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Original Research Article

Immuno-Modulatory Activity of Medicinal Herbs

Studies On Immuno-Modulatory Activity of Selected Medicinal Herbs (In-Silico Approach)

Preenon Bagchi[1*] and Ajit Kar[2]

[1] *Institute of Biosciences and Technology, MGM University, Chh. Sambhajinagar, India*

[2] *Vasishth Academy of Advanced Studies and Research, Sarvasumana Association, Bengaluru, India*

Abstract: In recent years, there has been a tremendous development of biotechnological, pharmacological, and medical techniques that can be implemented in the functional modulation of the immune system components. Immuno-modulation is a technique wherein we consider the combined effect of several Ayurvedic herbs. Significant early research studies demonstrate immune modulation. The current trend reported an increase in immuno-modulation studies by ayurvedic herbs. Immuno-modulation has attracted much attention because it directly applies to basic research and clinical therapy. Immuno-modulation is gaining significance as a new mode of treatment in pharmacy. The combined effects of several herbs are taken as treatment in immune modulation. In this work, we try to understand the immune modulatory properties of certain ayurvedic herbs. We have taken the FASTQC genome sequence of selected ayurvedic herbs for this study. After quality checking of the sequences, we joined both reads (forward and reverse) using the FASTQ interlaced. It is significant since we need to study the complete sequence (both the reads). Using Velvet software, we identified the K-mers. We took the k-mer coverage to understand the association of the herbs using clustering studies. To understand the immune modulation, we performed principal component analysis and visualized the analysis using a Scree plot and scatter plot. The clustering results of the k-mers are significant in understanding immune modulation. The association results proved the immune modulation property of the ayurvedic herbs.

Keywords: Immuno-modulation, K-mer, FASTQ interlaced, principle component analysis, scatter plot

***Corresponding Author**

Preenon Bagchi , Institute of Biosciences and Technology, MGM University, Chh. Sambhajinagar, India

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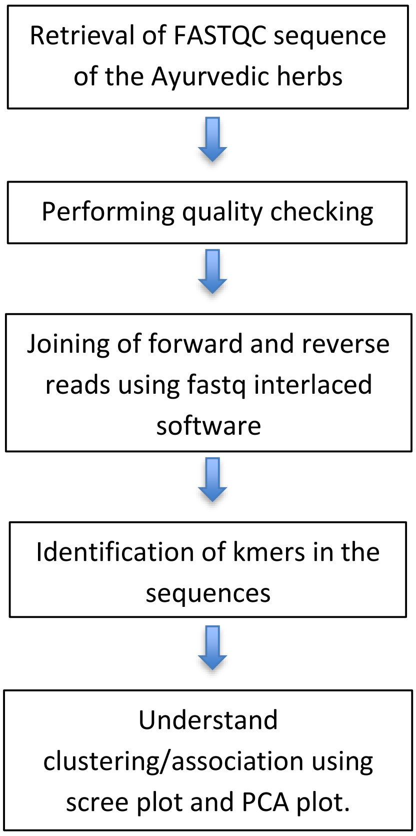
1. INTRODUCTION

An immuno-modulator is generally defined as a biological or synthetic substance that can stimulate, suppress, or modulate the human immune system, including both innate and adaptive arms of the immune responses. Specific immunity is seen to evolve as a highly sophisticated defense mechanism of higher organisms[1]. As a result of deficiencies in the immune system, recurrent infections, autoimmunity, and susceptibility to malignancy results. In humans, the immune response comprises two major mechanisms: the cell-mediated immune system, which includes phagocytes, macrophages, T lymphocytes, and granulocytes, and the humoral immune system, which includes antibodies produced by β lymphocytes. Mature β lymphocytes can be found in the bone marrow, lymphoid tissue, spleen, and to a lesser extent in the bloodstream[1, 2]. The major function of β lymphocytes is to produce antibodies and interact with T cells. The initial (primary) immune response develops over 8 to 14 days. This response includes the generation of "memory" β and T cells, which provide a long-term system for rapid immune response upon subsequent antigen exposure (secondary response). It allows the development of immune T cells and the antibody acting between 1 to 3 days of antigen exposure for rapid control or destruction (modulation) of the immune stimulus[2]. Bioinformatics is now routinely applied to experiments in genomics that rely on sophisticated computations. Genomics is the technology behind studying the full complement of genetic information in an organism's genome; bioinformatics has also grown some new disciplines: functional genomics, structural genomics, and evolutionary genomics. It is to be noted that multiple genetic and environmental factors contribute to the development of autoimmune diseases. Auto-immune diseases generally result from the failure of immune tolerance, the process by which the immune system recognizes and accepts self-tissue[3]. However, an association of specific gene polymorphisms is noted with autoimmune diseases. In Ayurveda, to maintain immune homeostasis under different conditions of impaired immune responses, several medicinal plants are used[4]. In the present study, specific phytocompounds are selected from the following promising plants: *Azadirachta indica, Ocimum sanctum, Tinospora cordifolia, Asparagus racemosus, Andrographis paniculata, Aegle marmelos, Aloe vera, Commiphora mukul, Vitex negundo, Allium sativum, Carica papaya, Moringa oleifera, Cinnamomum tamala, Curcuma longa, Berberis aristata, Syzygium aromanticism, Solanum xanthocarpum, Emblica officinalis, Pluchea lanceolata, Plumbago zeylanica, Ficus benghalensis, Withania somnifera, Centella Asiatica, Hemidesmus indicus, Rubia cordifolia, Phyllanthus niruri, Glycyrrhiza Alabama, Piper longum, Swertia chirayata, Glycosmis pentaphylla, Achyranthes aspera and Tragia involucrata*. To understand their association, we took the help of k-mers, which are substrings of length k within a biological sequence. A study by Kurtz et al., 2008, tried to focus on the role of k-mer frequencies in annotating plant genomes.[5] K-mers counting, which are the substrings of length k in DNA sequence data, is an essential technique and component of many bioinformatics methods, including transcriptome and metagenome assembly. The principle component analysis technique is used to ascertain the immune-modulation efficacy of the herbs.

2. METHODOLOGY



Flow chart: Association studies

In this work, the genome sequence of the plants was taken from the SRA database. The genome sequences were fed to the galaxy. After checking the quality of the sequences using the tool FASTQC (FASTQC tool checks the quality of the raw sequence; the tool is made to pinpoint potential problems in high throughput sequencing datasets.), we use FASTQ interlacer[6] to join the forward and reverse reads. Next, we assembled the forward and read with Velveth[7]. Velvet fragments the reads of the reads into k-mers, i.e., fragments of length k. We are required to input a value of k (k-mer size) for the assembly process. It is known that small k-mers have greater connectivity, but larger k-mers have better specificity. In the galaxy platform, our velvet software generates a confits file; further, a stats file is generated. Each of the files has five output files. From the velvet output file, we got the Stats file from which we selected the k-mer coverage for preparing our data frame. Further, we performed principal component analysis using a scree plot.

3. RESULTS

The genome sequence of the plants was downloaded from the SRA database (https://www.ncbi.nlm.nih.gov/sra) (Table 1).

Table 1: Plants used with the SRA accession number

Sl. No.	Plant Name	Accession Number
1	Asparagus racemosus	SRR13286700.1
2	Commiphora mukul	NA
3	Cinnamomum tamala	SRR19510836
4	Berberis aristata	NA
5	Syzygium aromaticiu	NA
6	Solanum xanthocarpum	NA
7	Emblica officinalis	NA
8	Pluchea lanceolata	NA
9	Hemidesmus indicus	NA
10	Rubia cordifolia	NA
11	Swertia chirayata	NA
12	Glycyrrhiza glabara,	NA
13	Tragia involucrata	NA
14	Azadirachta indica	SRR16605974.1.1
15	Andrographis paniculata	SRR12791806.1.1
16	Aegle marmelos	SRR7268533.1.1
17	Moringa oleifera	ERR7622174.1.1
18	Curcuma longa,	SRR15115090.1.1
19	Aloe vera	SRR5167034.1.1
20	Plumbago zeylanica	SRR7134476.1.1
21	Centella asiatica,	SRR9650293.1
22	Phyllanthus niruri	ERR7618146.1.1
23	Piper longum	SRR16889194.1
24	Carica papaya	ERR7622169
25	Allium sativum	SRR1219644.1.1
26	Vitex negundo	SRR13684571.1.1
27	Tinospora cordifolia	SRR446984.1
28	Ocimum sanctum	SRR14090688.1.1
29	Ficus benghalensis	SRR11470435.1.1
30	Withania somnifera	SRR9845564.1.1
31	Glycosmis pentaphylla	ERR7618720.1.1
32	Achyranthes aspera	SRR8666210.1.1

NA-Not available

The above genome sequences were uploaded to the galaxy platform (usegalaxy.org). The forward and reverse strands of the sequences were merged using fast interlacing. Further, we retrieved the k-mer coverage using velvet, as shown in Table 2.

Table 2: Plant with their K-mer coverage in a data frame

Sl. No.	Plant Name	V1	V2	V3	V4	V5
1	Withania	19	253.11725	96.619114	57.321746	221.42692
2	Ficus	18	121.94085	129.351319	109.259698	106.50602
3	Ocimum	17	239.73220	186.630081	119.392097	134.05260
4	Tinospora	16	73.55567	59.462419	43.462303	30.56573
5	Vitex	15	154.23704	114.013541	49.201852	85.37760
6	Allium	14	58.17121	929.936058	566.189702	0.00000
7	Carica	13	205.64015	202.577487	133.192327	165.52877
8	Glycosmis	12	183.15823	433.723742	251.531656	383.99967

9	Achyranthes	11	124.76223	202.075662	142.259158	127.85333
10	Piper	10	994.16979	4.110896	4.100216	4.89456
11	Phyllanthus	9	195.71499	10.202864	9.968410	11.69327
12	Centella	8	1796.00000	20.249288	20.249288	5327.33333
13	Plumbago	7	251.17151	105.170627	94.885459	249.10301
14	Aloe	6	304.56775	201.361078	181.818460	648.23862
15	Curcuma	5	149.52846	120.630055	81.634945	175.42570
16	Moringa	4	172.80000	121.110234	121.110234	154.40000
17	Aegle	3	479.65725	115.982181	113.359038	1317.05839
18	Azadirachta	2	1501.39000	647.784575	646.455397	16294.66000
19	Andrographis	1	589.84848	151.134918	151.134918	4537.87879

The kmer coverage (taken as a data frame as in Table 2) was uploaded in R software. We got clusters and biplots using the scatter and scree plot. To get Scree Plot in R, we used the factoextra package, and analysis was done using the prcomp() function.

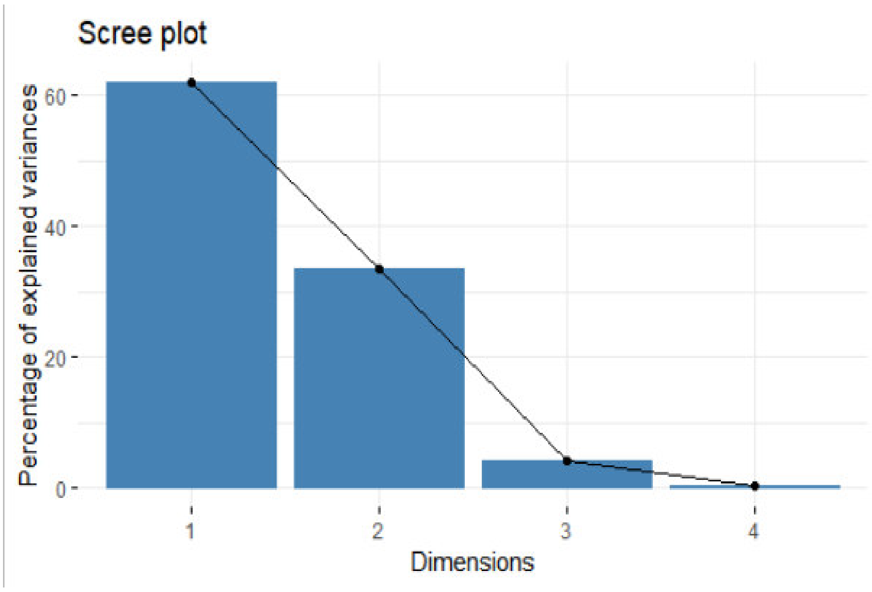


Fig. 1: Graph of individual plants. Similar plants are grouped.

Figure 1 shows a close association between the k-mers in the individual plants, and they are grouped.

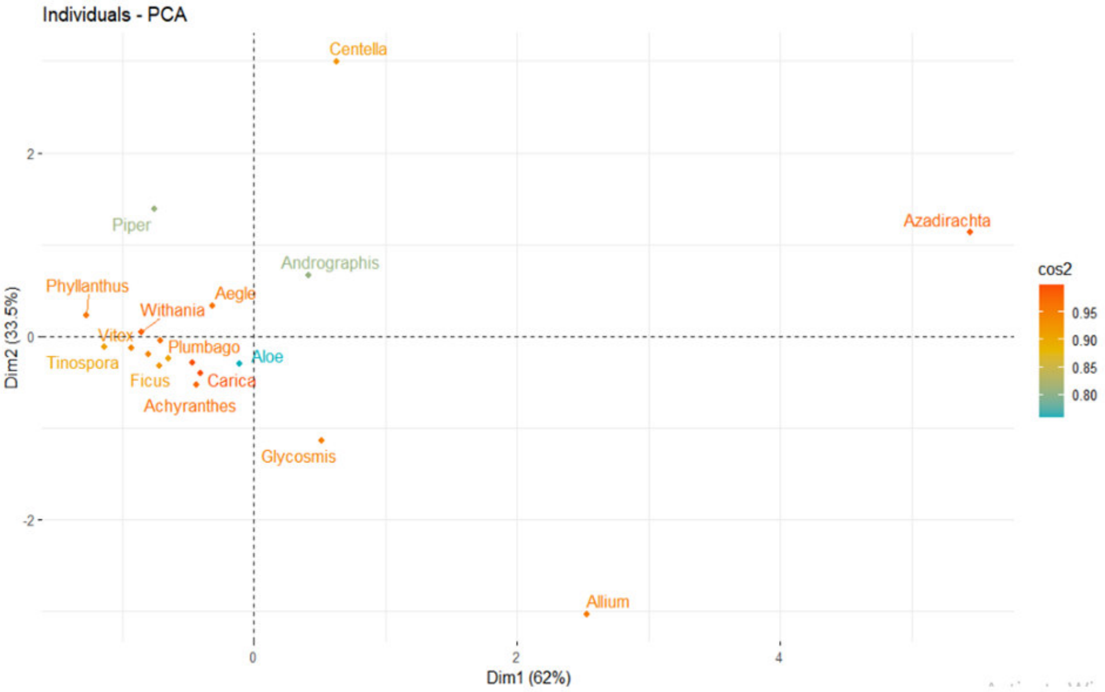


Fig. 2: Graph of variables. Positive correlated variables point to the same side of the plot. Negative correlated variables point to opposite sides of the graph.

Figure 2 Shows the formation of clusters among the herbs except Allum, Centella, and Azadirachta.

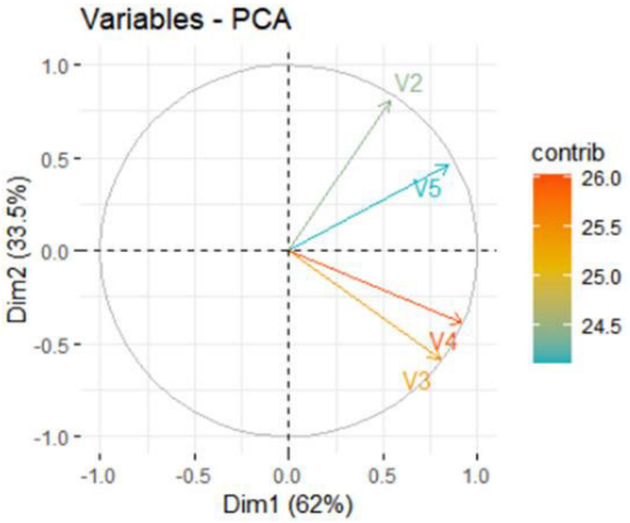


Fig. 3: Biplot of individuals and variables

Figure 3 shows the association between the kmers of the individual herbs.

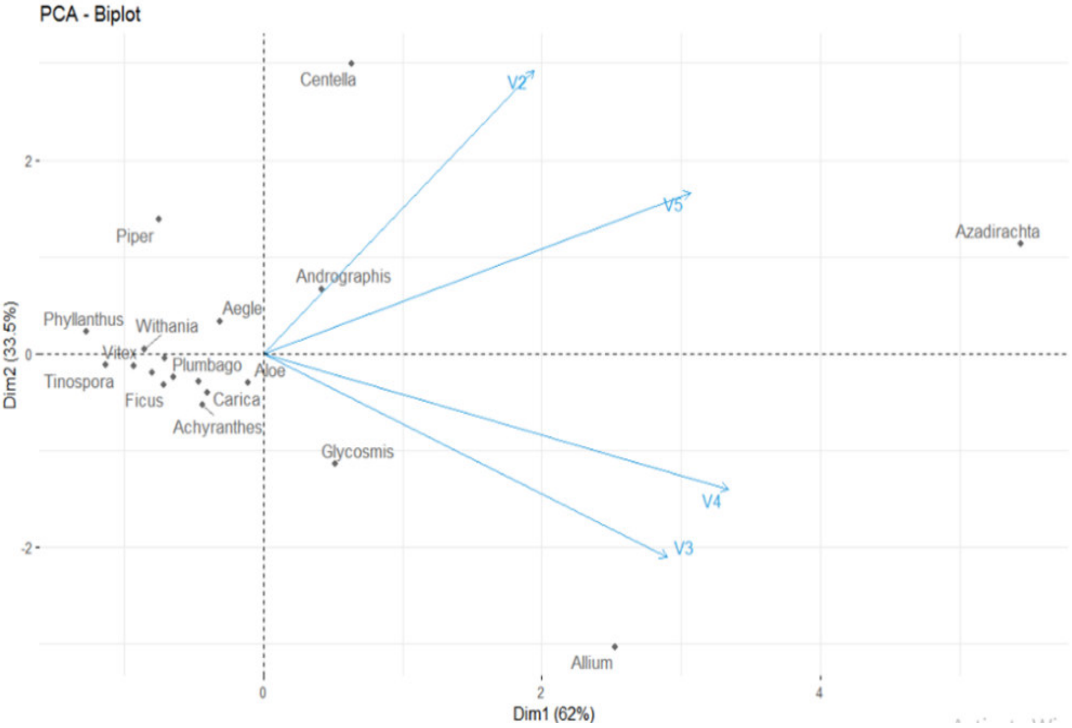


Fig. 4: Graph of individual plants, including the supplementary plants

Figure 4 shows the principal component, the k-mer coverage, and the cluster among the herbs except Allum, Centella, and Azadirachta.

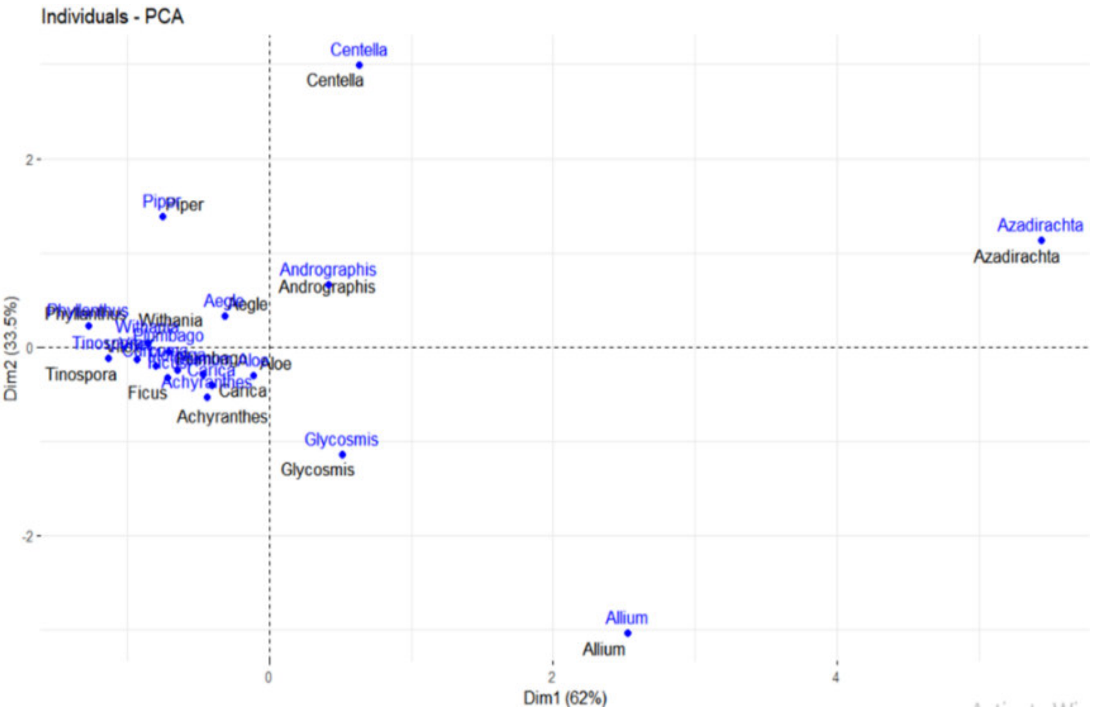


Fig. 5: Individual PCA

Figure 5 depicts the individual PCA and cluster formed among the plants except for Allum, Centella, and Azadirachta.

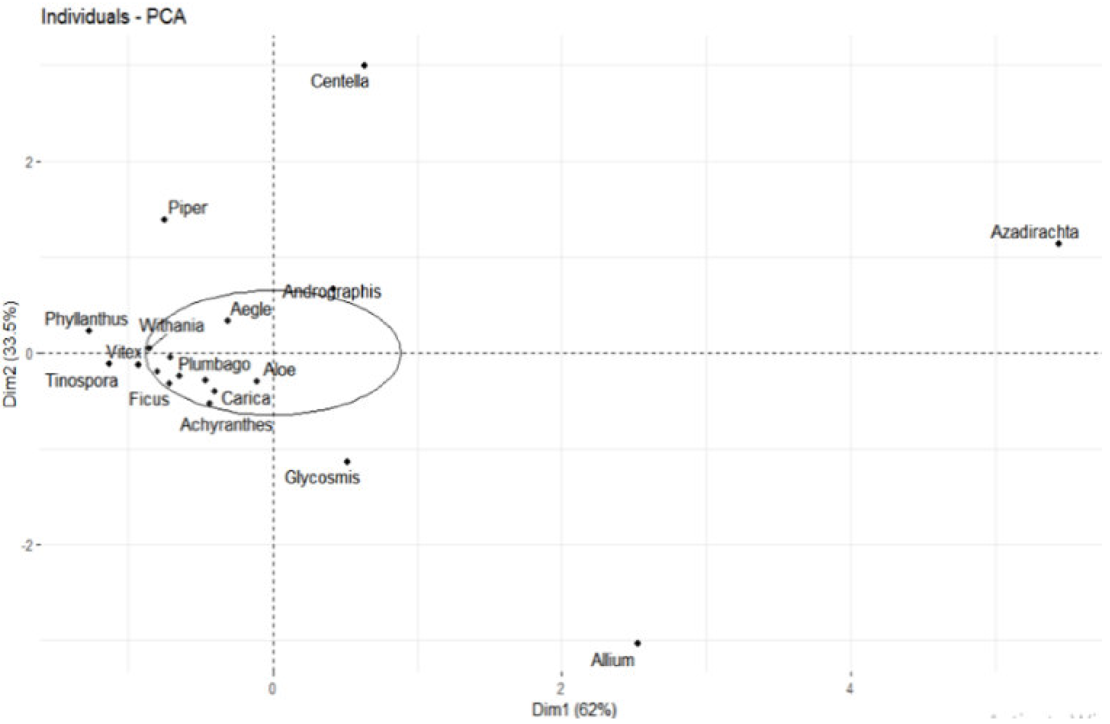


Fig. 6: Scatter Plot & Correlations

The figure depicts the scatterplot and cluster formed among the plants except for Allum, Centella, and Azadirachta.

4. DISCUSSION

The selected herbs (figures 1-6) (except Allum, Centella, and Azadirachta) are closely associatedThis analysis was as per the work of Bussi, et.al., 2021 who performed large-scale k-mer-based analysis of the informational properties of genomes[8]. Further, an unpublished study by Voichek and Weigel also tried identifying the role of k-mer in identifying the genetic variants in plants without considering the complete genome[9]. Orozco-Arias et al., 2021 explored the role of k-mers in building a machine-learning model to classify plant genomes[10]. The above work correlates with our work, which features k-mers in understanding the immune-modulation property in medicinal herbs. Further, Karikari et.al., 2023 also tried to understand the genome-wide association in plants using k-mer[11]. We used scree plot, a graphic plot showing the explained variance (or eigenvalues) having k-mer coverage in our work defined by the principal component to understand the correlation principal component. Work by Sarkar et al., 2021 determined the k-mer density in a DNA sequence by

PCR analysis[12]. Usually, we considered the first few components as the principal components and defined the overall variance in our work[13-16]. These plots (fig 1 to 6) shows the variance captured by the principal component from the data. It proves the k-mer association in our work and is validated by the principle component [17-21]. The y-axis in the plot shows the explained variance, which prominently predicts the amount of variation.

5. CONCLUSION

Understanding gene regulations in homeostasis provides new insights into the pathogenesis and treatment of mental disorders. This study aimed to combine gene expression (GE) microarray, next-generation sequencing (NGS), and bioinformatics to explore genetic regulations associated with mental disorders. The Kmer association depicted in our work highlights the principal component that shows the association. Immune response is a host's immediate reaction to encountering an invasive foreign entity. Specific phytocompounds could interact with a specific host's immune cells (in innate or adaptive arms) body's defense mechanism and maintain immune homeostasis under different conditions of impaired immune responses.

6. ACKNOWLEDGEMENT

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7. AUTHORS' CONTRIBUTION STATEMENT

Bagchi P. worked on sequence retrieval, k-mer formation, and association studies. Kar A gave the ayurvedic herb information. Further, gave the idea of this work.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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