International Journal of Plant Biology & Research

Research Article

Screening of Phytochemicals and *In silico* Approach through Drug Design of *Centella asiatica*

Soubhagyalaxmi Sahoo*, Subhashree Subhasmita Raut, and Debasrita Das

Tectona Biotech Resource Centre, India

***Corresponding author**
Soubhagyalaxmi Sahoo, Tectona Biotech Resource Centre (TBRC), Bhubaneswar, Odisha, India, Tel: 9337524676; Email: souvagya.tbrc@gmail.com

Submitted: 05 October 2019

Accepted: 11 December 2019

Published: 11 December 2019

ISSN: 2333-6668

Keywords
- Tb
- Blast
- Kegg
- String
- Malacard
- Mitarbase

Abstract

Centellaasiatica is a valuable medicinal herbaceous aromatic creeper which has been valued for centuries in ayurvedic medicine. Phytochemical analysis of *Centellaasiatica* plant extracts revealed the presence of various biochemical compounds such as alkaloids, flavonoids, glycosides, triterpenoids and saponins etc. Since triterpenoids and flavonoids have remarkable anti-inflammatory activity also Phyto compound possess a wide range of activities, which may help in protection against tuberculosis diseases. In this study, the molecular docking was applied to explore the binding mechanism and to correlate its docking score with the activity of plant derived compounds and also identified targets were validated through some other databases to select the better ones and design the drug. This study reveals that NOD2 is a better potential drug target for tuberculosis and Quercetin is the best ligand to inhibit NOD2 and might be prevent the disease Tuberculosis. Our result can be useful for the design and development of novel compounds having better inhibitory activity against several type of Tuberculosis. This potential agent will be a promising candidate can further be validated in research for its proper function. This work would help to design a potent lead molecule against any other disease.

INTRODUCTION

A Tuberculosis (TB) is an infectious disease, spreads through the air. Currently, one third of the world's population is infected with tuberculosis and each year there are 2 - 3 million deaths worldwide caused by the disease [1]. TB is a leading cause of death among people with human immunodeficiency virus (HIV). Individuals infected with HIV are very susceptible to TB and often develop this disease before other manifestations of AIDS become apparent .It has been made to enrich the knowledge of antimycobacterial activity of *Centella asiatica* plant extract against Mycobacterium tuberculosis [2].The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants [3]. Hence the sensitivity study of bacterial strains to the plant *Centella asiatica* was evaluated. Centella has been used as a wound-healing agent and a constituent of a brain tonic for the mentally challenged [4]. It has also been reported to be useful in the treatment of inflammations, diarrhea, asthma, tuberculosis and various skin lesions and ailments like leprosy, lupus, psoriasis and keloid [5]. In addition, numerous clinical reports verify the ulcer-preventive and antidepressive sedative effects of *C. asiatica* preparations, as well as their ability to improve venous insufficiency and microangiopathy [6]. Oil is known to be strong antimicrobial and antitumor agents [7]. The essential oil of Centella showed a broad spectrum of antibacterial activities against Gram-positive *(Bacillus subtilis, Staphylococcus aureus*) and Gram-negative (*Escherichia coli, Pseudomonas aeruginosa, Shigellasonnei*) organisms. Activity against Gram-positive bacteria was greater than against Gram negatives. Germacrene compounds in the essential, an attempt has been made to enrich the knowledge of antimycobacterial activity of *Centella asiatica* plant extract against Mycobacterium tuberculosis [2].

In silico drug design can take part considerably in all stages of drug development from the preclinical discovery stage to late stage clinical development. Its exploitation in drug development helps in the selection of only a potent lead molecule and. *In silico* methods have been of great importance in target identification and in prediction of novel drugs. So through *in silico* approach we can design a new lead compound of *Centella asiatica* plant that can be helpful for human being as curing disease [8,9].

MATERIALS AND METHODS

Selection of target protein

To analyse the involvement of accrued target genes in unique metabolic pathways of Tuberculosis the pathway analysis was executed through KEGG (Kyoto Encyclopedia of Genes and

Genomes) database (https://www.kegg.jp/), which is a resource for understanding high-level functions and utilities of the biological system and the pathway map represents the molecular interaction network diagram to explore the genomic relationship between the genes and the species. From this study 106 numbers of genes were selected for further studies.

Protein network enrichment

The Search Tool for the Retrieval of Interacting Genes (http://string.embl.de/) database is an online tool designed to construct a PPI network and analyse the functional interactions between proteins. Thus here the use of STRING was justified with enrich the protein-protein network between the collected target genes.

Validation

MalaCards (http://www.malacards.org/#) is an integrated database of human maladies and their annotations, modelled on the architecture of human genes. The MalaCards disease database integrates both specialized and general disease lists, including rare diseases, genetic diseases, complex disorders and more. In this present study MalaCards was used to identify the targeted genes from the selected genes through KEGG and STRING database, which were highly involved in causing tuberculosis.

Protein preparation

The selected target genes of tuberculosis were analysed in UniProt (www.uniprot.org) database to know their sub cellular localization and retrieve the protein information of related genes. BLASTp used for protein database using protein query that was used to carry out the study by analysing the most similar structure with the target protein by sequence alignment process for further analysis.

Screening of target miRNAs

After the functional annotation this study came to investigate the associated target miRNAs from miRTarBase, which is the experimentally validated microRNA-target interactions database http://mirtarbase.mbc.nctu.edu.tw/php/index.php) and provides the most updated collection of more validated miRNA information in contrast to previously developed databases.

Selection of ligand

Pubchem (https://pubchem.ncbi.nlm.nih.gov/) is designed to provide information on biological activities of small molecules, generally those with molecular weight less than 500 Daltons. The plant *C.asiatica* has been used as brain tonic, and to treat chronic diseases and mental disorders. From brief literature survey, the Phytocompunds that is present in good quantity in the plant *C.asiatica* having selected for docking analysis.

Preparation of ligand

Open Babel is computer software, a chemical expert system mainly used to interconvert chemical file formats. The three dimensional structures of shortlisted 3 phytocompounds were downloaded from Pubchem in SDF format. The SDF files were then converted to PDB format using Open Babel converter and further used for molecular docking analysis.

Molecular docking

Computational docking can be used to predict bound conformations and free energies of binding for small molecule ligands to macromolecular targets. Docking analysis was done for the two target proteins with six different phyto compounds that are present in the plant *P. amboinicus* using Auto Dock tools. From this study an optimized conformation for both the protein and ligand and relative orientation between protein and ligand such that the free energy of the overall system was minimized. Data from the docking results of all docking processes were tabulated and best confirmations were selected. All the images were taken using PyMol (www.pymol.org) software after in computational approaches.

Ligplot

LIGPLOT, are shown as dotted lines. Hydrophobic contacts are represented as spline curves that outline the hydrophobic parts of the ligand and are labelled by the contacting amino acid residues from the protein. Here, ligplot analysis was done to study the interaction between the proteins, ligand with different amino acid residues and also analysed the hydrogen bonds between the amino acids (Figure 1).

RESULT AND DISCUSSION

Retrieval of target genes

In total 106 number of target genes of tuberculosis were identified in KEGG pathway analysis, which were important genes involved in pathway to cause tuberculosis disease (Figure 2).

PPI Analysis

The Search Tool for the Retrieval of Interacting Genes (http://string.embl.de/) database is an online tool designed to construct a PPI network and analyze the functional interactions between proteins. Functional association, the 106 numbers of genes, which were retrieved from KEGG pathway database were subjected to STRING analysis to show the interaction rate between the targeted genes which was based on functions of respective proteins. There were total 62 numbers of genes

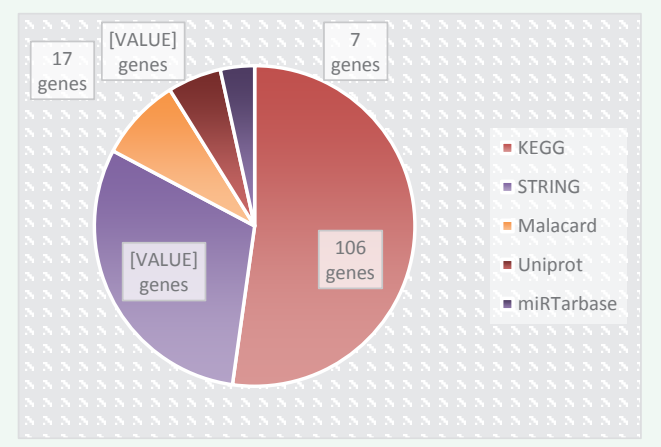

Figure 1 Schematic diagram of resulted genes.

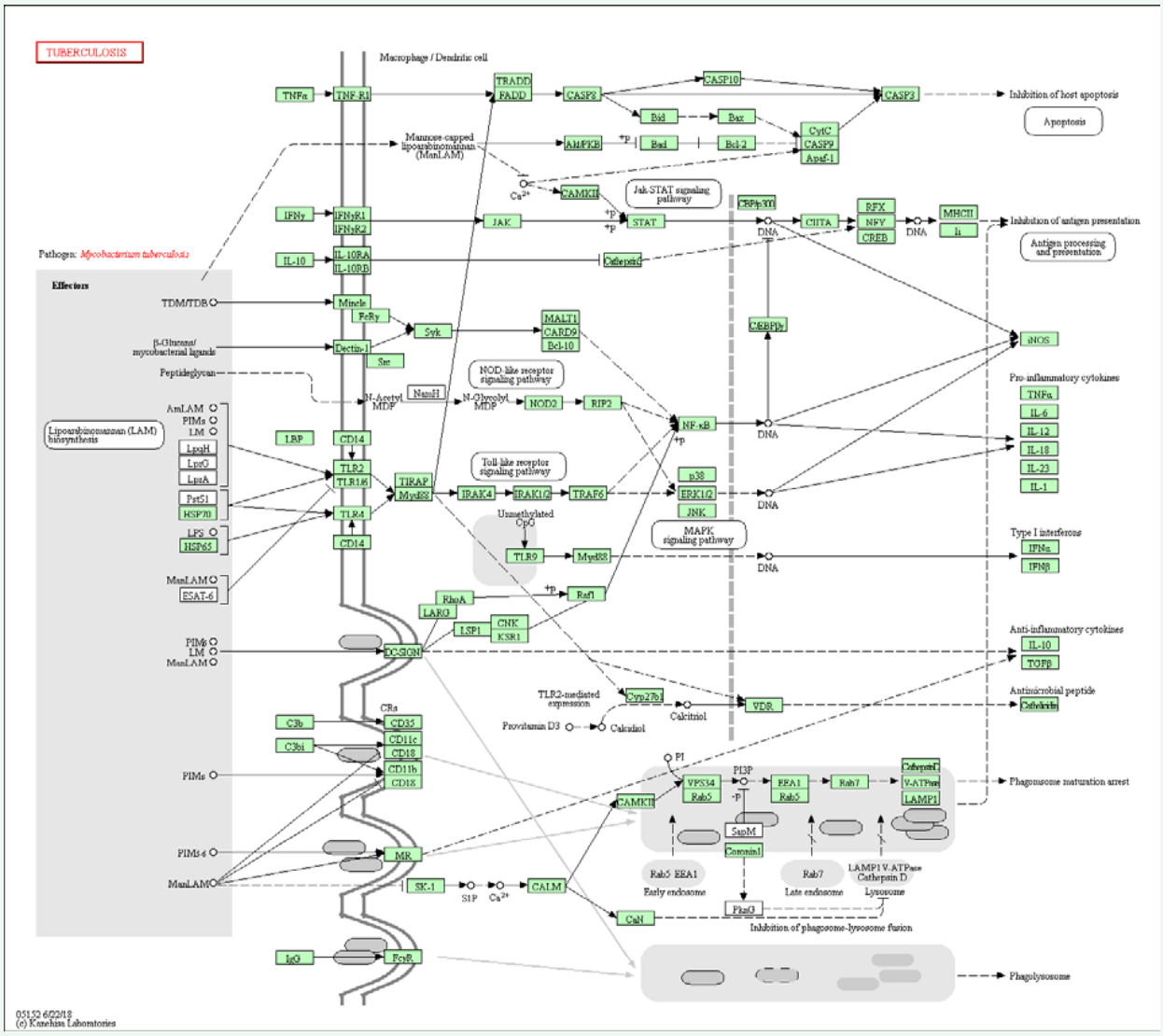

Figure 2 The Metabolic pathway of Tuberculosis.

were having interaction network out of 106 number of genes. The network was built based on the highest confidence score 0. 900. There were total 63 numbers of nodes, 143 numbers of edges, average node degree was 4.54, average local clustering co efficient was 0.436 and the PPI enrichment P value was < 1.0e-16 (Figure 3). This network might be provides the important drug targets for tuberculosis.

Validation

After the collection of target genes of tuberculosis, the validation was required to screen the good target for further analysis, so, for the validation all collected target genes, which were got from protein-protein interaction network through STRING analysis, were verified in MalaCards. As MalaCards is the human disease gene database, all the 62 genes of STRING result were searched in MalaCards database one by one to check the involvement of these genes in tuberculosis, as the reports present in MalaCards database are literature supported data sets, so the analysis through MalaCards is important. Thus, there were total 17 numbers of genes were identified through MalaCards search as those were involve in tuberculosis and also the MalaCard IDs were collected for all 17 number of selected genes for future analysis and review (Table 1).

The resulted 17 genes through MalaCards validation process were analysed or more validated through UniProt database, as it was mentioned above that UniProt is the collection of protein information database. So, The 6 numbers of genes were discarded because those genes were found as antigens and receptors and there were total 11 numbers of genes were considered as potential drug targets for tuberculosis out of 17 genes after UniProt database validation. Also the protein information like protein product names, amino acid length, UniProt IDs etc. of all the respective target genes were collected from UniProt for future prospectus (Table 2).

Further, binding affinity was explored using miRTarbase web server. So, in this present study the 11 selected putative drug targets for tuberculosis were searched in miRTarBase to

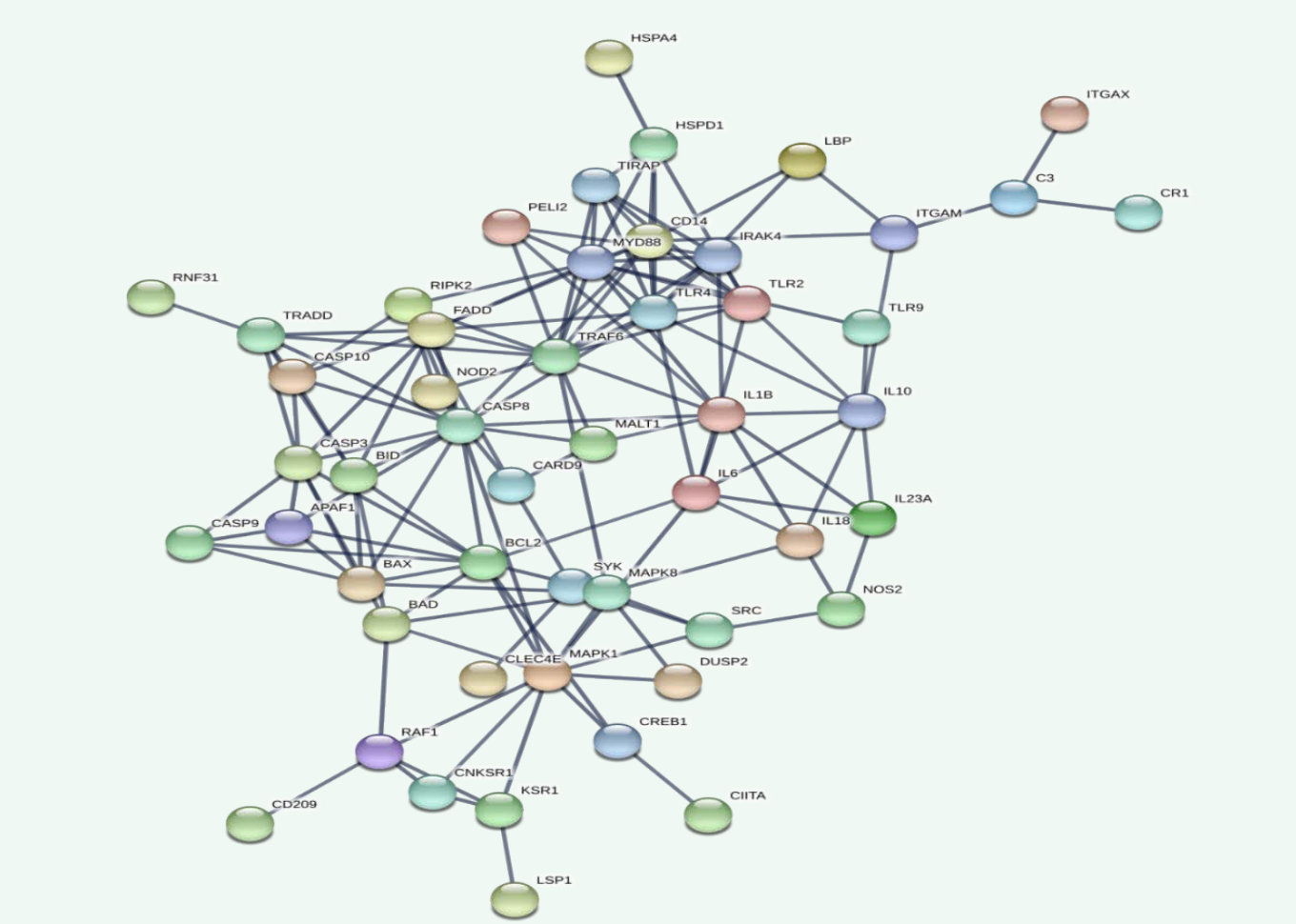

Figure 3 Protein- Protein interaction network built through STRING.

Table 1: List of Target genes validated through MalaCards database.

SL.NO	GENE NAME	MCID
1	CIITA	TBR010
2	FCGR3A	TBR010
3	CD209	TBR010
4	IL18	TBR010
5	BID	TBR010
6	IL6	TBR010
7	NOD2	TBR010
8	IL1B	TBR010
9	ITGAM	TBR010
10	IL10	TBR010
11	TLR4	TBR010
12	CD14	TBR010
13	TLR9	TBR010
14	TLR2	TBR010
15	MYD88	TBR010
16	HSPD1	TBR010
17	TIRAD	TBR010

collect their respective miRNAs. There were total 7 numbers of miRNAs were considered for 7 selected numbers of targeted genes which were collected based on the highest MFE score and its involvement in respective tuberculosis disease and also the strong evidence (Table 3).These miRNAs might be responsible to play an important role to cause disease, so the target miRNAs of target genes were also necessary to be inhibited by the putative drugs and subjected to further study.

Selection of natural compounds

As per literature survey, that are present in the plant *C.asiatica*that have great inhibitory effects against diabetes. The resultant phytochemicals i.e. kaempferol, Myricetin, Querecetin were analyzed and prepared through computational approach and hence downloaded from PubChem database of NCBI web server (Table 4). Then for docking analysis the collected three dimensional structures of ligands in SDF format were converted in to PDB format with the help of Open Babel (Figure 4) and then used in docking analysis.

Molecular docking analysis

The results of molecular docking were intended for energetically favorable binding poses for each three natural

Table 2: The protein information of target genes collected from UniProt.

SL. No	Gene NAME	Protein Product Name	Structure in PDB	UniProt-ID	Amino Acid length
1	FCGR3A	Low Affinity Immunoglobulin Fc Region Receptor Iii-A	PRESENT	P08637	254
2	IL18	Interlukin-18	PRESENT	Q14116	193
3	BID	Bh3-Interacting Domain Death Agonist	PRESENT	P55957	195
4	IL6	Interlukin-6	PRESENT	P05231	212
5	ITGAM	Integrin Alpha-M	PRESENT	P11215	1,152
6	IL10	Interleukin-10	PRESENT	P22301	178
7	MYD88	Interleukin-10	PRESENT	P22301	178
8	HSPD1	60 Kda Heat Shock Protein, Mitochondrial	PRESENT	P10809	573
9	NOD2	Nucleotide-Binding Oligomerization Domain Containing Protein-2	ABSENT	Q9HC29	1,040
10	CIITA	MHC Class II Transactivator	ABSENT	P33076	1.130
11	IL1B	Interleukin-1beta	ABSENT	P01584	269

Table 3: Collected Target miRNAs from miRTarBase with MFE score.

SI NO	GENE NAME	miRNA NAME	miRNA ID	POSITION	MFE SCORE
1	IL18	hsa-miR-346	MIRT004304	52-76	-28.30
2	BID	hsa-miR-492	MIRT006072	278-304	-13.90
3	IL6	hsa-miR-107	MIRT054777	1169-1191	-21.60
4	NOD_2	hsa-miR-122-5p	MIRT438655	511-531	-16.00
5	IL1B	hsa-miR-877-3p	MIRT734718	44-66	-14.80
6	IL10	hsa-miR-106a-5p	MIRT005082	625-647	-16.90
7	HSPD1	hsa-miR-1-3p	MIRT003976	221-242	-10.20

Table 4: The selected phytochemicals with its structural information collected from PubChem database.

Name of Phytochemicals	PubChem CID	Molecular Formula	Molecular Weight
Kaempferol	5280863	$C_{15}H_{10}O_6$	286.239 g/mol
Myricetin	5281672	$C_{15}H_{10}O_8$	318.237 g/mol
Quercetin	5280343	$C_{15}H_{10}O_7$	302.238 g/mol

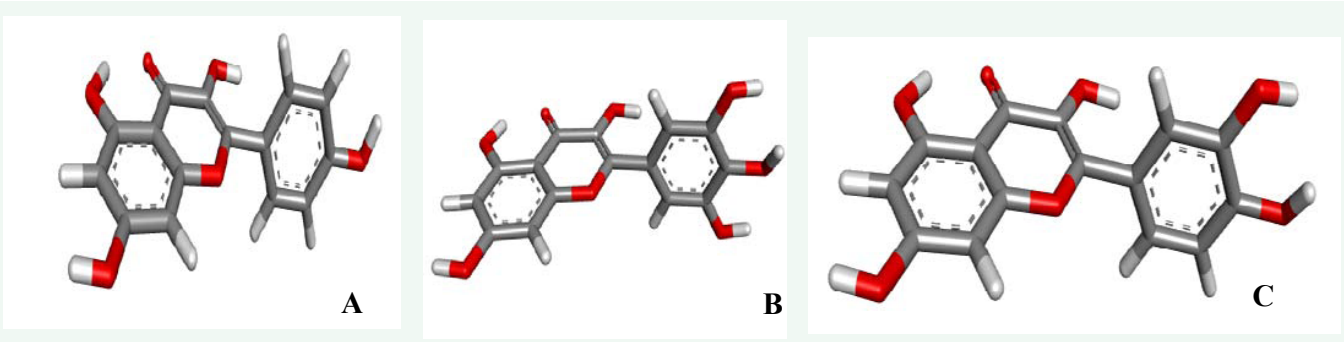

Figure 4 Structure of ligands (A) Kaempferol, (B) Myricitin, (C) Quercetin.

compounds proposed drug target of *C.asiatic*. Many studies have reported the association between **NOD2** polymorphisms and TB. The association between **NOD2** and tuberculosis (TB) risk has been reported widely, but the results of previous studies remained controversial and ambiguous. To assess the association between **NOD2** polymorphisms and TB risk, a meta-analysis was performed [10].The best binding interaction was seen by NOD2 protein with compound Kaempferol, Myricitin and Quercetin (Figure 5).

The binding energy of this docking result -7.45, -12.7, and

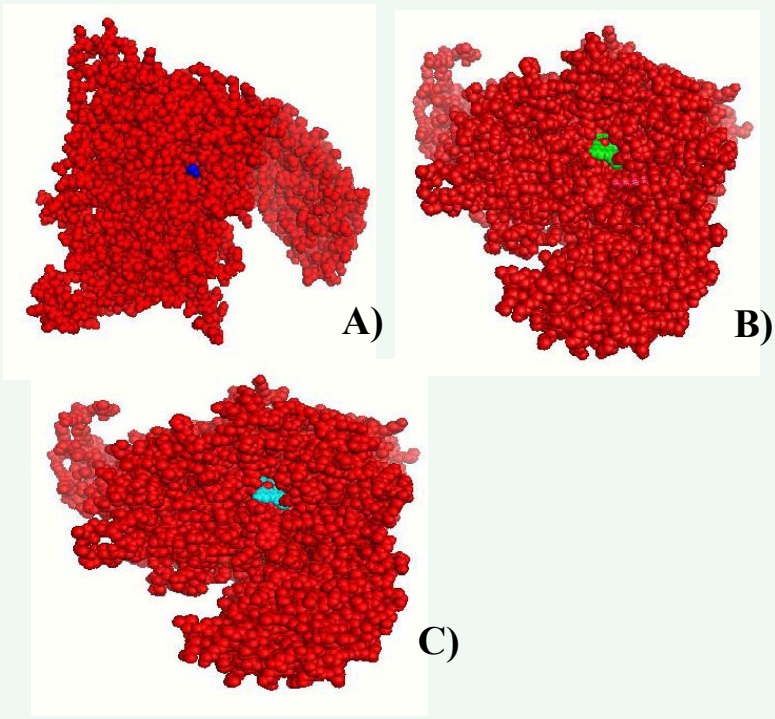

Figure 5 The best docking interaction between NOD2 withKaempferol, Myricetin&Quercetin.

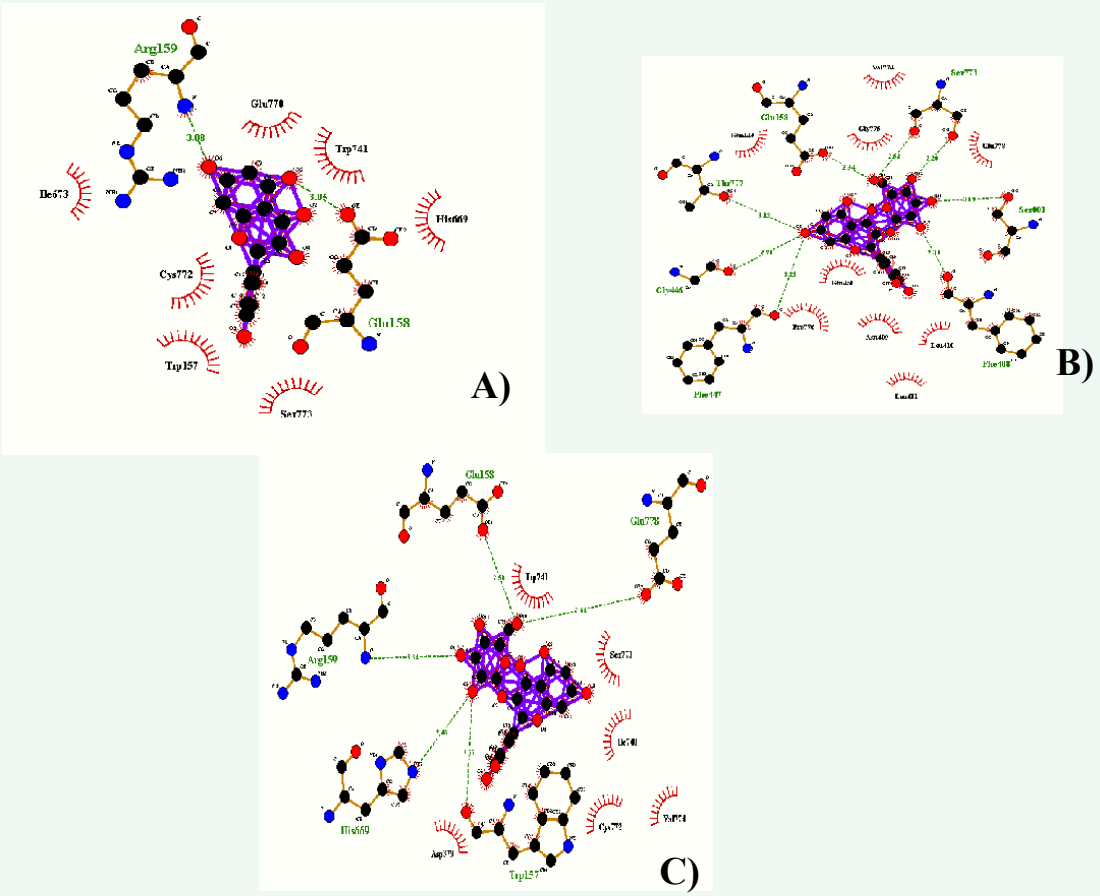

Figure 6 The ligplot analysis of docking interaction between NOD2 withKaempferol, Myricetin & Quercetin.

Table 5: Information obtained from docking calculation between selected natural compounds and drug target.

SL NO.	Phyto compound names	Gene Name	Binding energy	KI value	Inter molecular energy	Internal energy	Torsional energy	Unbound extended energy
1	Kaempferol	NOD2	-7.45	3.45 uM	-8.94	10.56	1.49	10.56
2	Myricetin		-12.7	490.23pM	-15.68	5.32	2.98	5.32
3	Quercetin		-12.75	450.57 pM	-15.73	5.11	2.98	5.11

-12.7 respectively, which was the best binding energy based on docking algorithms, resulted from all of the docking processes (Table 5). So this compound Quercetin could be used as an inhibitor to mutate and NOD2 gene as it has good binding affinity towards this gene. In docking results , amino acids residue of target NOD2 which undergo interaction with ligand Kaempferol , Myricetin and Quercetin were (arg159, Gla778, Trp741, His669, Glu158, Ser773, Trp157, Cys772, Ile673),(gly775, val774, ser773, glu778, ser801, phe408, leu411, leu410, asn409, gln450, pro776, phe447, gly446, thr777,glu449, glu158) and (glu158 ,trp741, glu773, ser773, Ile740, cys772, val774, trp157, asp379, his669, arg159) etc and the hydrogen bond was built between ligand with (Glu158 and Arg159) , (glu158, thr777, gly446, phe447, phe408, ser801, ser773) and (glu158, arg159, his669, trp157, glu778) respectively analysed through ligplot analysis as shown in Figure 6 (a,b,c).

In this study, *C. asiatica* is one of the important medicinal plants that have been used all over the world as unique sources of medicines and may constitute the most common human use of biodiversity. They are the richest bio-resource of traditional systems of medicine, modern medicines, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. Tuberculosis (TB) is a contagious disease. Like the common cold, it spreads through the air. Only people who are sick with TB in their lungs are infectious. It has been made to enhance the knowledge of antimycobacterial activity of *Centellaasiatica* plant extract against Mycobacterium tuberculosis. The association between **NOD2** and tuberculosis (TB) risk has been reported extensively, but the results of previous studies remained controversial and uncertain [10]. Despite the widespread use of an attenuated live vaccine and several antibiotics, there is more TB than ever before, requiring new vaccines and drugs and more specific and rapid diagnostics so the novel drug design should be needed for the better cure of the future disease management, but to design the new drug novel effective and validated drug targets also required. *In sillico* approach or computational methods to select targets, ligands in tuberculosis database and their refinement. This study reveals that NOD2 is a better potential drug target for tuberculosis and Quercetin is the best ligand to inhibit NOD2 and might be prevent the disease Tuberculosis.

CONCLUSION

In silico analysis provides the docking result, from which it was analysed that the compounds Kaempferol, Myricetin and quercetin were showed the 3 binding affinity i.e, -7.45, -12.61, ,-12.75 with the target protein NOD2 respectively, but out of these three only Quercetin showed the best binding affinity with target protein NOD2 i.e. -12.75 and bind with the protein perfectly. Also it had good active hydrogen bond interaction as compared to another compound. So it was concluded that Quercetin might be a good inhibitor against the target protein NOD2 to cure tuberculosis disease.

ACKNOWLEDGEMENT

We really thanks to Tectona Biotech Resource Centre (TBRC), Odisha, for providing necessary facilities, as well as we present our heartily gratitude to Dr.Shovan Kumar Mishra, Director of TBRC, for his endless support throughout this work.

REFERENCES

1. Chippada SC, Volluri SS, Srinivasa Rao B, Meena V. In vitro anti-inflammatory activity of methanolic extract of Centellaasiatica by HRBC membrane stabilisation. RASAYAN J Chem. 2011; 4: 457-460.
2. M. Suresh, Pradipta K Rath, A Panneerselvam, D Dhanasekaran, Nooruddin T. Anti-Mycobacterial Effect of Leaf Extract of Centellaasiatica (Mackinlayaceae). Res J. 2010; 872-876.
3. Dash BK, Faruquee HM, Sudhangshu KB, Md Khasrul A. "Antibacterial and Antifungal Activities of Several Extracts of Centellaasiatica L. against Some Human Pathogenic Microbes." Res J. 2011.
4. Zainol MK, Azizah Abdul Hamid, S. Yusof, Shukri Muse. "Antioxidative activity and total phenolic compounds of leaf, root and petiole of four accessions of Centellaasiatica (L.) Urban." Food Chem. 2003; 81: 575-581.
5. Ullah MO, Sultana S, Haque A. Antimicrobial, cytotoxic and antioxidant activity of Centellaasiatica. Eur J Sci Res. 2009; 260-264.
6. Zheng CJ, Qin LP. Chemical components of Centellaasiatica and their bioactivities. Chinese J Integrat Med. 2007; 348-351.
7. Cheng CL, Koo MW. Effects of Centellaasiatica on ethanol induced gastric mucosal lesions in rats. Life Sci. 2000; 67: 2647-2653.
8. Laskowski RA, Swindells MB. "LigPlot+: multiple ligand–protein interaction diagrams for drug discovery." J Chem Inf Model. 2011; 51: 2778-2786.
9. Šali Andrej, Tom L Blundell. "Comparative protein modelling by satisfaction of spatial restraints." J Mol Biol. 1993; 234: 779-815.
10. Wang C, Chen ZL, Pan ZF, Wei LL, Xu DD, Jiang TT. NOD2 polymorphisms and pulmonary tuberculosis susceptibility: a systematic review and meta-analysis. Int J Biol Sci. 2013; 10: 103-108.

Cite this article

Sahoo S, Raut SS, Das D (2019) Screening of Phytochemicals and In silico Approach through Drug Design of Centella asiatica. Int J Plant Biol Res 7(1): 1114.