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Original Article

In-vitro and in-silico analysis of spirulina extracts against rheumatoid arthritis disease: A therapeutic approach

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ABSTRACT

The current investigation of a combinatorial approach of insilico and invitro which was carried out to identify and explore the role of existing drugs and phytochemical compounds against potential targets of RA. In this study we got 150 genes related with the RA disease. From that 18 genes (proteins) were mostly related to 9 drugs to cure the disease. From the literature survey 8 Phytochemicals like Flavonoids, Alkaloids, Steroids, Lutein, Saponins, Tannin, Lycopene, and Alpha-Carotene were mostly present in Spirulina. These 4 proteins IL2RA, CFLAR, CASP8, and PTPN22 were mainly affected by Alkaloids, Flavonoids, and the Alpha-Carotene. Then the antibacterial activity of *S. platensis* was evaluated in different concentration of solvent extract (Methanol & Ethyl acetate) against targeted bacterial species (*S. aureus* and *E.Coli*). Toxicity of the solvent extract was tested by Paper disc diffusion method. Methanol extract gave the highest zone of inhibition than Ethyl acetate extract for the both bacteria E.Coli and *S. aureus.* FRAP-assay had been used to determine the antioxidant activity as it was simple and quick and the result shown that the Ethyl acetate extraction was more efficient than the Methanol extraction.

Key Words: -Rheumatoid arthritis (RA); S. platensis; Methanol & Ethyl acetate; S. aureus and E.Coli; FRAP-assay; Spirulina.

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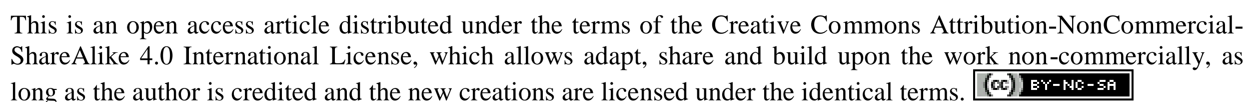
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INTRODUCTION

Rheumatoid arthritis (RA) is a common chronic and systemic autoimmune disorder characterized by inflammation of the synovial joints, destruction of cartilage and bone; it involves a complicated pathogenesis, with pathological changes in multiple targets[1,2]. RA affects about 1% of the world population, in a female/male ratio of 3/1. The disease can occur at any age, its incidence increasing with age[3]. Rheumatoid arthritis (RA) has been well characterized by symmetric polyarthritis affecting several joints, accompanying synovial hyperplasia, consequently leading to joint destruction and deformity, loss of function, and reduced quality of life. The prevalence of major rheumatic disease in adult range from 24% in China and Indonesia, but 45% in Philippines, Chile and rural Africa and affecting 0.5-1% of the entire human population[4]. Numerous studies have indicated that local release of lysosomal enzyme mediates atleast part acute and chronic inflammation in joints and in the synovial fluid in RA[5]. Currently steroids, non-steroidal anti-inflammatory drugs (NSAIDs),disease-modifying antirheumatic drugs (DMARDs) and immunosuppressant drugs are used in the relief of inflammation in RA and are often associated with severe adverse effect, the most common being gastro intestinal bleeding and peptic ulcers[6]. For this reason, more effective and safe drug with minimal side effects is required. Natural products are abundant source of pharmacologically active compounds, many of which have become important human drug[7]. *Spirulina platensis* is a blue green alga having diverse biological activity, due to high content of highly digestible proteins, vitamins, beta-carotene, phycocyanin and other pigments[8]. Early interest in *S. platensis* focused mainly on its potential as a source of protein and vitamins, but recently more attention has been made to study its therapeutic use, and a number of published reports suggest beneficial effects of this microalgae in acute allergic rhinitis[9], anti-cardiotoxic[10],anti-hepatotoxic[11] and its anti-nephrotoxic effects[12]. Keeping in view the nutritive and pharmacological properties of *S. platensis*, present investigation was undertaken to assess the immune modulatory effect of *S. platensis* against CIA.

MATERIALS AND METHODS

Collection, Isolation and Purification of *Spirulina*: Spirulina was collected from commercially available resources in the form of capsules. These capsules were detached to get spirulina in powder form quantifying approximately 5 gm in wt. In addition spirulina the capsules also contained other constituents that were needed to be removed to get the pure culture of spirulina. As per the literature reference CFTRI liquid medium was prepared for spirulina culture.

Preparation of CFTRI - Medium:
Materials and equipment: Double Distilled Water (DDH_2O), Measuring Cylinder, conical flask, Bacteriological Chemicals, Laboratory Scales, spatula , 1N NaOH Solution, 1N HCl Solution, PH Indicator Paper Or PH Meter, Gloves, Dispenser, culture tubes, Test Tube Caps, Test Tube Basket, Slanting Stage, Autoclave, Incubator, inoculation loop, needle, brush, petriplate, spirulina capsules, digital balance.

Process of Preparation
1. Measured the components (chemicals) of the medium (CFTRI-medium) into a flask containing 9/10 volume of the DDH_2O. Used a clean spatula for every measurement. Dissolved the solid components and make the final volume for 1-liter.
2. 5gm of Spirulina powder was dissolved in 1 liter of CFTRI-medium
3. The culture media was kept under observation for 10-15 days at 2400 lux light (2-bar lights) with room temperature.
4. Algal filaments were picked by the use of a glass micropipette into one drop of medium on a microscope slide and were examined under microscope.
5. Based on the growth the algal sample was transfer to the Solid CFTRI-medium by addition of 1% agar (1gm in 100ml of CFTRI-liquid medium) for its pure culture.The flasks containing agar-agar and CFTRI- liquid medium were autoclaved at 121°C.
6. PH (5.6) of the medium was checked with an indicator paper or with a pH meter and adjusts to the proper value with NaOH or HCl solution.
7. Poured the medium into the culture tubes and petriplates. Each tube contains about 5-6 ml medium, closed them with caps and places them into a test tube basket.
8. The culture tubes were placed onto a slanting stage to let the medium solidify in the tubes.
9. The prepared media could be stored for 1-2 weeks at room temperature or longer in a refrigerator.

The components were mixed one by one in distilled water. Then mixed them properly and prepare 1 litre of solution. Then maintained the PH 5.6 of the solution and prepare the media.

Sample Preparation for Antioxidant and Anti-bacterial Activity:-

Preparation of Ethyl acetate & Methanol extracts*:* Five gram of spray dried *Spirulina*

powder were taken in each two conical flasks and dissolved with 75ml of Ethyl acetate & Methanol respectively**.** Both the flasks were heated at 77^0C in the micro oven for 3-4 minutes followed by transfer to centrifuge tubes and centrifuge at 10,000 rpm for 15 minutes. Filtered through Whatmann filter paper (6mm) and kept for 24 hours for evaporation. The mixture was shaken frequently during first 6 hours & allowed to stand for the next 18 hours.

Anti-bacterial Activity of Spirulina: Agar well Diffusion Assay: *In vitro* antibacterial activity of different crude extracts of *Spirulina platensis* was evaluated using the agar well diffusion assay. 150 ml of double distilled water mixed with 5.7 gm of Muller Hinton Agar (MHA) mixed well by boiling in micro oven (2-3 mints) and autoclave for 15 mints at 15 atm pressure. Then 20 ml of MHA was poured in sterile petridishes (90 mm) and prepare 6 petridishes. This was allowed to solidify and then individual plates were marked for the organisms inoculated. 100 µl of respective bacterial extracts (E.coli & Staphylococcus Aureus) were pipetted into the well in assay plates. Plates were incubated overnight at 37°C and all the plates were observed for the zone of inhibition, diameter of these zones were measured in millimeters. All the tests were performed under sterile conditions and repeated three times.

Anti-oxidant Activity of Spirulina:-

The **Ferric Reducing Antioxidant Powe**r (FRAP) assay was done according to Benzie and Strain (1996) with some modifications. The stock solutions included 300mM acetate buffer (3.1 g C2H3NaO2 _ 3H2O and 16mL C2H4O2), pH 3.6, 10mM TPTZ (2, 4, 6- tripyridyl-s-triazine) solution (30 ml required) in 40mM HCl, and 20mM FeCl3 _ 6H2O solution. The fresh working solution was prepared by mixing 25mL acetate buffer, 2.5mL TPTZ solution, and 2.5mL FeCl3 _ 6H2O solution and then dissolve in 10ml distilled water .

Prepare a stock 10mM of Ascorbic acid weight 0.0176g ascorbic acid and make up to a final volume of 10ml using methanol. Then make fresh on a day of assay in a new centrifuge tube for 30 min in the dark condition. Readings of the colored product [ferrous tripyridyltriazine complex] were then taken at 594 nm.

Ascorbic assay was diluted as follows:

Add 20 µl stock + 980 µl dH_2O - Test tube 1
100 µl from T.t 1 + 900 µl dH_2O - Test tube 2
100 µl from T.t 2 + 900 µl dH_2O - Test tube 3
100 µl from T.t 3 + 900 µl dH_2O - Test tube 4
100 µl from T.t 4 + 900 µl dH_2O - Test tube 5
100 µl from T.t 5 + 900 µl dH_2O - Test tube 6
200 µl sample 1 + 800 µl dH_2O - Test tube 7
200 µl sample 2 + 800 µl dH_2O - Test tube 8

Mining of genes associated with RA from GWAS Catalog: Disease search for "**Rheumatoid arthritis**" with a p-value 0.05 was performed to retrieve GWAS studies on RA from GWAS Catalog (http://www.genome.gov/gwastudies/ currently https://www.ebi.ac.uk/gwas/). A total of 39 GWAS studies resulted in a total of 150 unique genes mapped to discrete genomic locations of human genome. The corresponding protein sequences encoded by these genes were obtained from UniProtKB database [13].

Functional annotation and GO association of RA genes by DAVID: The functional annotation of a total of 150 genes was performed through Gene Ontology (GO) analysis which describes the functions along the three categories *viz*., molecular functions (MF), biological processes (BP) and the cellular components (CC). The Database for Annotation, Visualization and Integrated Discovery (DAVID) Bioinformatics Resources 6.7 (http://david.abcc.ncifcrf.gov/) was used for GO term annotation (i.e., the common vocabulary for the functional description of genes and gene products) annotation. Finally to find the statistically significant GO terms of the genes, GO term enrichment analysis was performed. The DAVID parameters were filtered to reduce the false positives and the output was taken into account after applying multiple testing correction (p-values <0.05), fold change and False Discovery Rate (FDR). Genes from significantly enriched biological processes were termed as key genes and were used for network construction [14-15].

Generation of gene network and its interactions using STRING database: A total of 147 genes obtained from significantly enriched biological processes were termed as key genes and were used for network construction of RA were analyzed through STRING database [16-17].

Gene-disease association study through Web Gestalt:

WebGestalt(http://bioinfo.vanderbilt.edu/webgestalt/) was used for further functional categorisation of 147 BP genes including gene–phenotype association, gene–disease association and Drug association analysis. Further interactive phenotype ontology associated with RA genes was elucidated. Organism Homo sapiens was selected against select organism of interest column, hsapiens_gene symbol was selected at Select gene ID type, and outcome of DAVID functional analysis BP gene list consisting of 147 genes was uploaded in the Upload gene list column. The following entries such as Statistical Method/test: hyper geometric, Multiple Test Adjustment: BH, Significance Level: Top 10 and 0.05, Minimum Number of Genes for a Category: 2 were selected [18-19].

UniProt-(http://www.uniprot.org/):
The Universal Protein Resource (UniProt) was a comprehensive resource for protein sequence and annotation data. The corresponding protein sequences encoded by these genes were retrieved from UniProtKB database [20-22].

Analysis of Natural Variant: Natural variants were otherwise called as Single Nucleotide Polymorphisms (SNPs) that was a variation in a single nucleotide that occured at a specific position in the genome, where each variation was present to some appreciable degree within a population. Information about the natural variants of the genes namely TNFRSF9, IL2RA, PTPN22, CD40, RAG1, CSF2, CD83, CASP8, CFLAR, IL2RB, TYK2, PRKCQ (reported genes of GWAS) were retrieved from Uniprot database. The rsIds and its respective mutated/substitued position were being identified and pursued for analyses of the respective mutated position using SIFT, SNP & GO, PANTHER, and Ployphen2.0 to find out the effect of mutation on the protein function and structure. The gene and its corresponding rsIDs were represented in.

Retrieval of Drugs and proteins: The Structure Data Format (SDF) 3D structure of the reported drugs were retrieved from the NCBI PubChem [23-24] database (http://www.ncbi.nlm.nih.gov/pccompound/) along with its PubChem ID, Molecular weight and Molecular formula. The compounds were converted into pdb format structure using the PyMol [25] (academic version) tool and Discovery Studio v4.1 visulizer tools [26]. The structures of the corresponding proteins of reported genes were retrieved from PDB Protein Data Bank (PDB) [27]. The unknown structures were predicted using tool RaptorX [28-29] web servers due to unavailability at PDB Protein Data Bank (PDB).

Prediction of binding site: Structural and active site studies prediction of the proteins were done by using CASTP (Computed Atlas of Surface Topography of Proteins) at http://cast.engr.uic.edu [30].

RESULTS AND DISCUSSION

Preparation of Culture media (CFTRI-Liquid medium): After preparing the liquid medium of 1liter, 5gm of powdered Spirulina was added with it .Then it was kept at room temperature with 2400 lux light .After 10-15 days of observation the growth of the species was shown in the **Figure 1**.

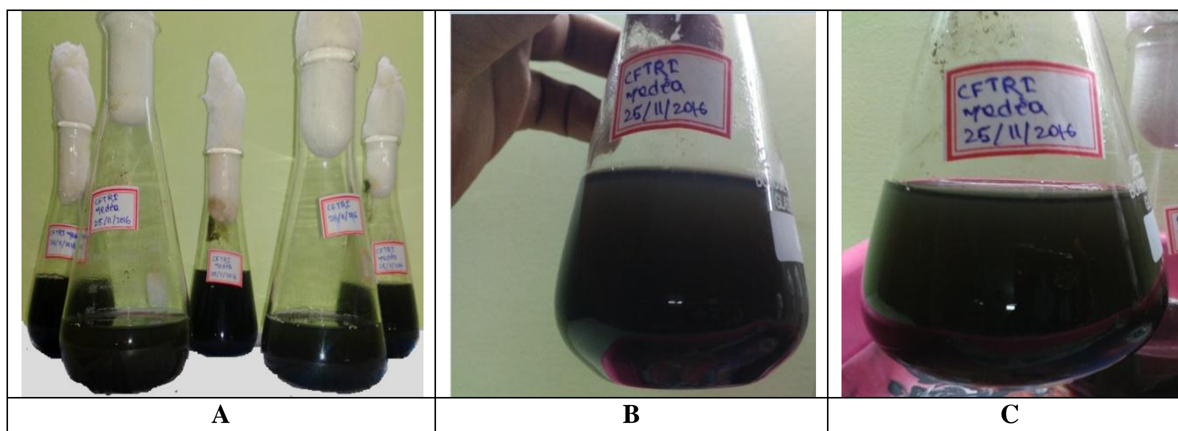

Fig.1:-Preparation of Culture media (CFTRI-Liquid medium)

Preparation of Solid CFTRI- medium and growth of Spirulina species: The growth on the liquid medium was observed, and then it was transferred into CFTRI-solid medium by adding 1gm of agar in 100ml of CFTRI-liquid medium. After preparing the solid media in pettriplates , the Spirulina species was kept on the media by spreading method. Then all the pettriplates kept at room temperature with 2400 lux light .Then the growth of the species was observed for 15-20 days. It was seen that every day the growth rate was 0.1-0.2 cm which was shown in **Figure 2.**

Microscopic observation of Spirulina in CFTRI medium: The growth of the Spirulina species on both solid and liquid medium was observed in light microscope. After taking the sample on the slide with a few amount of water, it showed the movement and the growth of the Spirulina species. The figures of the observation were given below in **Figure 3.**

Anti-Bacterial: The results obtained from the present study were recorded and analyzed using different solvents against ***Staphylococcus aureus*** *and* ***E.coli***. It was clear from the study that the diameter of the inhibition zone varies with the type of the solvent used and hence varies in antibacterial activity. The antibacterial activity of ***S. platensis*** was evaluated in different concentration of solvent

extract (Methanol & Ethyl acetate) against targeted bacterial species (***S. aureus*** **and** ***E.Coli***). Toxicity of the solvent extract had been tested by Paper disc diffusion method. Methanol extract gave the highest zone of inhibition of 2.76cm in diameter followed by Ethyl acetate extract which gave zone of inhibition of about 1.26 cm in diameter for bacteria E.Coli and Methanol extract gave the highest zone of inhibition of 3.63cm in diameter followed by Ethyl acetate extract which gave zone of inhibition of about 1.50 cm in diameter for bacteria ***S. aureus*** **(Table.1).**

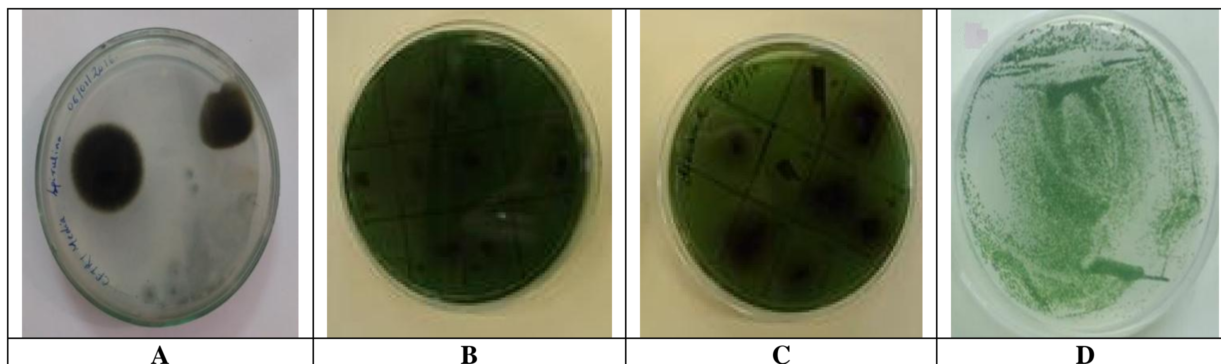

Fig.2:-Preparation of Solid CFTRI- medium and growth of Spirulina species in it

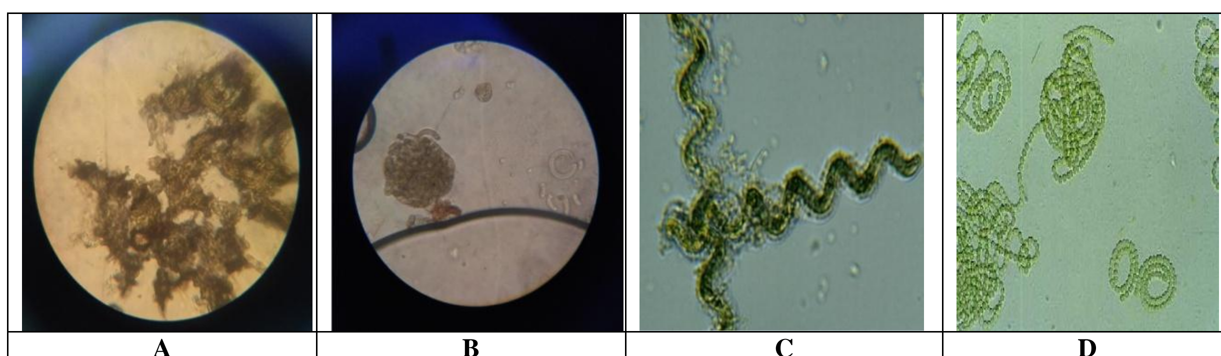

Fig.3:-Microscopic observation of Spirulina in CFTRI medium

Table.1:- The antibacterial activity of *S. platensis* at different concentrations.

Serial No	*Bacterial Species*	*Concentration(ml)*	*Ethyl Acetate Extract*	*MethanolExtract*
1	*E.coli* (***Escherichia coli***)	0.2	1.26	2.16
		0.4	1.8	1
		0.6	0.73	2.76
2	**Staphylococcus Aureus** (*S. aureus*)	0.2	1.5	3.63
		0.4	1.43	2.13
		0.6	0.5	2.76

After spreading the bacterial culture on the MHA media in different pettriplates, then the Toxicity of the solvent extract had been tested by Paper disc diffusion method and the zone of inhibition was calculated .The figure of the inhibition was shown below in **Figure 4.**

Anti-Oxidant activity of Spirulina extracts by FRAP assay: FRAP-assay had been used to determine the antioxidant activity as it is simple and quick. Higher FRAP values gives higher antioxidant activity. FRAP value is based on reducing ferric ion, where the antioxidants are reducing agent.

In present study, the results showed that FRAP values of Ethyl acetate was higher than the Methanol extract which are taken in Test tube 8 and 7 respectively. This showed that the Ethyl acetate extraction was more efficient than the Methanol extraction. **(Table 2)**

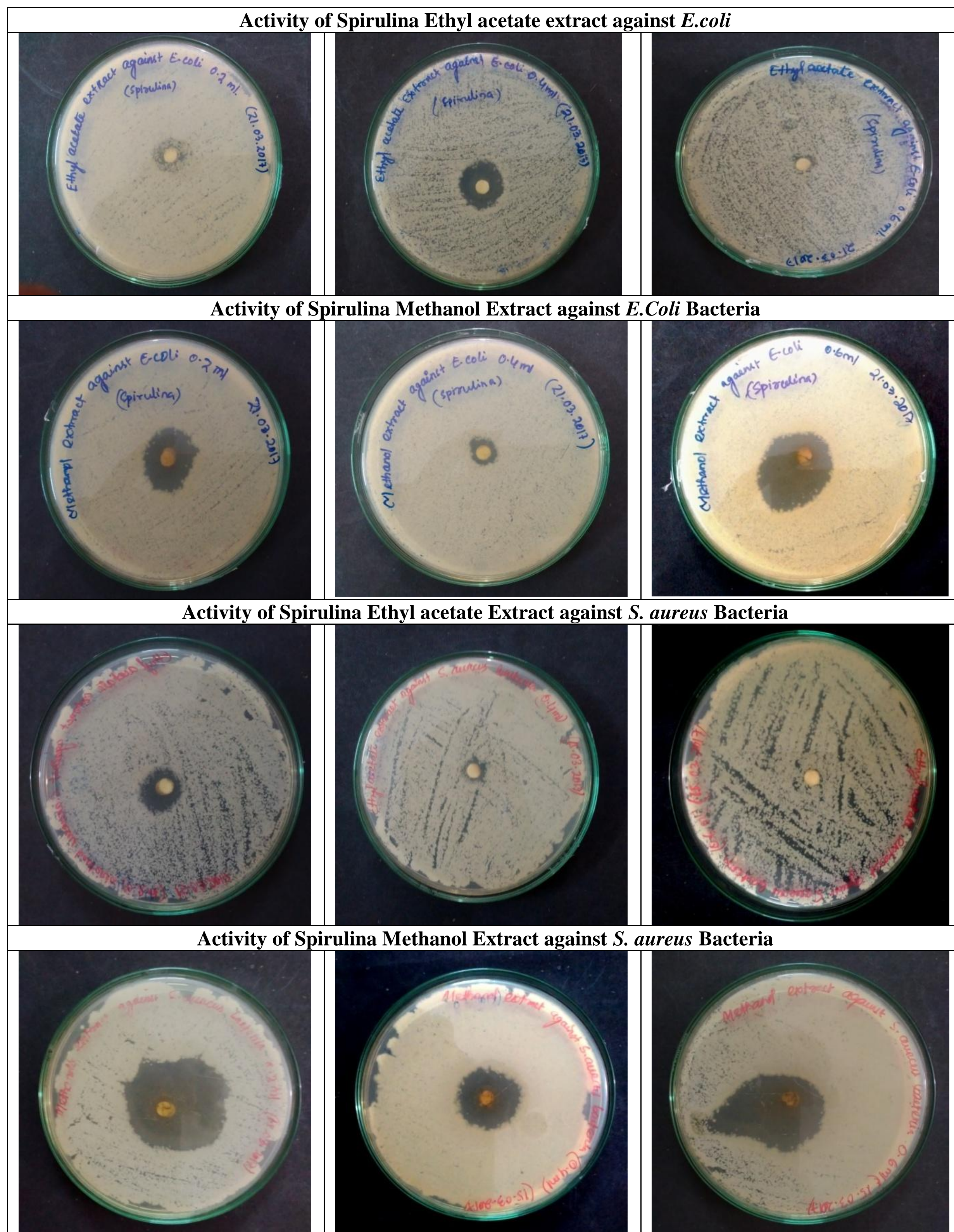

Fig.4:- Zone of inhibition of *S. platensis*

GWAS analysis: The GWAS studies reported 39 studies of rheumatoid arthritis with a total of 150 unique genes with p-value 0.05 mapped to discrete genomic locations of human genome. The list of 150 unique genes which were related to RA disease with P-value 0.05 was represented at **Table.3**. These genes were mostly associated with the Rheumatoid arthritis disease.

DAVID Analysis: The DAVID bioinformatics functional enrichment analysis reported 150 genes and 147 GO terms for biological processes (BP),

120 genes and 49 GO terms for molecular functions (MF) and 135 genes and 35 GO terms for cellular components (CC). Based on the essential role of biological processes, 147 genes and 125 GO terms obtained from significantly enriched biological processes were termed as key genes that were used for network construction of RA. These genes played a vital role for causing the RA disease. The results were represented in **Table.4**.

Table.2:- Anti oxidant activity of *S. platensis*

Standard concentration	Ascorbic acid solution Solution	Distilled water	Volume of FRAP reagent		O.D at 594nm
(mM)	(ml)	(ml)	(ml)		
0.1	1	9	3	Incubate at 37°C for 30 minutes	2.186
0.2	2	8	3		2.034
0.4	4	6	3		0.371
0.6	6	4	3		0.267
0.8	8	2	3		0.191
1.0	10	0	3		0.177
S1	200µl	9.8	3		0.388
S2	200 µl	9.8	3		0.737

The results of the antioxidant activity were taken in a graph below.

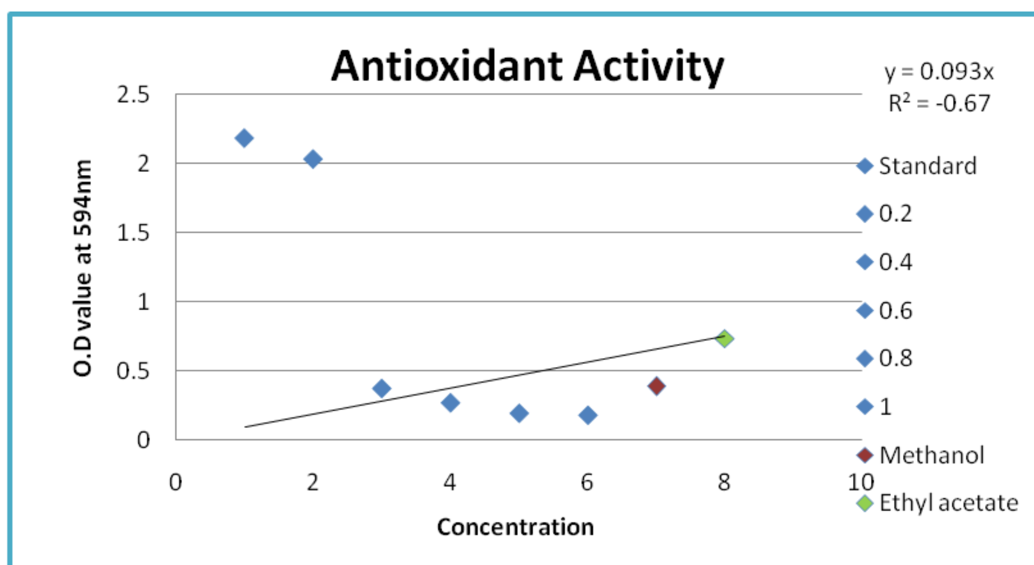

Fig.5:-Graph showing standard and sample curve for total Antioxidant activity

Table.3:- 39 studies of rheumatoid arthritis with a total of 150 unique genes

TNFAIP3	IGFBP1	EOMES	ZNF438
OLIG3	IRF5	FADS1	DPP4
TRAF1-C5	LDHAL3	FADS2	HLA
PTPN22	KCNIP4	FADS3	HLA-DQA2
CD40	GPR125	FCRL3	HLA-DQA1
PRKCQ	GMCL1L	GATA3	HLA locus
TNFIP3	GATSL3	GRHL2	HLA-DQB1
BLK	ATM	IFNGR2	C6orf10
TRHDE	RCAN1	CSF3	FCGR2A
ARHGEF3	P2RY10	IL20RB	MHC
AIRE	TRAF1	IL2RB	APOM
PFKL	CDK5RAP2	IL3	
PADI4	IL2RA	IL6R	
B3GNT2	KIF5A	IRAK1	

ANXA3	SALL3	IRF4	
CSF2	MMEL1	JAZF1	
CD83	TNFRSF14	LBH	
ARID5B	CDK6	LOC100506023	
PDE2A	CCL21	LOC145837	
ARAP1	PIP4K2C	LOC339442	
PLD4	C5	MED1	
PTPN2	NFKBIE	MTF1	
ETS1	PXK	INPP5B	
FLI1	SH2B3	PLCL2	
GCH1	IKZF3	AHNAK2	
PRKCH	UBASH3A	PPIL4	
ZNF774	ARL15	PVT1	
PRKCB1	ACOXL	RAD51B	
IRF8	ATG5	RASGRP1	
CCR6	C1QBP	RUNX1	
SPRED2	C4orf52	LOC100506403	
IL6ST	CCL19	SFTPD	
ANKRD55	CD2	PTPN11	
C5orf30	CD226	SYNGR1	
RBPJ	CD28	TAGAP	
AFF3	CD5	TEC	
CD247	CDK2	TNFRSF9	
IL2	CDK4	TPD52	
IL21	CEP57	TRAF6	
BATF	CASP8	RAG1	
STAT4	CFLAR	RAG2	
CTLA4	CLNK	TXNDC11	
POU3F1	COG6	TYK2	
KIF3	CXCR5	YDJC	
RTKN2	ABHD6	UBE2L3	
RPS12P4	DNASE1L3	WDFY4	

Table.4:- Genes associated with biological processes (BP) of RA.

HLA-DQB1	EOMES	KIF5A	EOMES
CSF3	GRHL2	TNFRSF9	AFF3
CSF2	BATF	C5	PIP4K2C
IL3	STAT4	PXK	PTPN22
IL2RA	FLI1	IRF5	OLIG3
HLA-DRB1	REL	CD40	ARID5B
RAG1	MTF1	RAD51B	JAZF1
CTLA4	POU3F1	CDK2	RAD51B
CCL19	RUNX1	C1QBP	ETS1
TNFRSF14	MED1	RASGRP1	FADS1
CLNK	TRAF1	PTPN2	ARHGEF3
CD40	IRAK1	TPD52	TNFAIP3
IL21	IL2RB	LBH	IL21
HLA-DQA2	TRHDE	CDK5RAP2	HLA-DRB1
HLA-DQA1	PRKCH	SALL3	IRF8
TNFRSF9	RCAN1	GATA3	PLD4
CCR6	CDK4	CASP8	RBPJ
C1QBP	ATM	CCL21	
CXCR5	SALL3	GCH1	
ETS1	CD83	IL6ST	
CCL21	TAGAP	IL20RB	
IRF8	PDE2A	ATG5	
AIRE	RTKN2	CSF2	
IL2	SH2B3	ARHGEF3	

CD247	IGFBP1	UBASH3A	
CD5	CD226	TEC	
DPP4	INPP5B	FADS1	
CD28	ARAP1	FADS3	
PTPN11	IL6ST	FADS2	
IRF5	IL6R	CFLAR	
IRF4	CD83	TYK2	
IFNGR2	PRKCQ	ATG5	
PRKCQ	RAG1	BLK	
GATA3	RAG2	PADI4	
PTPN22	SFTPD	FLI1	
TRAF6	IL3	CCR6	
IL2RA	IRAK1	PFKL	
IL20RB	CFLAR	PLCL2	
RASGRP1	CASP8	TYK2	
RBPJ	PTPN2	CDK4	
BATF	TNFAIP3	ANXA3	
CSF3	IL2RB	CDK6	
IKZF3	CD2	IKZF3	

STRING Analysis: A total of 147 genes obtained from significantly enriched biological processes are termed as key genes that were carried out for network construction of RA through STRING database. The result of the string was represented in **Figure.6**. The RA network of STRING database reported the genes namely **CXCR5, CCL19, CCL21, IL21, IL2RB, STAT4,CD40,IL6ST, IL2RA, CTLA4,IKZF3, HLA-DQA2, HLA-DQB1, TRAF1, IRF4, GATA3, CD40, PTPN11, CDK6, TNFRSF14, TRAF6, RUNX1, TNFRSF9, RAG1, RAG2, ATM, CDK4, CDK6, PAD14, OLIG3, BLK, IRAK1, PRKCH** and **ETS1** at the core region of the network. These genes may be said to play a key in RA as well as can be differentially expressed in RA disease.

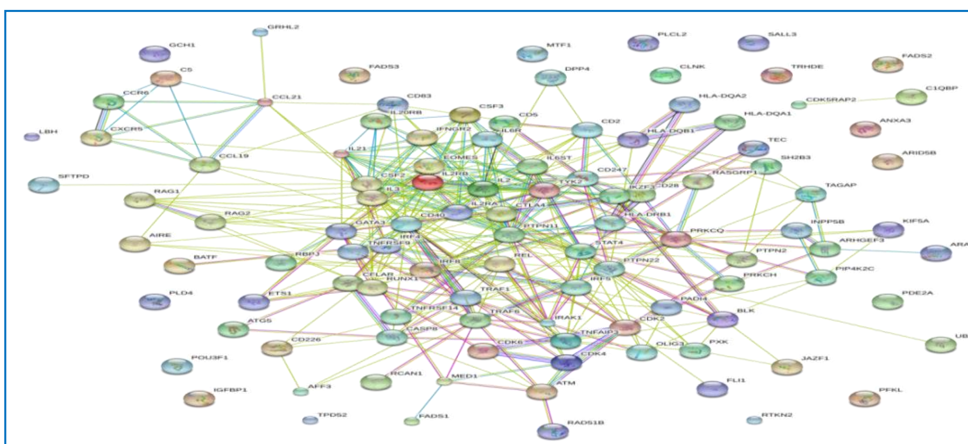

Fig.6:- Network construction of RA using STRING database

Web Gestalt analysis: Web Gestalt represented various graphs from the input of 147 BP genes like BP-BP graph, BP-CC graph and BP-MF graph. The PHEWAS (Phenome wide association studies) was carried out in Web Gestalt by considering PHEWAS BP-BH (Top 10) and BP-Bonferoni of 147 genes. Both the graphs represented variations among them this may be due to difference in statistical terms. The Drug association analysis of WebGestalt reported 9 drugs interacted with 18 genes or its corresponding proteins. The results of WebGestalt pertaining drugs against RA and its corresponding genes/proteins were cross checked by literature survey**. (Table.5)** The Structure Data Format (SDF) 3D structure of the reported drugs were retrieved from the NCBI PubChem database (http://www.ncbi.nlm.nih.gov/ pccompound/) along with its PubChem ID, Molecular weight and Molecular formula. The compounds were converted into pdb format structure using the PyMol (academic version) tool, Discovery Studio v4.1 visulizer tools and online SMILES translator web server https://cactus.nci.nih.gov/translate/) as per requirement.

Table.5:- The Drug association analysis of WebGestalt

S.L NO	DRUG	TOTAL NO OF GENE	Entrez Gene	GENE SYMBOL
1	immune globulin	12	3123	HLADRB1
			1235	CCR6
			3604	TNFRSF9
			3119	HLADQB1
			3559	IL2RA
			26191	PTPN22
			958	CD40
			5896	RAG1
			1437	CSF2
			708	C1QBP
			9308	CD83
			59067	IL21
2	lumiracoxib	2	3123	HLADRB1
			3119	HLADQB1
3	vorinostat	2	841	CASP8
			8837	CFLAR
4	prednisone	2	3559	IL2RA
			958	CD40
5	chloroquine	2	3560	IL2RB
			9474	ATG5
6	ribavirin	2	3119	HLADQB1
			7297	TYK2
7	podofilox	2	841	CASP8
			8837	CFLAR
8	phosphatidylserine	2	5588	PRKCQ
			841	CASP8
9	etoposide	2	841	CASP8
			8837	CFLAR

The detail about the drugs, DRUG Name, PubChem CID, Molecular Formula, Molecular Weight and its corresponding Target was reported in **Table.6.** These details about the Drugs were derived from the PubChem database.

Table.6:- Description of drugs associated with disease RA

S.L NO	DRUG NAME	PUBCHEM ID	MOLECULAR FORMULA	MOLECULAR WEIGHT (g/mol)	CHEMICAL NAME
1	immune globulin	119	$C_4H_9NO_2$	103.121 g/mol	4-aminobutyric acid; 4-Aminobutanoic acid; GABA; Gamma-aminobutyric acid; 56-12-2; Piperidic acid
2	Lumiracoxib	151166	$C_{15}H_{13}ClFNO_2$	293.722 g/mol	Lumiracoxib; Prexige; 220991-20-8; COX-189; Joicela; COX 189
3	Vorinostat	5311	$C_{14}H_{20}N_2O_3$	264.325 g/mol	Vorinostat; 149647-78-9; SAHA; Suberoylanilidehydroxamic acid; Zolinza; N-hydroxy-N'-phenyloctanediamide
4	Prednisone	5865	$C_{21}H_{26}O_5$	358.434 g/mol	Prednisone; 53-03-2; Deltasone; Prednisonum; Orasone; Prednison
5	Chloroquine	2719	$C_{18}H_{26}ClN_3$	319.877 g/mol	Chloroquine; Aralen; Chlorochin; Chloraquine; Reumachlor; Capquin
6	Ribavirin	37542	$C_8H_{12}N_4O_5$	244.207 g/mol	Ribavirin; 36791-04-5;

					Tribavirin; Rebetol; Virazole; Ribamide
7	Podofilox	10607	$C_{22}H_{22}O_8$	414.41 g/mol	Podofilox; 518-28-5; Condylox; Condyline; Podophyllinic acid lactone; Podofilox [USAN]
8	Phosphatidylserine	6323481	$C_{13}H_{24}NO_{10}P$	385.306 g/mol	Phosphatidylserine; Phosphatidyl-L-serine; LifeExtension PS Caps; (2S)-2-amino-3-[[(2R)-2-butanoyloxy-3-propanoyloxypropoxy]-hydroxyphosphoryl]oxypropanoic acid; Distearoylphosphatidylserine; Ptd-L-Ser
9	Etoposide	36462	$C_{29}H_{32}O_{13}$	588.562 g/mol	Etoposide; VePesid; Lastet; Toposar; Trans-Etoposide; VP-16

The structures of the corresponding proteins of reported 12 genes were retrieved from PDB Protein Data Bank (PDB).The region of mutation gave the position of the disordered gene sequence which caused the disease. This database also gave the molecular weight of the drug compound (Drugs). The UniProt IDs and details about the structure of these 12 genes were reported at **Table.8**. The ligands(Drugs) , whose structure was not present in the database would be predicted from online modelling software like **RaptroX** mentioned in **Table.7.**

Table.7:- Details of Modelled structure

S.L No	Protein Name	Template Name(Chain)	P-Values	Tool Used for Modelling	UniProt IDs
1	CCR6	5lwe(A)	4.54e-06	RaptroX	P51684
2	CD83	5mix(A)	9.45e-06	RaptroX	Q01151
3	HLADRB1	3o6f(B)	5.47e-07	RaptroX	Q07493
4	RAG1	3jbw(A)	1.51e-30	RaptroX	P15918
5	TNFRSF9	4yn0(A)	3.39e-06	RaptroX	Q07011

Natural Variant: The natural variants of corresponding proteins were obtained from UniProtKb database. The effect of mutation/substitution in non-synonymous SNPs (nsSNPs)on structure and function of various protein was analysed through online tools like **SIFT, SNP & GO, PANTHER** and **Ployphen2.0** to find out the consensus results. The result depicted that the genes which are deleterious/ diseased and effecting the structure and function of the genes may be the cause for RA disease. The results were represented in **Table.8**. In the table the deleterious / probably damage (PD)/probably beginning (PB) and Disease levelled rs Ids of the proteins were the genes related to cause the disease.

Table.8:-The list of non-synonymous SNPs (nsSNPs) analysed by sequence based *in silico* methods.

S.L NO	PROTEIN NAME	rs IDs	RESULTS			
			SIFT	PANTHER	SNPs&GO	PolyPhen-2
1	TNFRSF9	rs9657963	Deleterious	PD/PB	Neutral	Benign
		rs9657965	Deleterious	PD/PB	Neutral	Benign
		rs9657979	Deleterious	PD/PB	Neutral	Benign
		rs776878260	Deleterious	PD/PB	Neutral	PD
2	IL2RA	rs796051888	Deleterious	PD/PB	Disease	PD
		rs796051887	Deleterious	PD/PB	Disease	PD
		rs12722712	Deleterious	PD/PB	Neutral	PD
3	PTPN22	rs33996649	Deleterious	PD/PB	Neutral	Benign
		rs2476601	Deleterious	PD/PB	Neutral	Benign

4	CD40	rs750234130	Deleterious	PD/PB	Neutral	Benign
		rs28931586	Deleterious	PD/PB	Disease	PD
		rs11569321	Deleterious	PD/PB	Neutral	Benign
		rs11086998	Deleterious	PD/PB	Neutral	Benign
5	RAG1	rs1801203	Deleterious	PD/PB	Disease	PD
		rs4151027	Deleterious	PD/PB	Disease	Benign
		rs199474683	Deleterious	PD/PB	Disease	Benign
		rs4151029	Deleterious	PD/PB	Disease	Benign
		rs3740955	Deleterious	PD/PB	Disease	Benign
		rs4151030	Deleterious	PD/PB	Disease	PD
		rs121918568	Deleterious	PD/PB	Disease	PD
		rs121918571	Deleterious	PD/PB	Disease	PD
		rs104894289	Deleterious	PD/PB	Disease	PD
		rs104894291	Deleterious	PD/PB	Disease	PD
		rs104894291	Deleterious	PD/PB	Disease	PD
		rs199474682	Deleterious	PD/PB	Disease	PD
		rs199474684	Deleterious	PD/PB	Disease	PD
		rs104894292	Deleterious	PD/PB	Disease	PD
		rs199474679	Deleterious	PD/PB	Disease	PD
		rs141524540	Deleterious	PD/PB	Disease	PD
		rs199474685	Deleterious	PD/PB	Disease	PD
		rs4151031	Deleterious	PD/PB	Disease	PD
		rs199474677	Deleterious	PD/PB	Disease	PD
		rs199474678	Deleterious	PD/PB	Disease	PD
		rs199474686	Deleterious	PD/PB	Disease	PD
		rs104894298	Deleterious	PD/PB	Disease	PD
		rs193922461	Deleterious	PD/PB	Disease	PD
		rs4151032	Deleterious	PD/PB	Disease	PD
		rs199474681	Deleterious	PD/PB	Disease	PD
		rs104894285	Deleterious	PD/PB	Disease	PD
		rs104894284	Deleterious	PD/PB	Disease	Benign
		rs199474688	Deleterious	PD/PB	Disease	PD
		rs199474680	Deleterious	PD/PB	Disease	PD
		rs199474689	Deleterious	PD/PB	Disease	PD
		rs199474676	Deleterious	PD/PB	Disease	PD
		rs28933392	Deleterious	PD/PB	Disease	PD
		rs104894286	Deleterious	PD/PB	Disease	PD
		rs199474687	Deleterious	PD/PB	Disease	PD
		rs121918569	Deleterious	PD/PB	Disease	PD
		rs2227973	Deleterious	PD/PB	Disease	PD
		rs104894287	Deleterious	PD/PB	Disease	PD
		rs199474690	Deleterious	PD/PB	Disease	Benign
		rs4151033	Deleterious	PD/PB	Disease	PD
		rs199474691	Deleterious	PD/PB	Disease	PD
		rs4151034	Deleterious	PD/PB	Disease	PD
		rs104894290	Deleterious	PD/PB	Disease	PD
		rs150739647	Deleterious	PD/PB	Disease	PD
		rs121918570	Deleterious	PD/PB	Disease	PD
		rs104894288	Deleterious	PD/PB	Disease	PD
6	CSF2	rs2069640	Deleterious	PD/PB	Neutral	Benign
		rs25882	Deleterious	PD/PB	Neutral	Benign
7	CD83	rs2230193	Deleterious	PD/PB	Neutral	Benign
8	CASP8	rs35976359	Deleterious	PD/PB	Neutral	Benign
		rs17860424	Deleterious	PD/PB	Neutral	PD
		rs1045485	Deleterious	PD/PB	Neutral	Benign
9	CFLAR	rs13424615	Deleterious	PD/PB	Neutral	Benign
10	IL2RB	rs57770674	Deleterious	PD/PB	Neutral	PD
		rs2228143	Deleterious	PD/PB	Disease	Benign

		rs228942	Deleterious	PD/PB	Neutral	Benign
11	TYK2	rs35163004	Deleterious	PD/PB	Neutral	Benign
		rs12720343	Deleterious	PD/PB	Neutral	Benign
		rs1049619	Deleterious	PD/PB	Disease	PD
		rs12720263	Deleterious	PD/PB	Neutral	Benign
		rs2304256	Deleterious	PD/PB	Neutral	Benign
		rs2304255	Deleterious	PD/PB	Neutral	Benign
		rs55956017	Deleterious	PD/PB	Neutral	Benign
		rs2304254	Deleterious	PD/PB	Disease	PD
		rs12720356	Deleterious	PD/PB	Disease	PD
		rs55882956	Deleterious	PD/PB	Disease	PD
		rs34046749	Deleterious	PD/PB	Neutral	PD
		rs35018800	Deleterious	PD/PB	Disease	PD
		rs34536443	Deleterious	PD/PB	Neutral	PD
		rs55886939	Deleterious	PD/PB	Neutral	Benign
12	PRKCQ	rs45590231	Deleterious	PD/PB	Neutral	Benign
		rs2236379	Deleterious	PD/PB	Neutral	Benign
		rs34524148	Deleterious	PD/PB	Neutral	Benign

PD: Probably Damaging ; PB: Probably Beginning

CONCLUSION

The current investigation was a combinatorial approach of insilico and invitro which was carried out to identify and explore the role of existing drugs and phytochemical compounds against potential targets of RA. In this study we got 150 genes related with the RA disease. From that 18 genes (proteins) were mostly related to 9 drugs to cure the disease. From the literature survey 8 Phytochemicals like Flavonoids, Alkaloids, Steroids, Lutein, Saponins, Tannin, Lycopene, Alpha-Carotene were mostly present in Spirulina. By predicting the structures of the drugs, phytochemicals and the proteins, the docking analysis was performed. From the docking study these 4 genes IL2RA, CFLAR, CASP8, and PTPN22 were mostly related proteins used for curing the RA disease. These 4 proteins were mainly affected by Alkaloids, Flavonoids, and the Alpha-Carotene. The drug compounds which may play a vital role for reducing the effect of RA on the body are immuno globulin, vorinostat, podofilox, Etoposide. Using these four drug compounds and three phytochemical compounds many drugs/medicines were formed for curing the Rheumatoid arthritis disease. In this study the comparison of the effect of the drug as well as phytochemical compounds were performed to reduce the effect of the disease causing genes from the harmful foreign particles by blocking the binding sites of the gene by the above drugs and phytochemical compounds. This was studied by doing the Docking analysis. Then the antibacterial activity of *S. platensis* was evaluated in different concentration of solvent extract (Methanol & Ethyl acetate) against targeted bacterial species (*S. aureus* and *E.Coli*). Toxicity of the solvent extract had been tested by Paper disc diffusion method. Methanol extract gave the highest zone of inhibition than Ethyl acetate extract for the both bacteriasE.Coli and *S. aureus.* FRAP-assay had been used to determine the antioxidant activity as it was simple and quick. From the result it was shown that the Ethyl acetate extraction was more efficient than the Methanol extraction.

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