 μm.

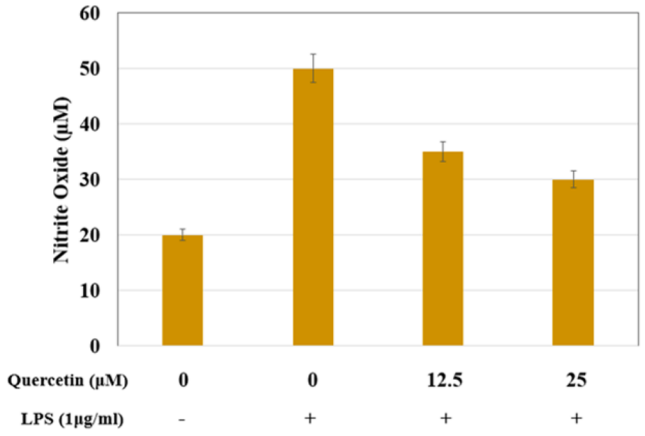

Figure 8. Effect of quercetin (QR) on nitric oxide production in RAW 264.7 mouse macrophage cells.

responsive genes and phytocompounds, and directly linked human targets. This is the first and foremost study, to perform *in silico* analysis for the selection of potential phytocompounds against anti-inflammation activity. Network pharmacology and cheminformatics approaches are the effective and easiest method for future research to identify the potential phytocompounds with significant curative properties against various human diseases/disorders including inflammatory diseases.

CONCLUSION

The pharmacological effects of *B. monnieri* are attributed to the presence of various biologically active compounds. In this present study, eight potential bioactive compounds were selected based on the molecular properties and bioactive scores. Several studies reported the pharmacological properties and phytocompounds abundance in the Bacopa. This study for the first time showed the interaction between the phytocompounds and human targets that is responsible for inflammation. Our findings strongly imply that phytocompounds of Bacopa are potential candidates for novel and desired compounds/drugs development to target neuroinflammation and have the crucial activity to treat various CNS disorders like Alzheimer's disease, schizophrenia, and depression.

The present study also hypothesizes that the identified potential phytocompounds from *B. monnieri* can be employed to regulate the gene-targets associated signaling pathways, which could ultimately lead to the treatment of inflammation responsive diseases. As expected, the obtained *in vitro* results revealed that the identified potential phytocompound effectively inhibits the expression of inflammation responsible genes such as *COX2* and *iNOS* in LPS-induced RAW264.7 cells. Nonetheless, *in vivo* analysis is mandatory for further confirmation of this hypothesis. Further, our comprehensive study also establishes the framework for future research on the treatment of the numerous CNS related diseases, the applications of system pharmacology in the development of novel and desired drugs from Indian traditional medicinal plants, and also identifies the druggable targets of many diseases.

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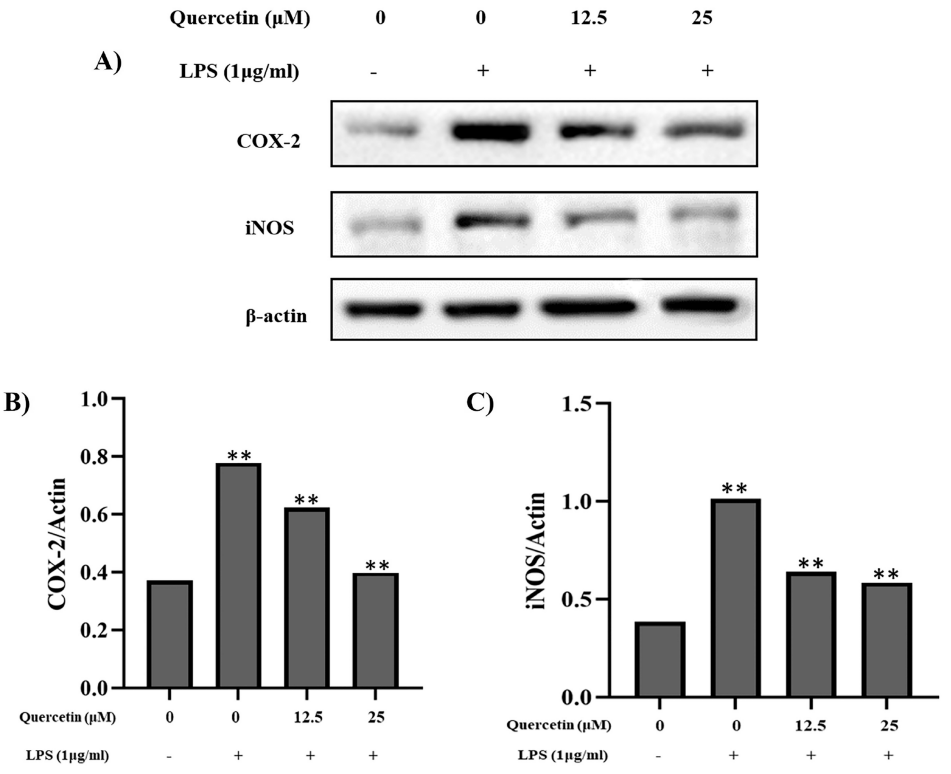

Figure 9. RAW 264.7 macrophage cells treated with 1 μg/mL of LPS containing quercetin (QR) (12.5 and 25 μM). (A) The protein expression of *COX-2* and *iNOS* was detected by Western blot assay (B) Changes in expression level of *COX-2* and (C) *iNOS*. Significance was determined by Dunnett's *t* test (**$P > 0.05$) using SPSS statistics software version 17.0.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c05318.

Information on phytocompounds and their canonical SMILES and structures (Table S1); pathway enrichment analysis of identified inflammation associated genes (Figure S1) (PDF)

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R.J.: conceptualization, methodology, validation, formal analysis, investigation, writing−original draft. P.M.: conceptualization, methodology, validation, formal analysis, investigation, writing−review and editing. S.A.: methodology, validation, formal analysis, investigation. H.S.: formal analysis, investigation, writing−review and editing. M.R.: conceptualization, methodology, writing−review and editing, supervision. All authors have read and approved the final manuscript.

Notes

The authors declare no competing financial interest.

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