Progress in Microbes and Molecular Biology

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Supplementary materials

Table S1. Study procedures and assessments for baseline screening and 6-week intervention phase.

Procedure	*Intervention phase*						
	Week 0	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**
	Visit 1	**Visit 2**	**Visit 3**	**Online**	**Visit 4**	**Online**	**Visit 5**
Consent Form	x						
DSM-5 Mental State Examination	x	x	x	x	x	x	x
Patient Health Questionnaire PHQ-9	x						
Blood Investigations (Full Blood Counts and Thyroid Function Test)	x						
Beck Depression Inventory BDI	x	x	x	x	x	x	x
Patient Global Impression- Severity of Illness PGI-S	x	x	x	x	x	x	x
Patient Global Impression- Global Improvement PGI-I			x	x	x	x	x
Clinical Global Impression- Severity of Illness CGI-S	x	x	x		x		x
Clinical Global Impression- Global Improvement CGI-I			x		x		x
Pharmacovigilance Form			x	x	x	x	x

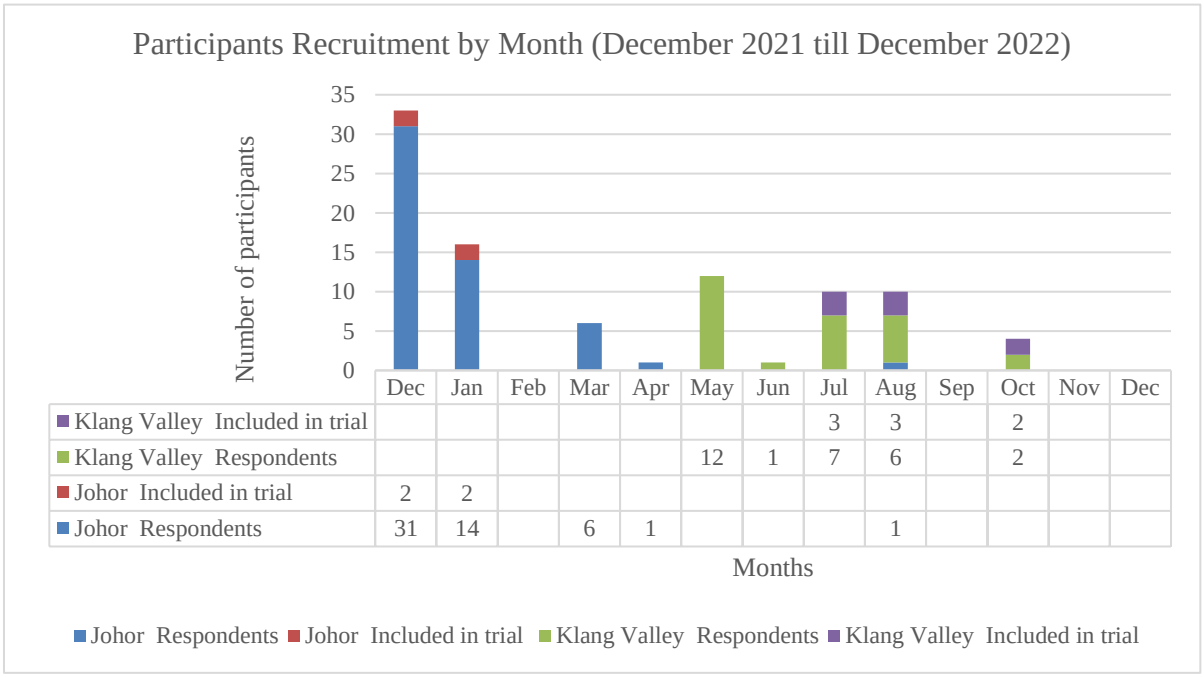

Figure S1. Participants recruitment by month (December 2021 till December 2022).

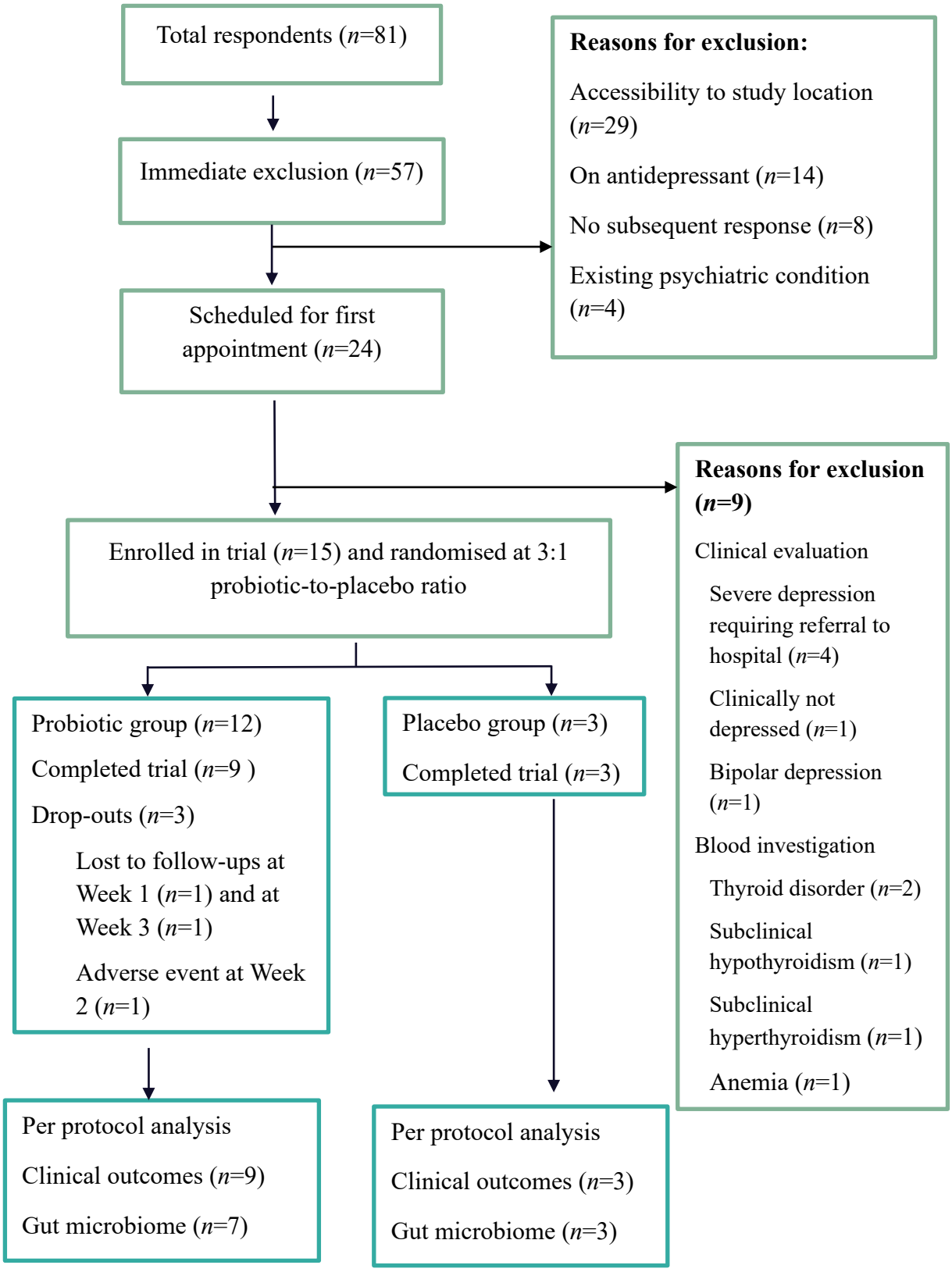

Figure S2. Patient recruitment flow (December 2021 till December 2022) showing recruitment stages, screening assessments, reasons for exclusions, and randomisation of included participants.

Table S2. Demographics and clinical profiles of participants in the probiotic (*n*=9) and placebo (*n*=3) groups.

	Probiotics (*n*=9)	Placebo (*n*=3)
Demographics		
Age, mean, range	32.33 (6.59), 23-42	28.66 (4.72), 25-34
Females, *n* (%)	8 (88.9)	2 (66.7)
Ethnicity, *n* (%)		
Malay	5 (55.6)	0
Chinese	2 (22.2)	3 (100)
Indian	2 (22.2)	0
Education, *n* (%)		
Less than high school	1 (11.1)	0
College/Diploma	1 (11.1)	0
Bachelor's degree	4 (44.4)	3 (100)
Master's Degree	3 (33.3)	0
Employment status, *n* (%)		
Employed full-time	6 (66.7)	1 (33.3)
Unemployed	1 (11.1)	0
Student	1 (11.1)	2 (66.7)
Self-employed	1 (11.1)	0
Non-psychiatric comorbidity, *n* (%)		
Nil	6 (66.7)	3 (100)
Hypertension, on oral medication	2 (22.2)	0
Endometriosis, on oral medication	1 (11.1)	0
Past psychiatric history, *n* (%)		
Nil	4 (44.4)	3 (100)
Previously diagnosed with MDD, on oral medication	3 (33.3)	0

	Probiotics (*n*=9)	Placebo (*n*=3)
Previously diagnosed with GAD, not on medication	1 (11.1)	0
Previously diagnosed with MDD and GAD, on oral medication	1 (11.1)	0
Regular diet, *n* (%)		
Malay cuisine	4 (44.4)	0
Ketogenic	1 (11.1)	0
No specific pattern	4 (44.4)	3 (100)
Exercise pattern, *n* (%)		
Exercise occasionally	5 (55.6)	1 (33.3)
Exercise regularly	1 (11.1)	2 (66.7)
Sedentary	3 (33.3)	0
Clinical measures, mean score		
PHQ-9	13.44 (6.26)	7.66 (2.08)
BDI (Total scores)	26.11 11.83)	13.66 (3.78)

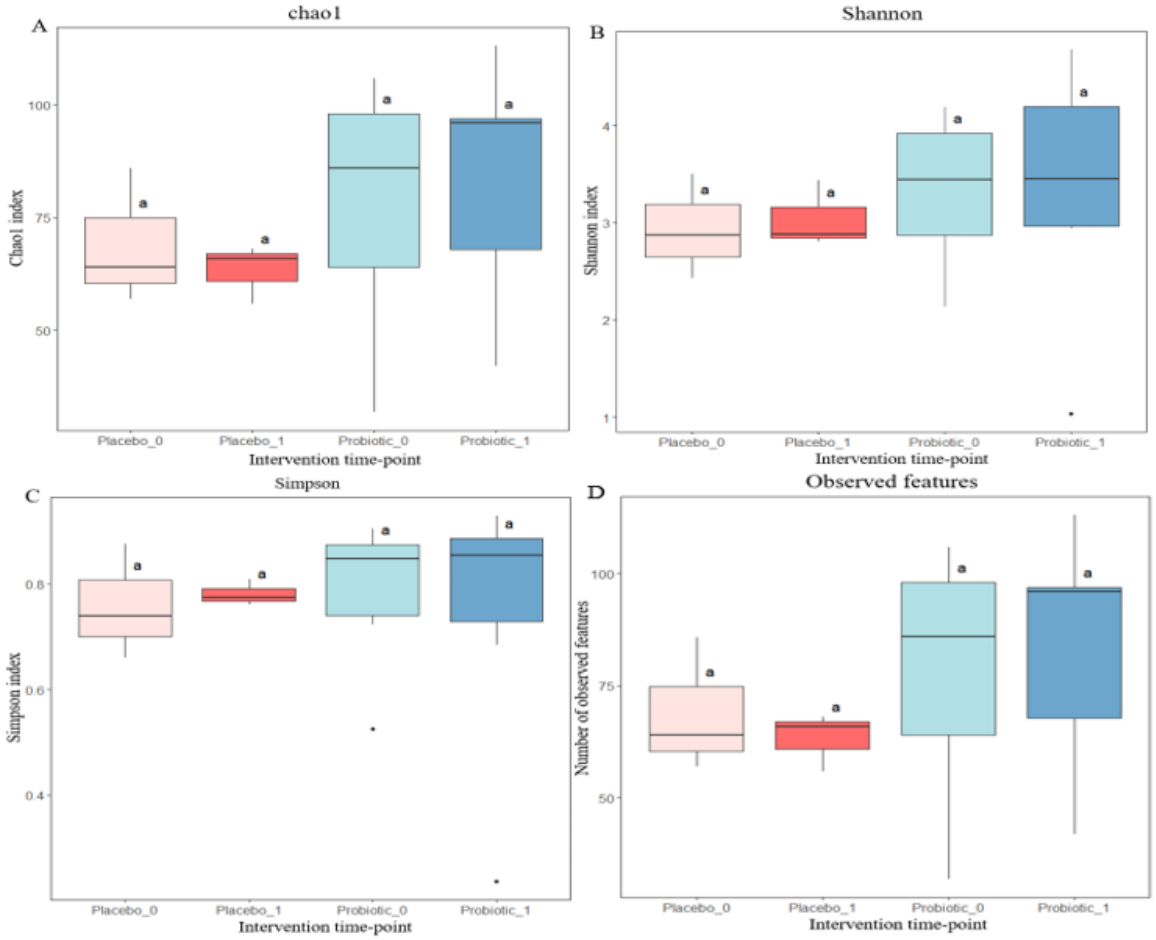

Figure S3. Box and whisker plots of alpha diversity indices showing the abundance and consistency before (_0) and after (_1) intervention within and between probiotics (*n*=7) and placebo (*n*=3) groups.

(A) The Chao-1 index reflects the amplicon sequence variants (ASV) abundance in samples, (B) Shannon and (C) Simpson indices reflect the diversity of ASV in samples, and (D) Observed features represent the total number of unique ASVs present in the samples. The greater the Chao, the higher the expected species richness of the microbiota; the smaller the Simpson index, the higher the diversity of the microbiota, and the greater the Shannon index, the higher the microbiota diversity. Whiskers represent the lowest and highest values within 1.5 times the IQR from the first and third quartiles, respectively. The superscript letter "a" indicates that groups sharing the same letter do not exhibit a statistically significant difference.

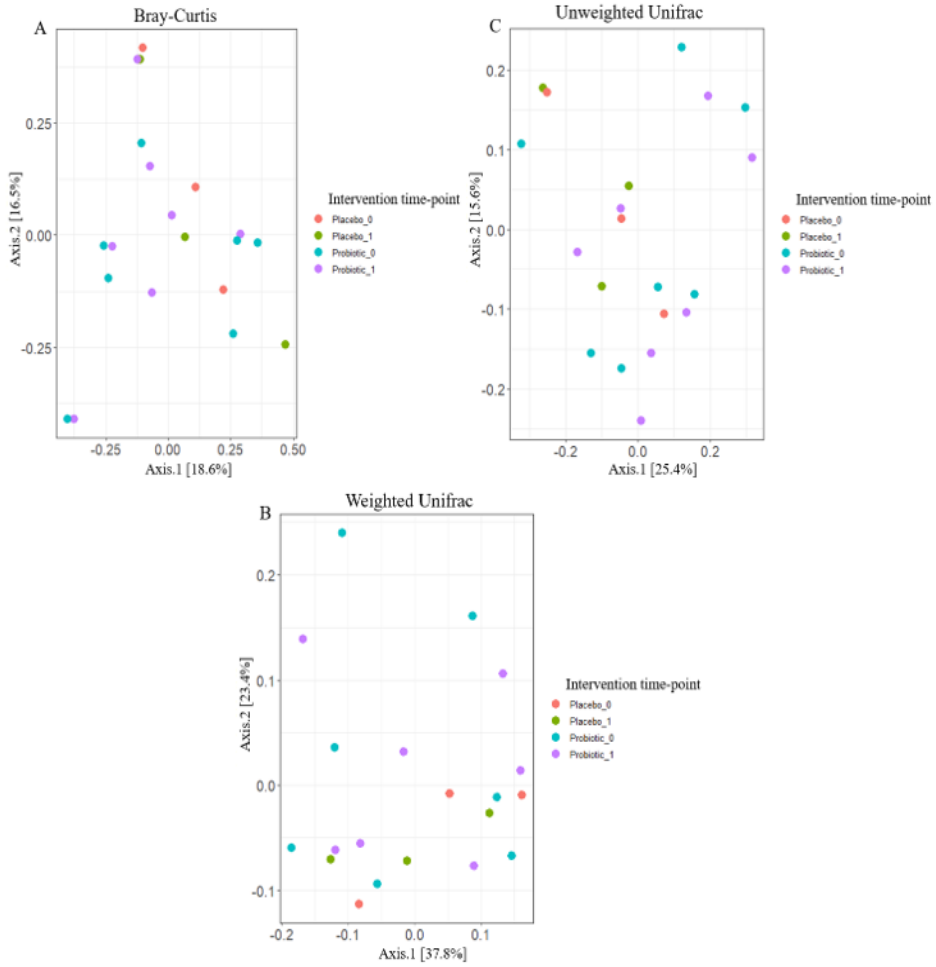

Figure S4. Principal Coordinate Analysis (PCoA) plots visualising the dissimilarity between the samples before (_0) and after intervention (_1) based on the distance matrix using the (A) Bray-Curtis, (B) Weighted Unifrac and (C) Unweighted Unifrac measures. The percentage of diversity explained by each coordinate is indicated in the axis.

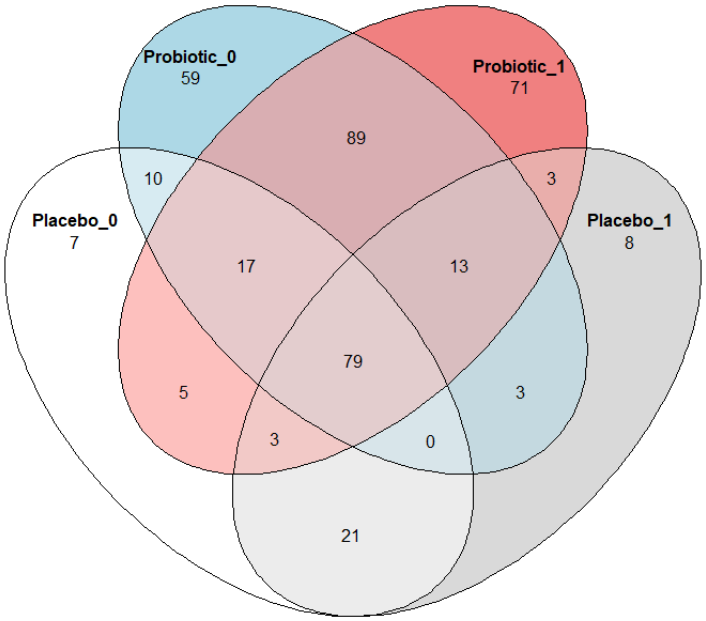

Figure S5. The Venn diagram shows the number of common and unique amplicon sequence variants (ASV) before (_0) and after (_1) intervention within and between probiotics (*n*=7) and placebo (*n*=3) groups.