

## Supplementary data

# Characterization of Metabolites in Plasma, Urine and Feces of Healthy Participants After Taking Brahmi Essence for Twelve Weeks Using LC-ESI-QTOF-MS metabolomic approach

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**Figure S2.** Scheme of sampling time points of biological samples throughout the study.

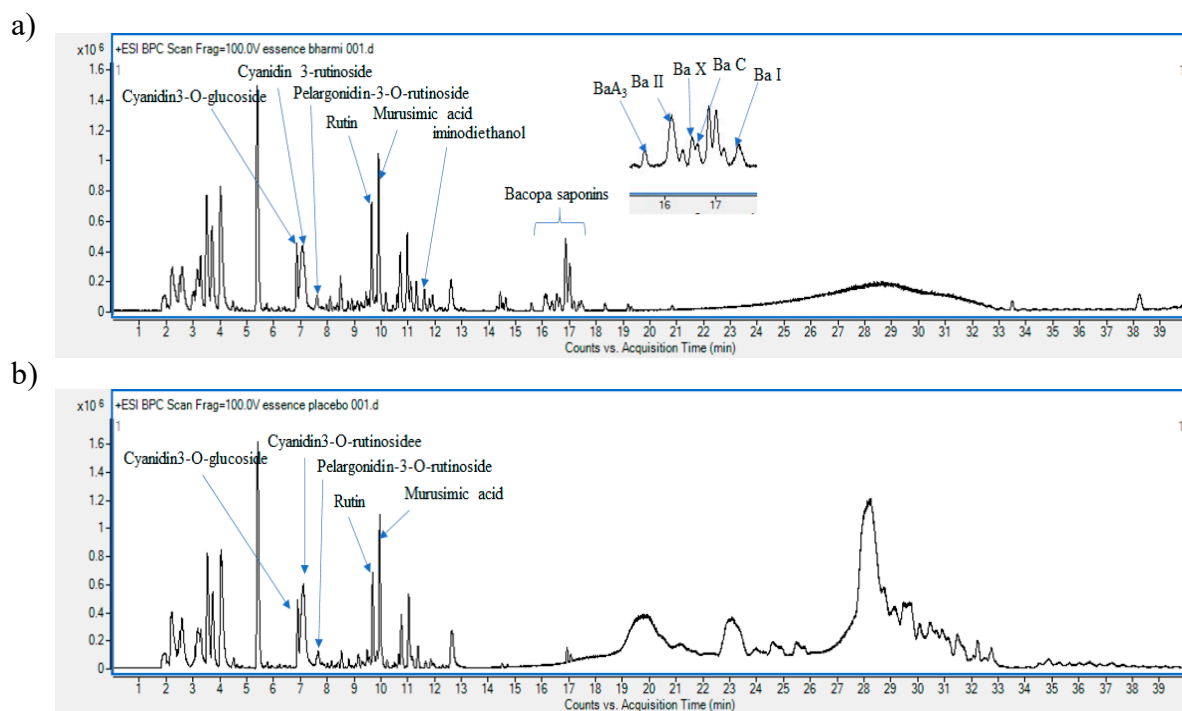
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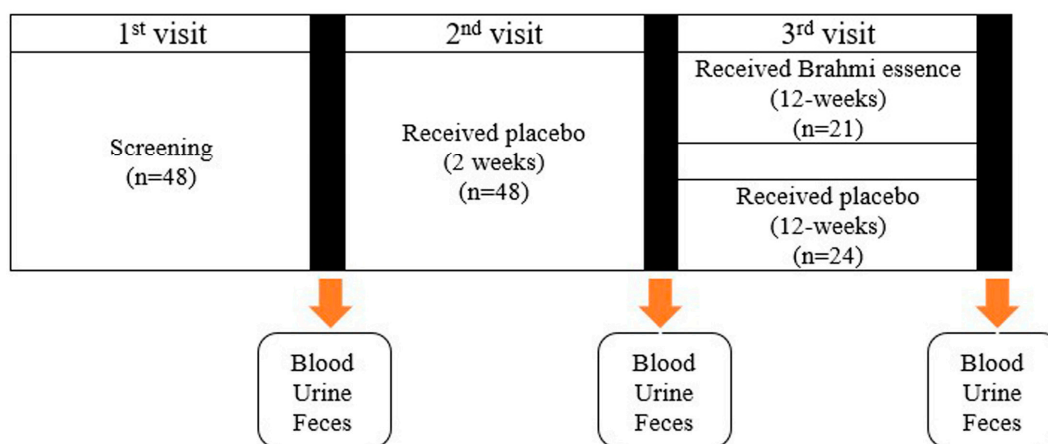
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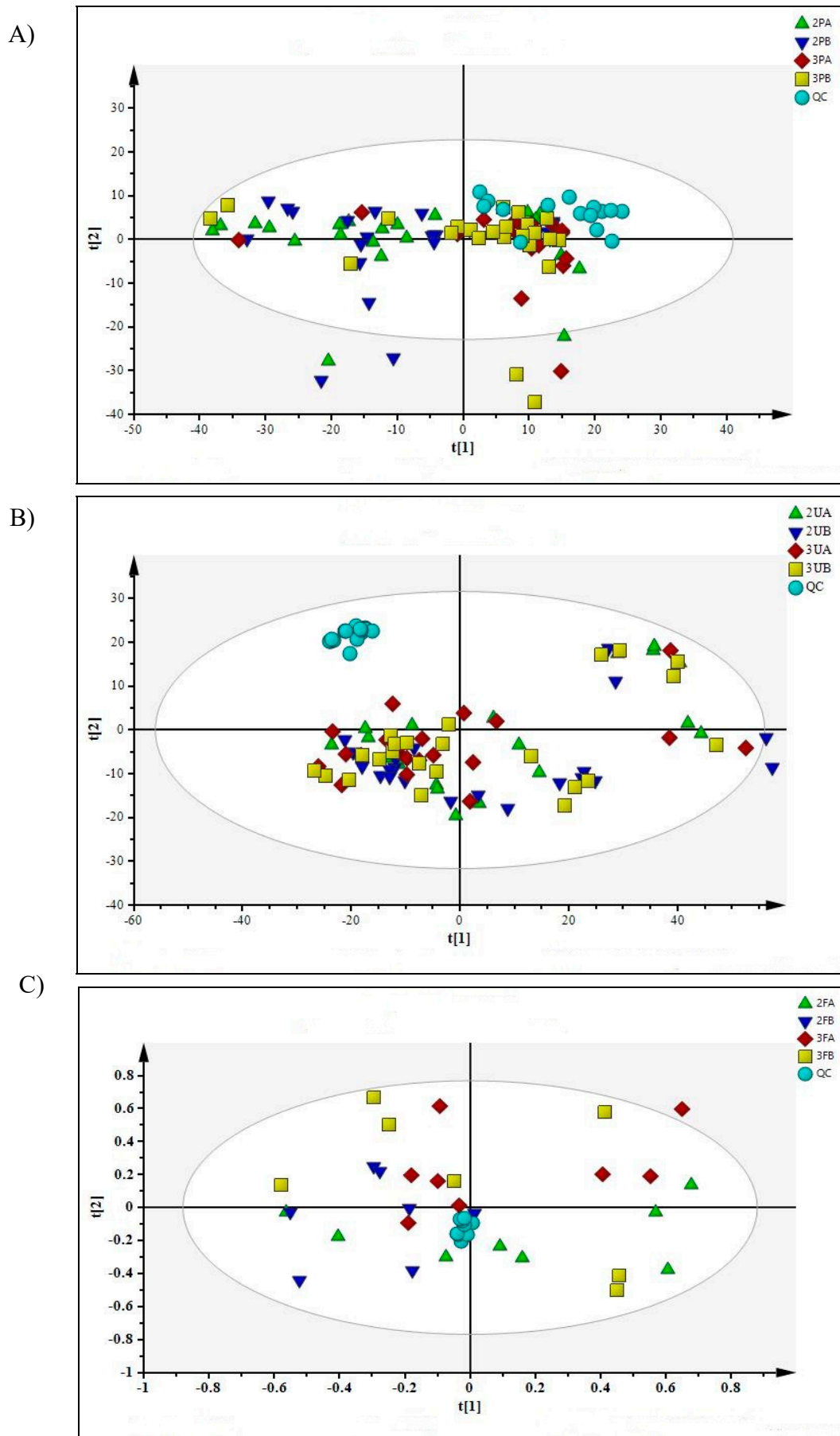
**Table S3.** p-value of metabolic pathway from enrichment analysis by using Metaboanalyst (a) plasma, (b) Urine, (c) feces Samples.



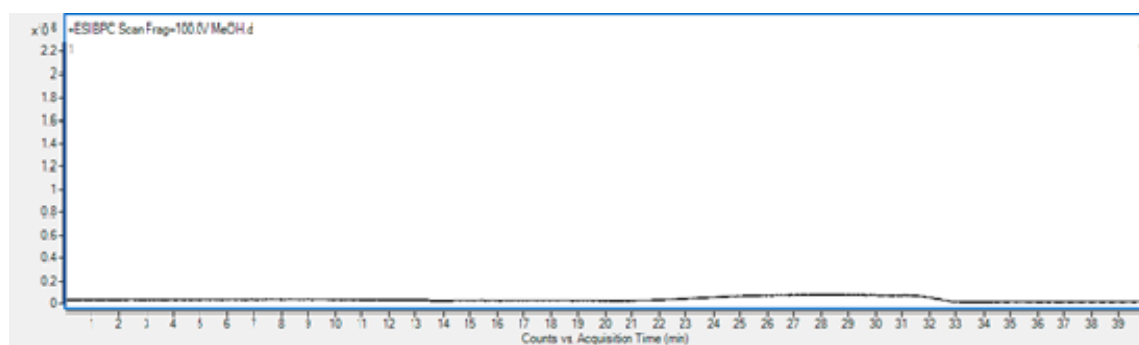
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**Figure S2:** Scheme of sampling time points of biological samples throughout the study.



**Figure S3:** The PCA score plots of A) plasma, B) urine and C) feces samples. The data used for PCA were normalized by total peak intensities of each samples.



**Figure S4:** The Base peak chromatogram of methanol (blank sample)

**Table S1.** Some tentatively identified compounds in Brahmi essence and placebo analyzed by positive mode LC-ESI-QTOF-MS.

Rt (min)	Detected m/z	Assigned ion	Tentatively identified compounds	Samples
7.0	449.1093	[M+H] <sup>+</sup>	Cyanidin 3-glucoside	Brahmi essence, placebo
7.1	595.1683	[M] <sup>+</sup>	Cyanidin 3-rutinoside	Brahmi essence, placebo
7.6	579.1693	[M] <sup>+</sup>	Pelargonidin3-O-rutinoside	Brahmi essence, placebo
9.7	611.1632	[M+H] <sup>+</sup>	Rutin	Brahmi essence, placebo
9.9	492.3182	[M+H] <sup>+</sup>	Murusimic acid	Brahmi essence, placebo
11.6	218.2126	[M+H] <sup>+</sup>	iminodiethanol	Brahmi essence
15.6	951.4984	[M+H] <sup>+</sup>	Bacoside A <sub>3</sub>	Brahmi essence
16.1	951.4954	[M+H] <sup>+</sup>	Bacopaside II	Brahmi essence
16.5	921.4855	[M+H] <sup>+</sup>	Bacopaside X	Brahmi essence
16.6	921.4850	[M+H] <sup>+</sup>	Bacopasaponin C	Brahmi essence
17.4	979.4601	[M+H] <sup>+</sup>	Bacopaside I	Brahmi essence

**Table S2.** The percent change of response time for **memory speed** of participants from placebo run-in (2nd visit) to after 12-weeks (3rd visit) in treatment group and the placebo group.

Group A		Group B	
Subjects	% change of response time	Subjects	% change of response time
1	6.37	1	20.36
2	-24.87	2	-4.30
3	-31.91	3	13.04
4	8.19	4	-4.27
5	1.36	5	-0.67
6	-2.71	6	-4.02
7	0.02	7	-9.87
8	-3.92	8	-16.86
9	4.27	9	6.97
10	-19.82	10	7.32
11	-22.22	11	8.03
12	4.24	12	-8.18
13	-4.32	13	7.93
14	-4.71	14	5.88
15	-30.72	15	-24.42
16	-3.31	16	-7.31
17	5.61	17	-9.37
18	-24.28	18	-13.28
19	-23.76	19	-0.35
20	0.50	20	-13.34
21	0.25	21	4.12
		22	17.46
		23	-12.03
		24	-7.70

**Table S3.** p-value of metabolic pathway from enrichment analysis by using Metaboanalyst (a) plasma, (b) Urine, (c) feces samples

(a)	Metabolic pathway	p value
	Aminoacyl-tRNA biosynthesis	5.99E-05
	Phenylalanine, tyrosine, and tryptophan biosynthesis	0.000538
	Valine, leucine, and isoleucine biosynthesis	0.00245
	Phenylalanine metabolism	0.0039
	Valine, leucine, and isoleucine degradation	0.057
	Ubiquinone and other terpenoid-quinone biosynthesis	0.0855
	Pantothenate and CoA biosynthesis	0.172
	Fatty acid degradation	0.324
	Tryptophan metabolism	0.337
	Tyrosine metabolism	0.344
(b)	Metabolic pathway	p value
	Phenylalanine, tyrosine, and tryptophan biosynthesis	0.0131
	Phenylalanine metabolism	0.0325
	Aminoacyl-tRNA biosynthesis	0.148
(c)	Metabolic pathway	P value
	Aminoacyl-tRNA biosynthesis	1.40E-08
	Phenylalanine, tyrosine, and tryptophan biosynthesis	0.000339
	Valine, leucine, and isoleucine biosynthesis	0.00156
	Phenylalanine metabolism	0.00248
	Valine, leucine, and isoleucine degradation	0.0376
	Ubiquinone and other terpenoid-quinone biosynthesis	0.0689
	Biotin metabolism	0.0763
	Lysine degradation	0.181
	Cysteine and methionine metabolism	0.232
	Tryptophan metabolism	0.28
	Tyrosine metabolism	0.286
	Fatty acid biosynthesis	0.315