A NETWORK PHARMACOLOGY OF BELUNTAS (*Pluchea Indica*) ON IMMUNITY CASES

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Abstrak

COVID-19 is classified as an outbreak related to the human immune system. The process of spreading was indeed quick, which made this outbreak a particularly dangerous pandemic. As of March 10, 2023, Indonesia had recorded 6,738,225 positive cases and 160,941 deaths from COVID-19 in 2020. The aim is to curb virus spread by boosting the immune system through antibody production. Studies suggest that certain Indonesian plants have immunomodulatory potential. This study aimed to determine the protein network associated with the body's immune system, which was activated by giving beluntas (*Pluchea Indica*). The research method used is descriptive in silico analysis using a online databases: KNApSAck, Dr. Duke, Pubchem, Swiss ADME, Swiss Target Prediction, Gene Cards, Venny, STRING, and KEGG. Based on the results of pharmacological network analysis, the *P. indica* contains 234 secondary metabolites, 126 of which have high bioavailability. Proteins associated with *P. indica* contain 1317 compounds, and those related to immunomodulators contain 1380 proteins. 340 proteins were found to have interacted with P. indica, all linked to immunomodulation, suggesting its potential for developing treatments for immune-related disorders through further research Based on KEGG Pathway analysis, there are five critical pathways in the immunomodulatory system, namely Th17 cell differentiation, IL-17 signaling pathway, T cell receptor signaling pathway, Fc epsilon RI signaling pathway, and TNF signaling pathway. There is 17 compound that can be an immunomodulator because it interacts with five critical pathways in the immunomodulator system.

***Kata Kunci**: Covid-19, Immunity, beluntas, Pluchea Indica, network pharmacology*

Background

COVID-19 (Corona Virus Disease 2019) is a case of initial immunity that appeared in Wuhan at the end of 2019 (Lena *et al*., 2023). The first COVID-19 case in Indonesia was reported on March 2, 2020. Until now, in Indonesia, there have been 6,738,225 positive people, and the death total is 160,941 as of March 10, 2023 (Johns Hopkins University, 2023). The spread of the COVID-19 virus happens directly or indirectly through droplet transmission or through contact with physical / objects that have direct contact with patients exposed to COVID-19 (Priani, 2021).

The immune system is a system whose job is to protect and defend the body from dangerous pathogens and even destroy the foreign cells that have entered the body (Priani, 2021). Studies that have been done previously show that certain plants can increase the immune system (Setiawan *et al*., 2021). One of them is *Pluchea Indica* (Nurhalimah, 2014; Sahara and Pristya, 2022). However, no explanatory information exists on how *P. indica* can increase immunity. Therefore, this research will find or demonstrate the mechanism of molecules that occur in humans when given the treatment of extract *P. indica*.

Testing activity of a compound can done through three approaches: in silico, in vitro, and in vivo tests. In vivo and in vitro tests require lots of time and cost compared with an in silico test (Makatita *et al*., 2020). The method used is a descriptive study using computer models (Adelina, 2018; Bajorath, 2015). The step in this method is prediction, hypothesis, and providing a new outlook on treatment in the field of medical and therapeutic (Bare *et al*., 2019). In-silico tests were carried out in networking pharmacology, a term used for the first time in 2007 (Hopkins, 2007). Networking pharmacology gives the basis for complex biology systems from the network perspective. We can understand the circumstances of health and disease in the body by determining and analyzing network biology and using it as a target for designing effective drug intervention methods (Wang *et al*., 2021). In silico test is scientifically valid, relatively new, and highly accurate *(Istyastono et al*., 2020)

Method

Tools

Several databases were used in this research, includes: Dr. Duke's Phytochmeical and Ethnobotanical Databases (https://phytochem.nal.usda.gov/pytochem/search), PubChem (https://pubchem.ncbi.nlm.nih.gov/), SwissADME (https://www.swissadme.ch/) SwissTargetPrediction (https://www.swisstargetprediction.ch/), GeneCards (https://www.genecards.org/), Venny (https://bioinfogp.cnb.csic.es/tools/venny/), StringDB (https://stringdb.org/), and KEGG (https://www.genome.jp/kegg/kegg1.html/).

Research Method

Identification of plant secondary metabolite compounds was obtained using the Dr. Duke's Phytochmeical and Ethnobotanical Databases, then searching for SMILES code for each compound using PubChem and entered to SwissADME to see bioavailability prediction by using Boiled-EGG method (Daina *et al*., 2017; Daina and Zoete, 2016). SwissTargetPrediction was used to predict the interaction of compounds with proteins targeted in research with compounds that pass the Boiled-EGG method (Daina *et al*., 2019). The target proteins of immunomodulators were searched using GeneCards (Stelzer *et al*., 2016). Then, look for intersections of proteins predicted to bind to compounds from plants using Venny (Oliveros J, 2015). The list of proteins that appear is then entered into StringDB (Szklarczyk *et al*., 2021). After that, we look for predictions of proteins that are interrelated with the immune system using KEGG (Kanehisa *et al*., 2023). To see which proteins interact most with pathways related to the immune system, we then looked at the secondary metabolite compounds of *P. indica* that interact with those proteins.

Result and Discussion

Identification of secondary metabolites of P. indica

Secondary metabolites of *P. indica* were obtained using Dr. Duke's Phytochemicals and Ethnobotanical Databases. This database was widely used to characterize plant bioactive compounds (Nguyen-Vo *et al.*, 2020). 234 compounds were identified in Dr. Duke's Phytochemicals and Ethnobotanical Databases (**Table 1**).

Table 1. A list of secondary metabolite of *P. indica*

No	Compound Name	Compound Code
1	(-)-Epicatechin	Molecule 1
2	(-)-Hydroxycitric Acid	Molecule 2
3	(+)-Syringaresinol	Molecule 3
4	(+)-Pinoresinol	Molecule 4
5	17-Epiazadiradione	Molecule 5
6	2-(2,4-Dihydroxyphenyl)-5,6-Methylenedioxybenzofuran	Molecule 6
7	2-Acetyl-Furan	Molecule 7
8	2-Hydroxy-3',4'-Dihydroxyacetophenone	Molecule 8
9	2-Methylanthraquinone	Molecule 9
10	2-Octene	Molecule 10
11	3,4-Dihydroxyphenyl-Acetate	Molecule 11
12	3,5-Dimethylphenol	Molecule 12
13	5-Dehydro-Avenasterol	Molecule 13
14	5-Methyl-Furfural	Molecule 14
15	Acalyphamide	Molecule 15
16	Alpha-Amyrin	Molecule 16
17	Alpha-Copaene	Molecule 17
18	Alpha-Humulene	Molecule 18
19	Alpha-Muurolene	Molecule 19
20	Alpha-Oxoglutaric-Acid	Molecule 20
21	Alpha-Phellandrene	Molecule 21
22	Alpha-Pinene	Molecule 22
23	Alpha-Terpineol	Molecule 23
24	Ambolic-Acid	Molecule 24
25	Ambonic-Acid	Molecule 25
26	Arabinose	Molecule 26
27	Arachidic-Acid	Molecule 27
28	Aromadendrene	Molecule 28
29	Ascorbic-Acid	Molecule 29
30	Aspartic-Acid	Molecule 30
31	Aurantiamide	Molecule 31
32	Azadirachtanin	Molecule 32
33	Azadirachtin	Molecule 33
34	Azadirachtol	Molecule 34
35	Azadiradione	Molecule 35
36	Azadirone	Molecule 36
37	Behenic-Acid	Molecule 37

38	Beta-Amyrin	Molecule 38
39	Beta-Amyrin-Acetate	Molecule 39
40	Beta-Carotene	Molecule 40
41	Beta-Caryophyllene	Molecule 41
42	Beta-Elemene	Molecule 42
43	Beta-Ionone	Molecule 43
44	Betanin	Molecule 44
45	Beta-Pinene	Molecule 45
46	Beta-Sitosterol	Molecule 46
47	Beta-Sitosterol-Beta-D-Glucoside	Molecule 47
48	Betulin	Molecule 48
49	Betulinic-Acid	Molecule 49
50	Campesterol	Molecule 50
51	Carotenoids	Molecule 51
52	Carvacrol	Molecule 52
53	Cellobiose	Molecule 53
54	Chlorogenic-Acid	Molecule 54
55	Cinnamaldehyde	Molecule 55
56	Citric-Acid	Molecule 56
57	Cysteine	Molecule 57
58	Cystine	Molecule 58
59	D-Arabinose	Molecule 59
60	Daucosterol	Molecule 60
61	Dehydroascorbic-Acid	Molecule 61
62	Delphinidin-3-Arabinoside	Molecule 62
63	Desacetylnimbin	Molecule 63
64	D-Galactose	Molecule 64
65	D-Glucose	Molecule 65
66	Dipentene	Molecule 66
67	Ducheside-A	Molecule 67
68	D-Xylose	Molecule 68
69	Ellagic-Acid	Molecule 69
70	Epoxyazadiradione	Molecule 70
71	Ethyl-Cinnamate	Molecule 71
72	Euscaphic-Acid	Molecule 72
73	Exiguaflavone-A	Molecule 73
74	Exiguaflavone-B	Molecule 74
75	Fructose	Molecule 75
76	Furfural	Molecule 76
77	Furfurol	Molecule 77
78	Galactose	Molecule 78
79	Galacturonic-Acid	Molecule 79
80	Gallic-Acid	Molecule 80
81	Gamma-Cadinene	Molecule 81
82	Gamma-Ionone	Molecule 82
83	Gamma-Sitosterol-Acetate	Molecule 83
84	Gedunin	Molecule 84

85	Geraniol	Molecule 85
86	Glutamic-Acid	Molecule 86
87	Glutaric-Acid	Molecule 87
88	Glyoxylic-Acid	Molecule 88
89	Histamine	Molecule 89
90	Histidine	Molecule 90
91	Hordenine	Molecule 91
92	Hydrocyanic-Acid	Molecule 92
93	Hyperoside	Molecule 93
94	Indicaxanthin	Molecule 94
95	Isoazadirolide	Molecule 95
96	Isobetanin	Molecule 96
97	Isoleucine	Molecule 97
98	Isomaltol	Molecule 98
99	Isomaltose	Molecule 99
100	Isomangiferolic-Acid	Molecule 100
101	Isonimbinolide	Molecule 101
102	Isoorientin	Molecule 102
103	Isorhamnetin	Molecule 103
104	Isovitexin	Molecule 104
105	Kaempferitrin	Molecule 105
106	Kaempferol	Molecule 106
107	L-(+)-Tartaric-Acid	Molecule 107
108	Lactic-Acid	Molecule 108
109	Lagerine	Molecule 109
110	Lauric-Acid	Molecule 110
111	Leucine	Molecule 111
112	Lignoceric-Acid	Molecule 112
113	Limonene	Molecule 113
114	Linalool	Molecule 114
115	Lupeol	Molecule 115
116	Luteolin	Molecule 116
117	Lysine	Molecule 117
118	Maackiain	Molecule 118
119	Malic-Acid	Molecule 119
120	Malonic Acid	Molecule 121
121	Mangiferic-Acid	Molecule 122
122	Mangiferol	Molecule 123
123	Mangiferolic Acid	Molecule 124
124	Mangiferonic Acid	Molecule 125
125	Mannose	Molecule 126
126	Meldenin	Molecule 127
127	Meliantriol	Molecule 128
128	Mescaline	Molecule 129
129	Methional	Molecule 130
130	Methionine	Molecule 131
131	Methyleneglutamic-Acid	Molecule 132

132	Methyleneglutamine	Molecule 133
133	Methyl-Furan	Molecule 134
134	Methyl-Gallate	Molecule 135
135	Methyl-Glutamic-Acid	Molecule 136
136	Methyl Salicylate	Molecule 137
137	Myrcene	Molecule 138
138	Myricetin	Molecule 139
139	Myristic-Acid	Molecule 140
140	Neo-Beta-Carotene-U	Molecule 141
141	Neobetanin	Molecule 142
142	Nerol	Molecule 143
143	Neryl-Acetate	Molecule 144
144	Niacin	Molecule 145
145	Nimbaflavone	Molecule 146
146	Nimbandiol	Molecule 147
147	Nimbin	Molecule 148
148	Nimbinene	Molecule 149
149	Nimbinin	Molecule 150
150	Nimbinone	Molecule 151
151	Nimbiol	Molecule 152
152	Nimbione	Molecule 153
153	Nimbocinolide	Molecule 154
154	Nimbolide	Molecule 155
155	Nimbolin-A	Molecule 156
156	Nimbolin-B	Molecule 157
157	Nimbosterol	Molecule 158
158	Nimocinol	Molecule 159
159	Nimolicinol	Molecule 160
160	Nimolinone	Molecule 161
161	N-Methylpyrrole	Molecule 162
162	N-Methyl-Tyramine	Molecule 163
163	Oleanolic-Acid	Molecule 164
164	Oleic-Acid	Molecule 165
165	Orientin	Molecule 166
166	Ovatodiolide	Molecule 167
167	Oxaloacetic-Acid	Molecule 168
168	Palmitic-Acid	Molecule 169
169	Palmitoleic-Acid	Molecule 170
170	Pantothenic-Acid	Molecule 171
171	P-Coumaric-Acid	Molecule 172
172	P-Cresol	Molecule 173
173	Pectin	Molecule 174
174	Penduletin	Molecule 175
175	Pentosan	Molecule 176
176	Petunidin-3-Arabinoside	Molecule 177
177	Phenyl-Acetaldehyde	Molecule 178
178	Phenylalanine	Molecule 179

179	Phorbic-Acid	Molecule 180
180	Phytin	Molecule 181
181	Pipecolinic-Acid	Molecule 182
182	Piperitone	Molecule 183
183	Piscidic-Acid	Molecule 184
184	Plucheoside A	Molecule 185
185	Pomolic-Acid	Molecule 186
186	Proline	Molecule 187
187	Pufa	Molecule 188
188	Pyrazines	Molecule 189
189	Pyridoxine	Molecule 190
190	Pyrrole	Molecule 191
191	Quebrachitol	Molecule 192
192	Quercetin	Molecule 193
193	Quercitrin	Molecule 194
194	Quinic-Acid	Molecule 195
195	Quisqualic-Acid	Molecule 196
196	Raffinose	Molecule 197
197	Rhamnose	Molecule 198
198	Riboflavin	Molecule 199
199	Rutin	Molecule 200
200	Safrole	Molecule 201
201	Salannin	Molecule 202
202	Salannolide	Molecule 203
203	Scillarenin	Molecule 204
204	Scilliglaucoside	Molecule 205
205	Scopoletin	Molecule 206
206	Shikimic-Acid	Molecule 207
207	Sorbose	Molecule 208
208	Stearic-Acid	Molecule 209
209	Steroids	Molecule 210
210	Stigmast-7-En-3-Beta-Ol	Molecule 211
211	Stigmasterol	Molecule 213
212	Succinic-Acid	Molecule 214
213	Succinimide	Molecule 215
214	Sugiol	Molecule 216
215	Tamarindienal	Molecule 217
216	Taraxasteryl-Acetate	Molecule 218
217	Tartaric-Acid	Molecule 219
218	Tectoquinone	Molecule 220
219	Terpinen-4-Ol	Molecule 221
220	Thiamin	Molecule 222
221	Threonine	Molecule 223
222	Tocopherol	Molecule 224
223	Triacetonamine	Molecule 225
224	Trigonelline	Molecule 226
225	Tryptophan	Molecule 227

226	Tylophorine	Molecule 228
227	Tyramine	Molecule 229
228	Uronic-Acid	Molecule 230
229	Ursolic-Acid	Molecule 231
230	Vepinin	Molecule 232
231	Vit-D	Molecule 233
232	Vitexin	Molecule 234
233	Xanthoxylin	Molecule 235
234	Xylose	Molecule 236

Bioavailabity Prediction of secondary metabolites of P. indica

Bioavailability is an important parameter for determining the amount and level of drug absorption in the body (Labibah and Rusdiana, 2022). Therefore, determining bioavailability is more important than stating whether a compound has medicinal potential. Bioavailability prediction was carried out using the SwissADME web server with the Boiled-EGG method (brain or intestinal estimated permeation method). This method was proposed as an accurate prediction model that accounts for the lipophilicity and polarity of small molecules (García-Beltrán *et al*., 2023; Panova *et al*., 2023). The Boiled-EGG model provides a rapid, intuitive, easily reproducible, yet statistically unprecedented powerful method for predicting passive gastrointestinal absorption and brain access of small molecules useful for drug discovery and development (Feng *et al*., 2020; Naveed *et al*., 2023). This method uses an image model (**Figure 1**) to classify compound absorption. On the other hand, the yolk area demonstrates the potential of compounds like Lagerine, Meliantriol, Neo-Beta-Carotene-U to cross the blood-brain barrier, as indicated by their wLogP and TPSA values that describe their lipophilicity and polarity (Daina and Zoete, 2016). Out of a total of 234 compounds, 126 have been found to have high bioavailability, while 109 compounds have been shown to have low bioavailability. (**Table 2**).

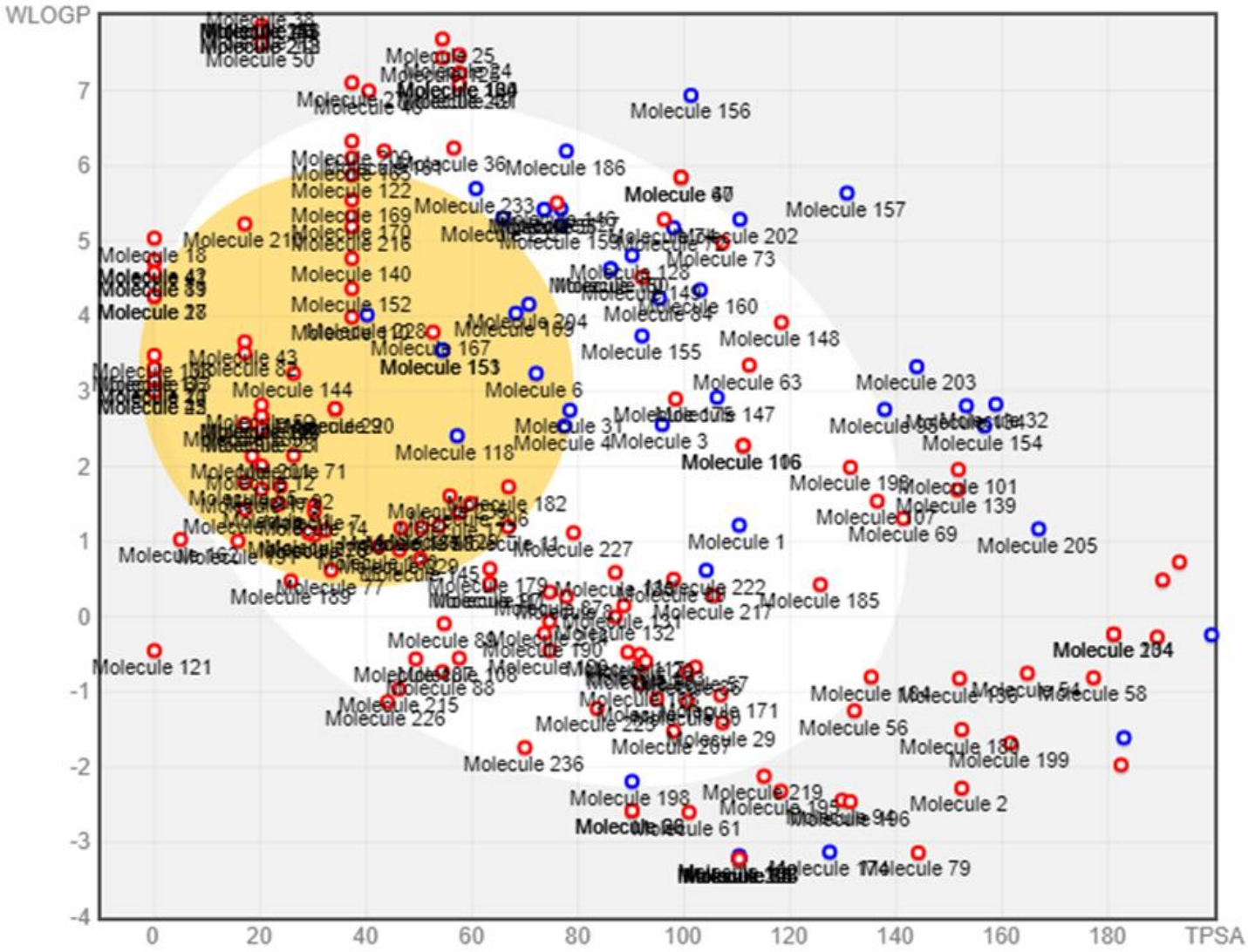

Figure 1. Bioavailability prediction of of the secondary metabolite of *P. indica* using BOILED-Egg method.

Table 2. Bioavailability prediction of the secondary metabolite of *P. indica* using BOILED-Egg

No.	Bioavailability Prediction	Total	Compound Code
1.	High	127	Mol 1, Mol 3, Mol 4, Mol 5, Mol 6, Mol 7, Mol 8, Mol 9, Mol 11, Mol 12, Mol 14, Mol 20, Mol 23, Mol 29, Mol 30, Mol 31, Mol 35, Mol 36, Mol 43, Mol 52, Mol 55, Mol 57, Mol 63, Mol 69, Mol 70, Mol 71, Mol 72, Mol 73, Mol 74, Mol 76, Mol 77, Mol 80, Mol 82, Mol 84, Mol 85, Mol 86, Mol 87, Mol 88, Mol 89, Mol 90, Mol 91, Mol 92, Mol 97, Mol 98, Mol 106, Mol 107, Mol 108, Mol 109, Mol 110, Mol 111, Mol 114, Mol 116, Mol 117, Mol 118, Mol 119, Mol 120, Mol 121, Mol 126, Mol 127, Mol 128, Mol 129, Mol 130, Mol 131, Mol 132, Mol 133, Mol 134, Mol 136, Mol 139, Mol 140, Mol 142, Mol 143, Mol 144, Mol 145, Mol 146, Mol 147, Mol 148, Mol 149, Mol 150, Mol 151, Mol 152, Mol 154, Mol 158, Mol 159, Mol 160, Mol 162, Mol 164, Mol 166, Mol 167, Mol 168, Mol 169, Mol 170, Mol 171, Mol 172, Mol 174, Mol 177, Mol 178, Mol 181, Mol 182, Mol 185, Mol 186, Mol 187, Mol 188, Mol 189, Mol 190, Mol 192, Mol 197, Mol 200, Mol 203, Mol 205, Mol 206, Mol 208, Mol 209, Mol 212, Mol 213, Mol 214, Mol 215, Mol 218, Mol 219, Mol 220, Mol 221, Mol 223, Mol 224, Mol 225, Mol 226, Mol 227, Mol 230, Mol 231, Mol 233, Mol 234
2.	Low	109	Mol 2, Mol 10, Mol 13, Mol 15, Mol 16, Mol 17, Mol 18, Mol 19, Mol 21, Mol 22, Mol 24, Mol 25, Mol 26, Mol 27, Mol 28, Mol 32, Mol 33, Mol 34, Mol 37, Mol 38, Mol 39, Mol 40, Mol 41, Mol 42, Mol 44, Mol 45, Mol 46, Mol 47, Mol 48, Mol 49, Mol 50, Mol 51, Mol 53, Mol 54, Mol 56, Mol 58, Mol 59, Mol 60, Mol 61, Mol 62, Mol 64, Mol 65, Mol 66, Mol 67, Mol 68, Mol 78, Mol 81, Mol 83, Mol 93, Mol 94, Mol 95, Mol 96, Mol 99, Mol 100, Mol 101, Mol 102, Mol 103, Mol 104, Mol 105, Mol 112, Mol 113, Mol 115, Mol 122, Mol 123, Mol 124, Mol 125, Mol 135, Mol 137, Mol 138, Mol 141, Mol 153, Mol 155, Mol 156, Mol 157, Mol 161, Mol 163, Mol 165, Mol 173, Mol 175, Mol 176, Mol 179, Mol 180, Mol 183, Mol 184, Mol 193, Mol 194, Mol 195, Mol 196, Mol 198, Mol 199, Mol 201, Mol 202, Mol 204, Mol 207, Mol 210, Mol 211, Mol 211, Mol 216, Mol 217, Mol 222, Mol 228, Mol 229, Mol 232

Immunomodulatory Proteins that Associated With Secondary Metabolites of P. indica

After obtaining the bioavailability prediction of each secondary metabolite compound of *P. indica*, the next step was target proteins prediction that can interact with the compounds carried out by SwissTargetPrediction (Lawal *et al*., 2021). The results show that 1,317 proteins were predicted to interact with secondary metabolites of *P. indica*. In order to obtain related proteins with immunomodulators, it was carried out by GeneCards (Safran *et al*., 2021). The results show that 1,340 related proteins were connected with immunomodulators.

Venny was used to find the intersection between secondary metabolites-linked and immunomodulator-linked proteins. Based on the interaction results, 161 immunomodulator-linked proteins were predicted for interaction with secondary metabolites of *P. indica*. From the interaction results, 304 immunomodulator-linked proteins were predicted to interact with secondary metabolites of *P. indica* (**Figure 2**).

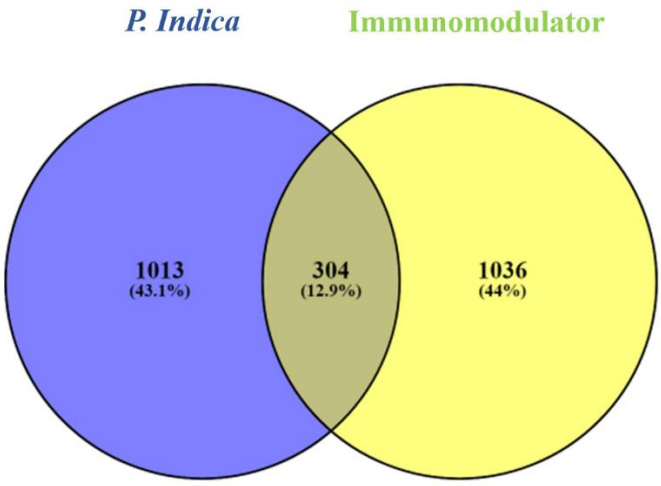

Figure 2. Venn diagram of protein that predicted linked with *P. indica* and immunomodulator-linked protein

Network Pharmacology Analysis

Protein obtained from the intersection Venn diagram was then analyzed using StringDB (**Figure 3**), which aims to make network interaction between selected proteins target. This step determines the connection between the selected protein and analyzes the biological pathways influenced by this protein (Veda *et al*., 2023). StringDB is a database with over nine million proteins that are known and predicted to integrate linkage functional data from various sources (Grabowski and Rappsilber, 2019; Jung *et al*., 2021). It delivers a very easy and fast way to see groups of related genes/proteins functionally (Grabowski and Rappsilber, 2019)

After that, KEGG enrichment analysis was carried out. From the analysis results, the pathways associated with the immunomodulator were searched, and five pathways were selected with the highest strength (Veda *et al*., 2023). KEGG (Kyoto Encyclopedia of Genes and Genomes) was used for bioinformatics research and education in drug development (Hehenberger, 2020) KEGG is a collection of pathway maps drawn manually, representing our knowledge about molecular interaction (Kanehisa *et al*., 2023).

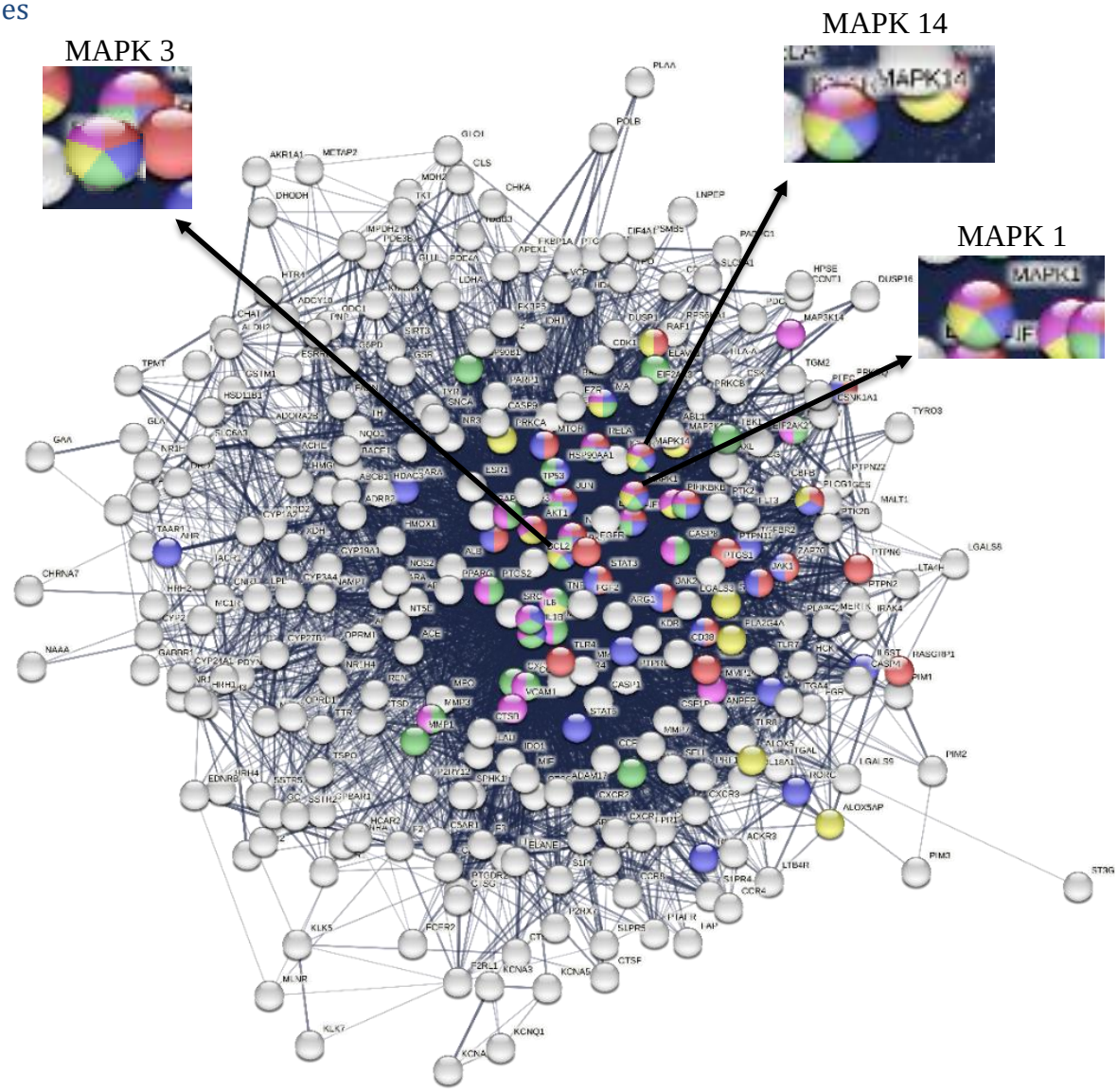

Figure 3. Network pharmacology prediction results using StringDB

Table 3. Immunomodulator related pathway with KEGG enrichment

No.	Pathways	Strength
1.	PD-L1 expression and PD-1 checkpoint pathway in cancer	1.31
2.	Th17 cell differentiation	1.3
3.	IL-17 signaling pathway	1.27
4.	Fc epsilon RI signaling pathway	1.18
5.	TNF signaling pathway	1.17

Based on KEGG pathway analysis, we obtained three proteins related to five immunomodulator-related pathways: MAPK1, MAPK3, and MAPK14. Then, we look for which compounds are predicted to interact with the target protein (**Table 4**). Some compounds can interact with more than one target protein. There are four compounds (mol 109, mol 127, mol 140, and mol 142) that can interact with MAPK1 and MAPK 3, 12 compounds (mol 5, mol 35, mol 36. Mol 43, mol 70, mol 84, mol 114, mol 129, mol 139, mol 149, mol 158, mol 230) between MAPK 1 and 14, and there are one compound (mol 85) between MAPK 3 and MAPK 14.

Table 4. A list of secondary metabolites can interact with protein that five of immunomodulator-related pathways

Proteins	Compound Code
MAPK1	Mol 4, mol 5, mol 23, mol 31, mol 35, mol 36, mol 43, mol 70, mol 74, mol 84, mol 109, mol 110, mol 114, mol 127, mol 129, mol 139, mol 140, mol 142, mol 149, mol 154, mol 158, mol 168, mol 171, mol 203, mol 215, mol 230
MAPK3	Mol 82, mol 85, mol 109, mol 121, mol 127, mol 140, mol142, mol 150, mol 164, mol 169, mol 182, mol 206
MAPK14	Mol 5, mol 35, mol 36, mol 43, mol 70, mol 71, mol 84, mol 85, mol 114, mol 126, mol 129, mol 139, mol 143, mol 147, mol 148, mol 149, mol 158, mol 160, mol 166, mol 168, mol 177, mol 181, mol 200, mol 230

Conclusion

According to the results of network pharmacology analysis conducted on beluntas (Pluchea Indica), it has been found that 17 different compounds play a significant role in the immune system. This is due to the fact that these compounds have been shown to interact with two proteins that are related to immunomodulatory pathways.

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