

XXX Symposium on
Bioinformatics and
Computer-Aided Drug
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Oral Presentation



NETWORK PHARMACOLOGY REVEALED THE POTENTIAL OF BITTER HONEY IN SUPPRESSION OF CEREBRAL MALARIA-INDUCED INFLAMMASOME

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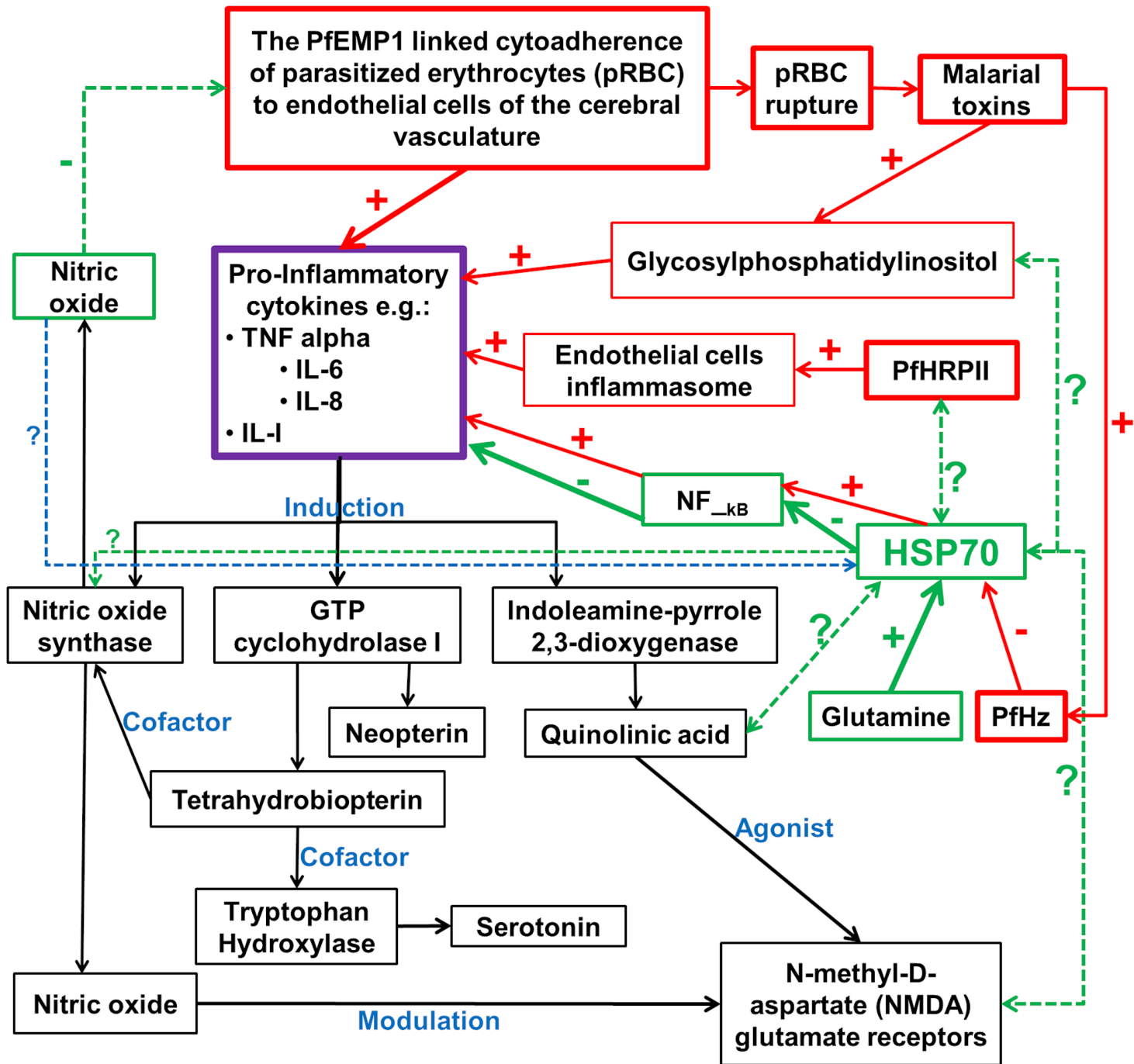


INTRODUCTION

- Human *Plasmodium falciparum* malaria, is one of the world's leading causes of death
- Cerebral malaria (CM) is a fatal complication of *P. falciparum* infection., known to be associated with permanent disabilities and/or deaths, especially among children

INTRODUCTION

- CM is associated with functional interplay between many molecular entities, including neurotransmitters and chaperones (Daniyan et al., 2022)
- The biological and physiological links between CM, inflammation, and inflammasome, testifies to the complexity of its pathology



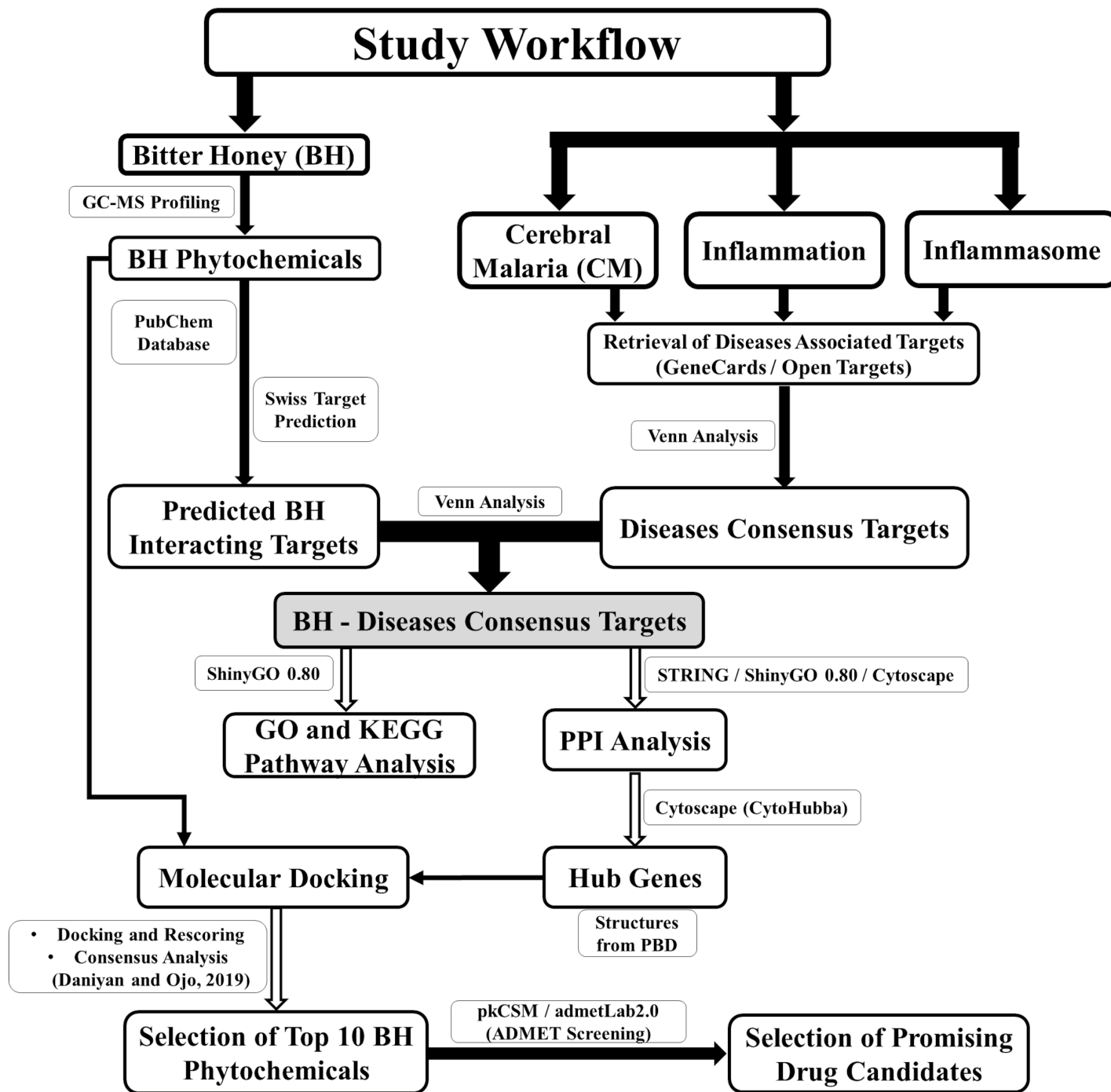
INTRODUCTION

- Natural Products are gaining improved patronage, potential due to:
 - Resistance to available and affordable drugs
 - Worsening economic crisis
 - Affordability and availability
 - Renewed interest in the integration of traditional with orthodox medicine
- Bitter Honey is one of the focal point due to its inherent biological properties

INTRODUCTION

- Previous work from our teams on the medicinal properties of bitter honey has established the following:
- Botanical and bioactive markers,
 - Inhibitory effects on pancreatic alpha-amylase activity
 - Anti-dyslipidaemia, cardio-protective, and ameliorative effects on hepatorenal damage in experimental diabetic rats.

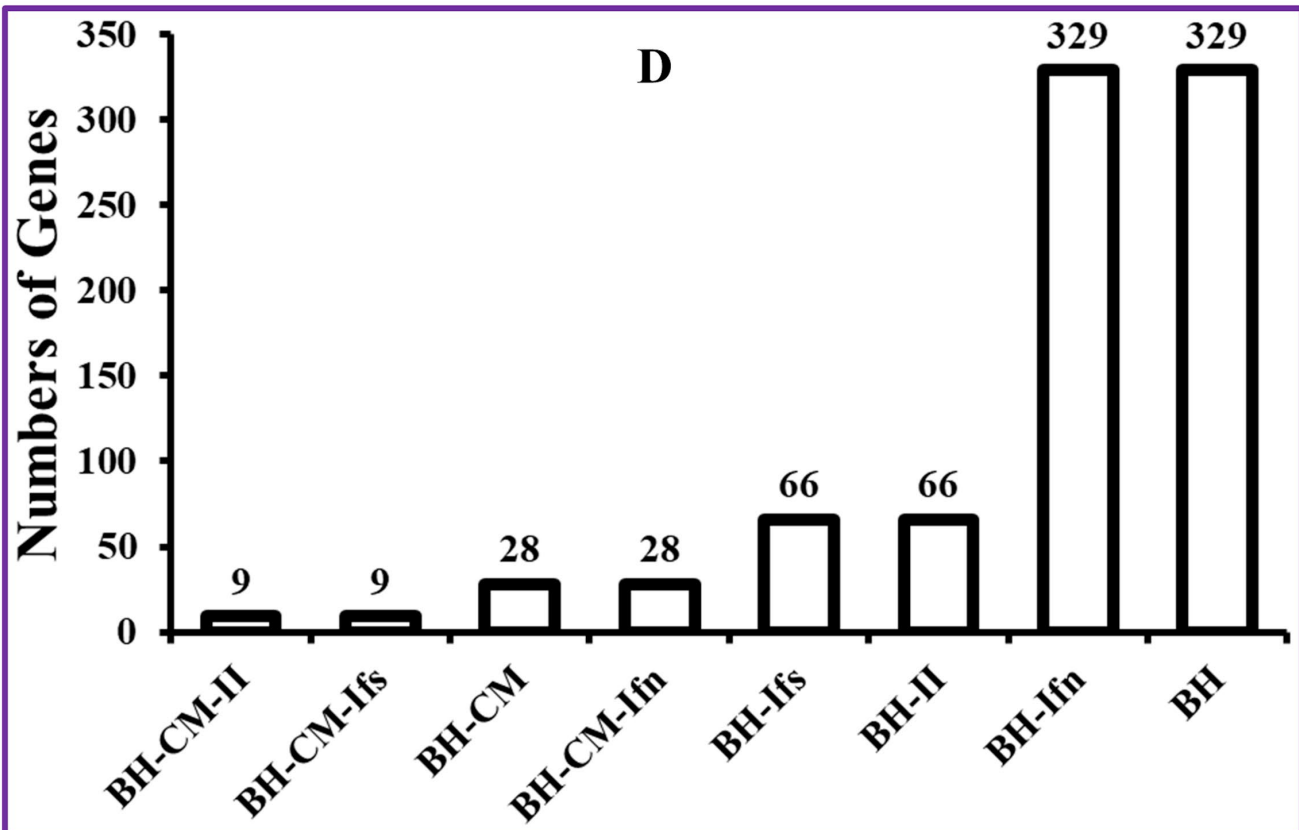
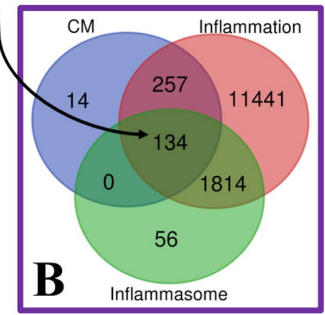
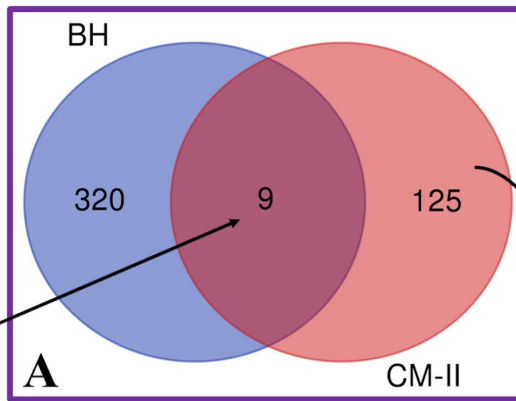
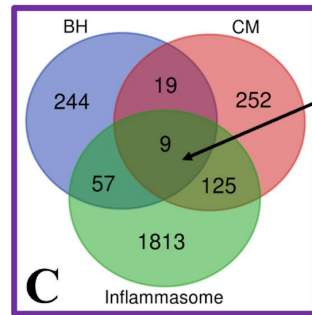
(Adeoye et al., 2022b, 2022a, 2022c, 2023)



RESULTS AND DISCUSSION

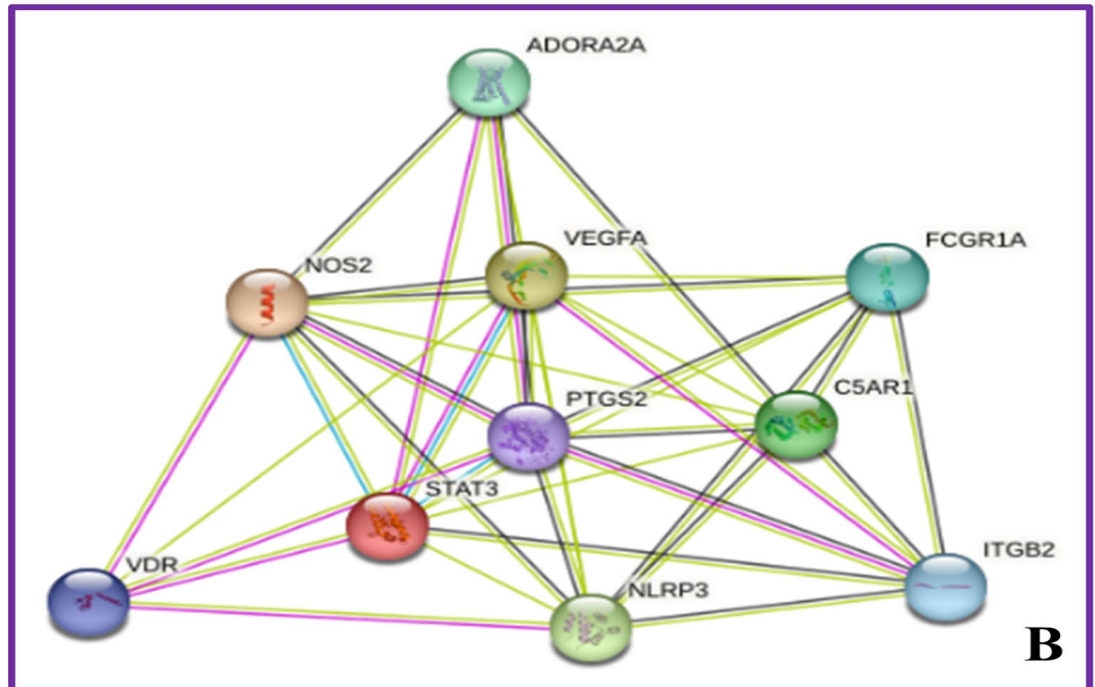
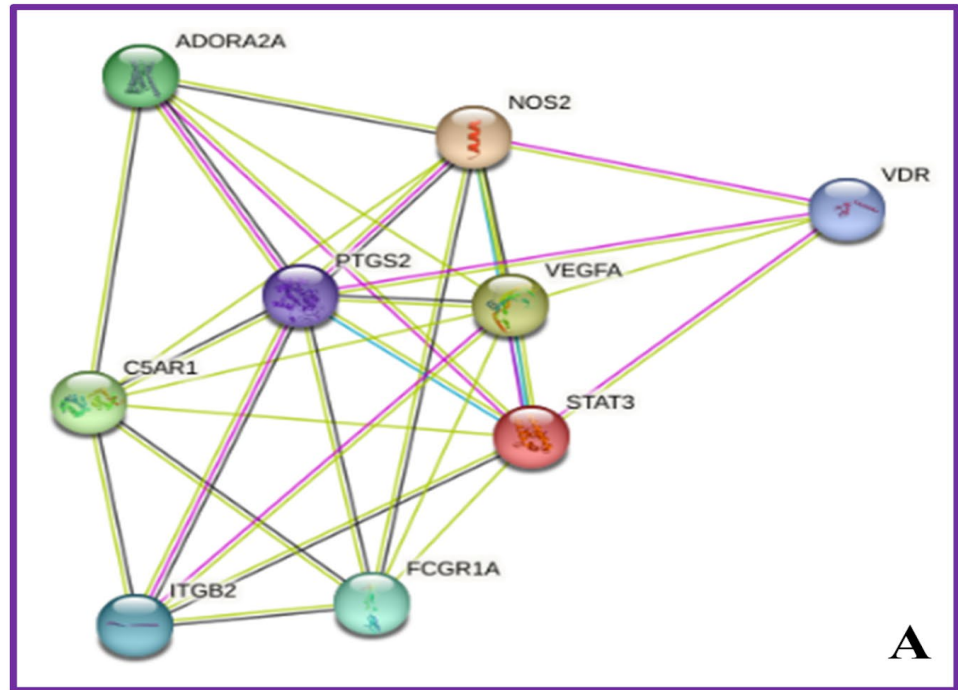
Comparative analysis of Target Genes associated with BH Phytochemicals

- BH – Bitter Honey Phytochemicals
- CM – Cerebral Malaria
- Ifs – Inflammasome
- Ifn – Inflammation
- If – Ifn-Ifs



Protein-Protein Interaction Analysis

- NLRP3 was included as functional control
- Interactions are both functional and physical
- The PPI Interactions:
 - 29 ($p = 2.64 \times 10^{-8}$)
 - 38 with NLRP3 ($p = 1.05 \times 10^{-11}$)



Frequencies of occurrence in biological functions with or without NLRP3

Genes	Numerical Frequencies				Percentage of Occurrence			
	Without NLRP3		With NLRP3		Without NLRP3		With NLRP3	
	All	Similar	All	Similar	All	Similar	All	Similar
BIOLOGICAL FUNCTIONS								
ADORA2A	79	70	84	70	54.11	52.24	51.85	52.24
C5AR1	88	81	100	81	60.27	60.45	61.73	60.45
FCGR1A	40	38	46	38	27.4	28.36	28.4	28.36
ITGB2	79	74	87	74	54.11	55.22	53.7	55.22
NOS2	67	65	79	65	45.89	48.51	48.77	48.51
PTGS2	104	95	112	95	71.23	70.9	69.14	70.9
STAT3	103	95	112	95	70.55	70.9	69.14	70.9
VEGFA	98	88	102	88	67.12	65.67	62.96	65.67
VDR	57	54	63	54	39.04	40.3	38.89	40.3
NLRP3*			87	58			53.7	43.28
TOTAL	146	134	162	134				
BF								
KEGG PATHWAYS								
	All	Best 20	All	Best 20	All	Best 20	All	Best 20
ADORA2A	7	2	7	2	7.07	10	7.07	10
C5AR1	6	3	6	3	6.06	15	6.06	15
FCGR1A	11	5	11	5	11.11	25	11.11	25
ITGB2	19	8	19	8	19.19	40	19.19	40
NOS2	19	7	19	7	19.19	35	19.19	35
PTGS2	21	7	21	7	21.21	35	21.21	35
STAT3	32	9	32	9	32.32	45	32.32	45
VDR	5	2	5	2	5.05	10	5.05	10
VEGFA	23	12	23	12	23.23	60	23.23	60
NLRP3*			5	1			5.21	5
TOTAL	96	20	96	20				
KEGG								

*Analysis of genes association with
Biological Functions and KEGG
Pathways with or without NLRP3*

Consensus Genes Association with Biological Functions			
Number of Consensus Genes	Number of Associated BF	Number of Associated BF with NLRP3 Association	% NLRP3 Association
9	8	8	100
8	14	11	78.57
7	13	8	61.54
6	15	8	53.33
5	17	10	58.82
4	23	8	34.78
3	21	4	19.05
2	23	0	0
Consensus Genes Association with KEGG Pathways			
Number of Consensus Genes	Number of Associated KEGG Pathways	Number of Associated KEGG Pathways with NLRP3 Association	% NLRP3 Association
4	3	0	0
3	9	0	0
2	20	2	10
1	64	3	4.69
0	0	6	0

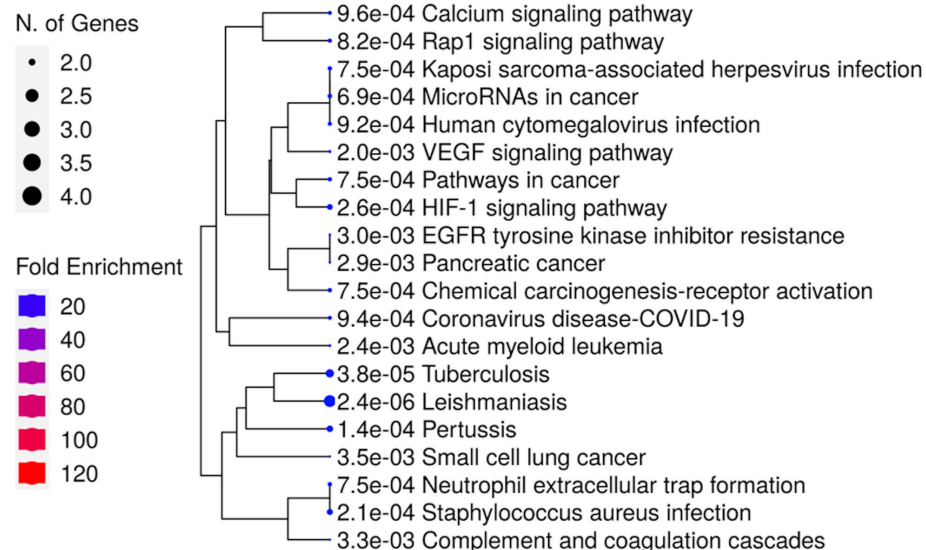
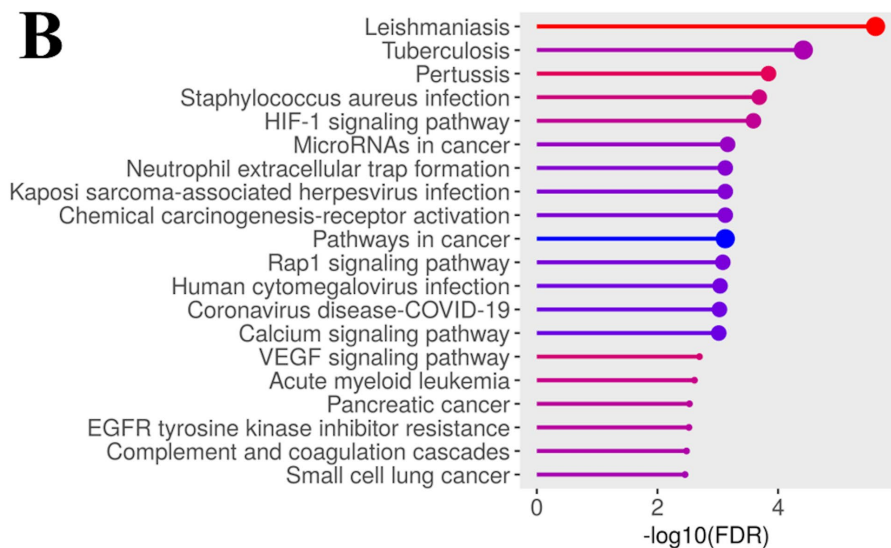
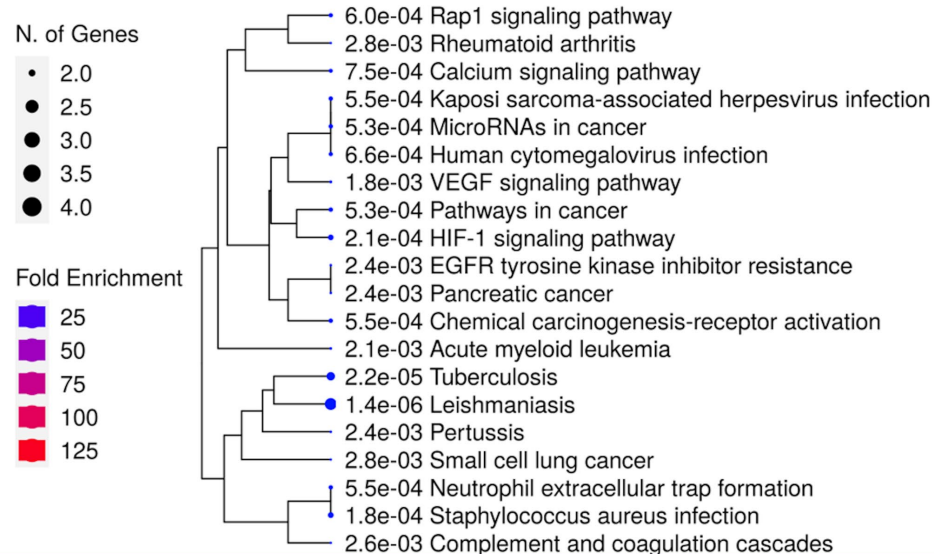
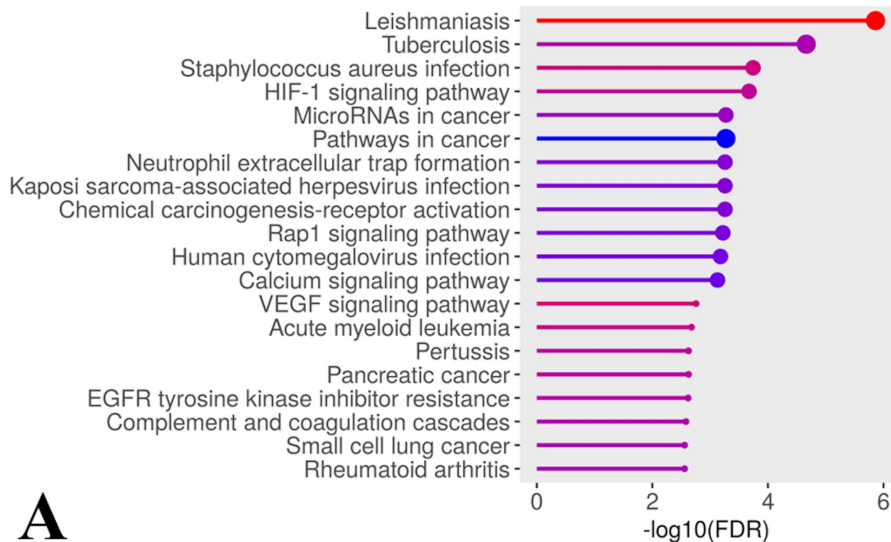
Therefore, it does appear that the presence of NLRP3 does not alter the target gene association in KEGG pathways.

Biological Functions that show association with all the consensus genes and NLRP3

S/No.	Biological Functions	Target Genes	P value	FDR
1	Positive regulation of the cellular process	ALL	1.25E-05	0.0029
2	Regulation of catalytic activity	ALL	6.06E-09	7.79E-05
3	Regulation of cellular metabolic process	ALL	3.42E-05	0.0055
4	Regulation of macromolecule metabolic process	ALL	4.35E-05	0.0065
5	Regulation of nitrogen compound metabolic process	ALL	1.88E-05	0.0040
6	Regulation of primary metabolic process	ALL	2.53E-05	0.0048
7	Response to organic substance	ALL	4.90E-08	0.00012
8	Signal transduction	ALL	3.73E-06	0.0013

ALL = ADORA2A, C5AR1, FCGR1A, ITGB2, NOS2, PTGS2, STAT3, VDR, VEGFA, and NLRP3

Enrichment Analysis and hierarchical clustering



Except for the replacement of Rheumatoid arthritis with COVID-19, the inclusion of NLRP3 did not significantly alter the KEGG enrichment

Network Analysis with Cytoscape (CytoHubba plugin)

- The consensus analysis of CytoHubba 12 scoring functions:
 - Identified 6 hub genes ranked (in parenthesis) as follows:
 - STAT3 (1), ITGB2 (2), FCGR1A (3), C5AR1 (4), PTGS2 (5), and NOS2 (6) when analyzed without NLRP3,
 - STAT3 (1), PTGS2 (2), C5AR1 (3), FCGR1A (4), NLRP3 (5), ITGB2 (6), and NOS2 (7) when analyzed with NLRP3

Identification of the most Promising BH Phytochemicals

- The top 10 phytochemicals by docking, rescoring and consensus analysis.

PHYTOCHEMICALS	ADORA2A	C5ARI	FCGRIA	ITGB2	NLRP3	NOS2	PTGS2	STAT3	VDR	VEGFA	Average	
											Score	Rank
Ergosta-5,22-dien-3-ol	1	1	1	3	1	2	3	2	6	2	2.2	1
Friedelan-3-one	4	2	2	1	2	4	1	3	7	1	2.6	2
Alpha-Amyrin	7	3	3	1	3	3	1	4	13	3	4.3	3
Aciphyllene	3	4	6	7	6	6	4	5	11	4	5.6	4
(E)-2-bromobutyloxychalcone	5	5	4	17	5	5	5	11	2	8	6.7	5
Beta-Guaiene	6	9	7	4	10	8	6	9	4	5	6.8	6
5-Acetamido-4,7-dioxo-4,7-dihydrobenzofurazan	15	10	5	4	8	11	8	6	7	6	8	7
Octahydronaphthalene	9	5	8	7	11	10	7	11	5	9	8.2	8
aR-Turmerone	7	7	9	9	8	9	9	14	10	7	8.9	9
1H-Cyclopropa[a]naphthalene	10	7	10	14	7	7	10	11	14	10	10	10
Control (Co-crystallized ligand)	2	25	19	12	4	1	25	1	1	24		

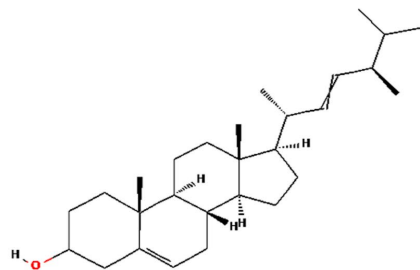
Identification of the most Promising BH Phytochemicals

- They showed good ADMET properties
- The top 3 (Ergosta-5,22-dien-3-ol, Friedelan-3-one, and Alpha-Amyrin) revealed a high probability for:
 - intestinal absorption,
 - CNS and BBB permeability,
 - very low potential for toxicity, and
 - shared similar structural backbones

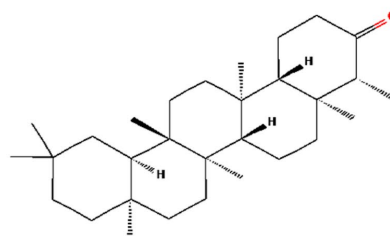
Identification of the most Promising BH Phytochemicals

Phytochemicals	Lipinski	Excretion	Pgn			Permeability				Toxicity			Rank
			INH	SUB	Caco2	I [#]	Skin	CNS	BBB	DILI	Ames	C*	
Ergosta-5,22-dien-3-ol	YES	Moderate	0.064	0.001	1.213	94.515	-2.819	-1.719	0.857	0.114	0.03	0.047	1
Friedelan-3-one	YES	High	0.086	0	1.236	97.452	-2.722	-1.471	0.484	0.022	0.038	0.012	2
Alpha-Amyrin	YES	High	0.181	0	1.327	94.156	-2.811	-1.809	0.762	0.012	0.011	0.017	3
Aciphyllene	YES	Moderate	0.2	0	1.408	94.084	-1.518	-2.182	0.884	0.394	0.015	0.602	4
(E)-2-bromobutyloxychalcone	YES	Low	0.991	0.001	1.288	93.522	-2.654	-1.382	0.109	0.745	0.653	0.744	5
Beta-Guaiene	YES	Moderate	0.922	0.001	1.416	94.704	-1.596	-2.149	0.377	0.729	0.011	0.583	6
5-Acetamido-4,7-dioxo-4,7-dihydrobenzofurazan	YES	Low	0.247	0.001	0.133	78.303	-2.88	-3.199	0.982	0.992	0.95	0.943	7
Octahydronaphthalene	YES	High	0.002	0	1.415	94.484	-1.434	-1.858	0.89	0.045	0.011	0.77	8
aR-Turmerone	YES	Moderate	0.766	0.002	1.667	95.352	-1.377	-1.707	0.202	0.259	0.015	0.475	9
1H-Cyclopropa[a]naphthalene	YES	Moderate	0.024	0.008	1.591	95.848	-1.728	-1.292	0.845	0.879	0.873	0.881	10

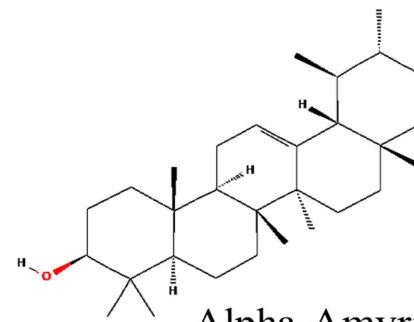
Pgn is P-glycoproteins INH is Inhibitor; SUB is Substrate; I[#] is Intestinal; CNS is Central Nervous System BBB is Blood Brain Barrier; DILI is Drug-induced Liver Injury; C* is Carcinogenicity. Pgn (INH and SUB), BBB, and Toxicity (DILI, Ames, and C*) are rated on a scale of probability of 0 (NO) to 1 (YES). Caco2 > 0.90, Intestinal > 90%, skin < -2.50, and CNS > -2 are regarded as great.



Ergosta-5,22-dien-3-ol

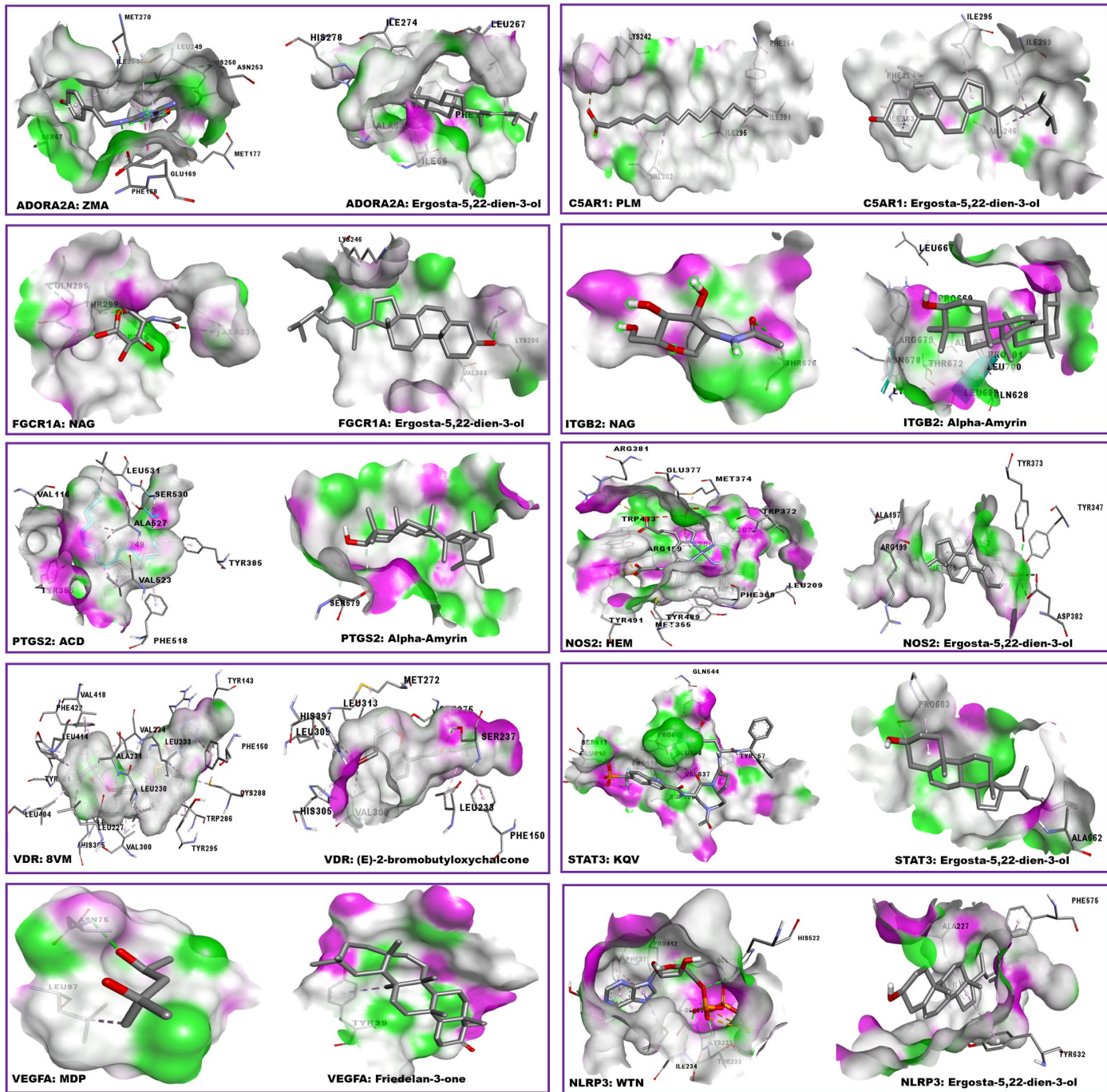


Friedelan-3-one



Alpha-Amyrin

The interaction analysis revealed that the best interaction with target proteins and NLRP3 are mainly with Ergosta-5,22-dien-3-ol, Friedelan-3-one, and Alpha-Amyrin



CONCLUSION

- The study has been able to:
 - Identified some biologically relevant phytochemicals from Bitter Honeys
 - Identified nine (9) BH-CM-II associated genes as potential targets of BH derived phytochemicals
 - Identified three (3) promising phytochemicals for further exploration and validation
 - Predict that BH has potential to suppress inflammasome-mediated cell death in cerebral malaria

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**THANK
YOU**