

Identification and Cytotoxic Evaluation of Nutmeg (*Myristica fragrans* Houtt.) and Its Adulterants

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Supplementary materials

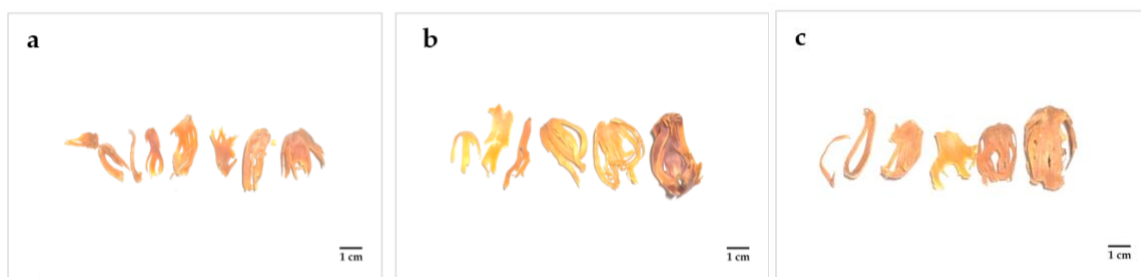


Figure S1 The arils of authentic nutmeg samples: AA1 (a), AA2 (b), and AA3 (c)

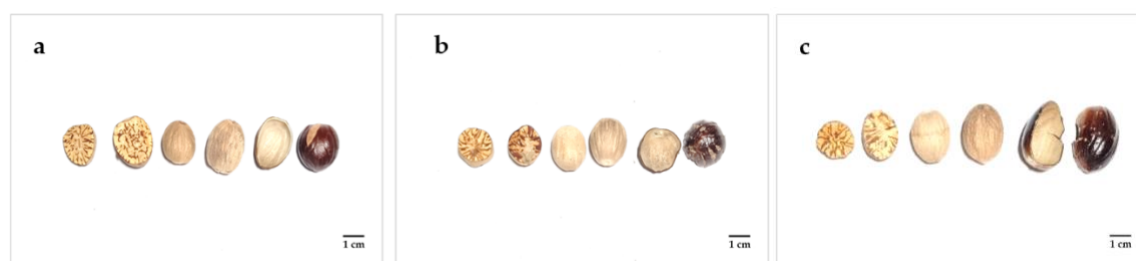


Figure S2 The seeds of authentic nutmeg samples: AS1 (a), AS2 (b), and AS3 (c)



Figure S3 The commercial dried arils and aril product of nutmeg: PA1 (a), PA2 (b), PA3 (c), PA4 (d), PA5 (e), PA6 (f), PA7 (g), PA8 (h), PA9 (i), and PA10 (j)

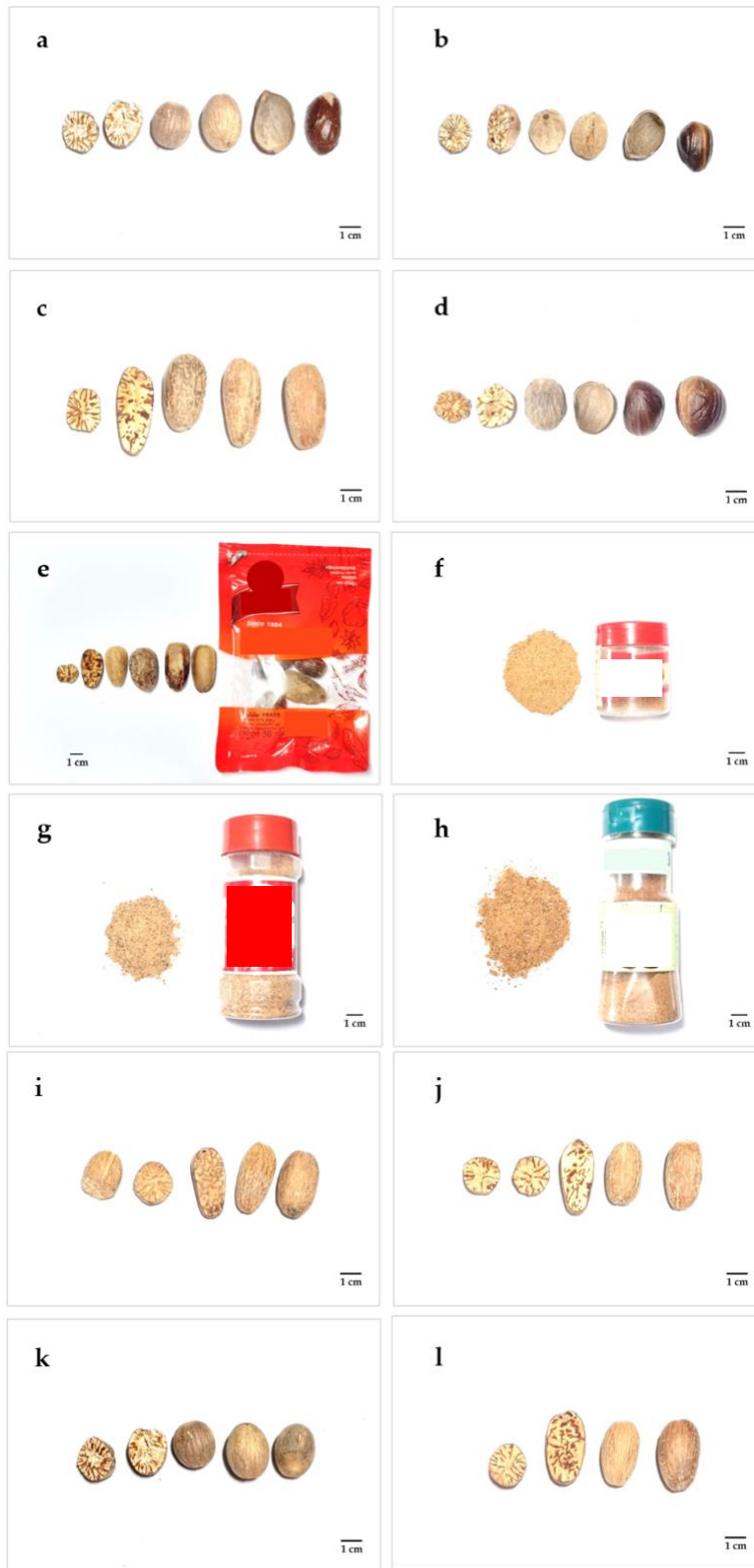


Figure S4 The commercial dried seeds and seed products of nutmeg: PS1 (a), PS2 (b), PS3 (c), PS4 (d), PS5 (e), PS6 (f), PS7 (g), PS8 (h), PS9 (i), PS10 (j), PS11 (k), and PS12 (l)

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      ....|....| ....|....| ....|....| ....|....| ....|....|
      10      20      30      40      50
M. fragrans      GGTGCTGTTG AAGCTCTATC AACAAATGGC TAATACTTCG GTATTAGTGT
globose-shaped nutmeg (PS1) GGTGCTGTTG AAGCTCTATC AACAAATGGC TAATACTTCG GTATTAGTGT
oval-shaped nutmeg (PS3)   GCTGCTGTTG AAGCTCTATC AACAAATGGC TAATACTTCG GTATTAGTGT

      ....|....| ....|....| ....|....| ....|....| ....|....|
      60      70      80      90     100
M. fragrans      ATACGAGTCA TTGAAGGATC AGATCAATAC CCAGAAGTGG GTATTGATCT
globose-shaped nutmeg (PS1) ATACGAGTCA TTGAAGGATC AGATCAATAC CCAGAAGTGG GTATTGATCT
oval-shaped nutmeg (PS3)   ATACGAGTCA TTGAAGGATC AGATCAATAC CCACTTCTGG GTATTGATCT

      ....|....| ....|....| ....|....| ....|....| ....|....|
      110     120     130     140     150
M. fragrans      GGTTCACTAG TGGTTTACTC ACATAAGATT TTTTTTTTTC AATTTCAGAA
globose-shaped nutmeg (PS1) GGTTCACTAG TGGTTTACTC ACATAAGATT TTTTTTTTTC AATTTCAGAA
oval-shaped nutmeg (PS3)   GGTTCACTAG TGGTTTACTC ACATAAGA-- TTTTTTTTTC AATTTAAGAA

      ....|....| ....|....| ....|....| ....|....| ....|....|
      160     170     180     190     200
M. fragrans      AATATTAATT ATTTGGTTCA TGATCGAACA TCATACC-TT TTTTTTTTAT
globose-shaped nutmeg (PS1) AATATTAATT ATTTGGTTCA TGATCGAACA TCATACC-TT TTTTTTTTAT
oval-shaped nutmeg (PS3)   AATATTAATT ATTTGGTTCA TGATCGAACA TCATACC-TT TTTTTTTTAT

      ....|....| ....|....| ....|....| ....|....| ....|....|
      210     220     230     240     250
M. fragrans      TTTAACTGAT CTTCAGTTTA TGTACATTCC TAAAATGCAT TTTTTCCTAT
globose-shaped nutmeg (PS1) TTTAACTGAT CTTCAGTTTA TGTACATTCC TAAAATGCAT TTTTTCCTAT
oval-shaped nutmeg (PS3)   TTTAACTGAT CTTCAGTTTA TGTACATTCC TAAAATGAAT TTTTTCCTAT

      ....|....| ....|....| ....|....| ....|....| ....|....|
      260     270     280     290     300
M. fragrans      TGTCAATTTT ATAAAGGGTA TAAATTAGTA TGAAAAAAAA AAAGAAAGAC
globose-shaped nutmeg (PS1) TGTCAATTTT ATAAAGGGTA TAAATTAGTA TGAAAAAAAA AAAGAAAGAC
oval-shaped nutmeg (PS3)   TGTCAATTTT ATAAAGGGTA TAAATTAGTA TG-AAAAAAA AAAGAAAGAC

      ....|....| ....|....| ....|....| ....|....| ....|....|
      310     320     330     340     350
M. fragrans      TAGTAATCTA AAATTTGTGT CTTTAGAAAA GACAAGGGGC GGATGTACCC
globose-shaped nutmeg (PS1) TAGTAATCTA AAATTTGTGT CTTTAGAAAA GACAAGGGGC GGATGTACCC
oval-shaped nutmeg (PS3)   TAGTAATCTA AAATTTGTGT CTTTAGAAAA GACAAGGGGC GGATGTAGCC

      ....|....| ..
      360
M. fragrans      AAGGGGATCA AG
globose-shaped nutmeg (PS1) AAGGGGATCA AG
oval-shaped nutmeg (PS3)   AAGTGGATCA AG

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Figure S5 Alignment of *trnH-psbA* region of authentic *M. fragrans*, commercial globose-shaped nutmeg (PS1), and oval-shaped nutmeg (PS3).

Table S1 Morphological characteristics and size measurements of arils and seeds of authentic *M. fragrans* and commercial nutmeg samples

Specimen Code	Sample Typologies	Color	Odor	Dimensions (cm)		
				Length	Width	Thickness
Arils						
Authentic samples						
AA1	Dried arils	Orange	pungent	2.60 ± 0.53	0.90 ± 0.51	0.05 ± 0.01
AA2	Dried arils	Yellowish orange	pungent	3.23 ± 0.60	0.75 ± 0.40	0.06 ± 0.01
AA3	Dried arils	Orange	pungent	3.10 ± 0.53	1.23 ± 0.76	0.04 ± 0.01
Commercial samples						
PA1	Dried arils	Yellowish orange	pungent	2.73 ± 0.75	0.70 ± 0.44	0.04 ± 0.01
PA2	Dried arils	Yellowish orange	pungent	2.77 ± 0.68	1.37 ± 0.68	0.04 ± 0.01
PA3	Dried arils	Yellowish orange	pungent	2.43 ± 0.20	0.88 ± 0.49	0.05 ± 0.01
PA4	Dried arils	Yellowish orange	pungent	3.95 ± 1.18	1.28 ± 0.19	0.06 ± 0.00
PA5	Aril Powder	Yellowish orange	pungent	-	-	-
PA6	Dried arils	Yellowish orange	pungent	2.63 ± 0.51	0.98 ± 0.49	0.05 ± 0.01
PA7	Dried arils	Orange	pungent	3.45 ± 0.88	1.03 ± 0.38	0.06 ± 0.01
PA8	Dried arils	Yellowish orange	pungent	3.68 ± 0.36	0.93 ± 0.23	0.06 ± 0.01
PA9	Dried arils	Yellowish orange	pungent	4.80 ± 0.18	1.00 ± 0.46	0.06 ± 0.01
PA10	Dried arils	Orange	pungent	3.13 ± 0.55	0.87 ± 0.55	0.05 ± 0.00
Seeds						
Authentic samples						
AS1	Dried seeds	bright brown	pungent	1.78 ± 0.48	1.40 ± 0.10	-
AS2	Dried seeds	bright brown	pungent	2.00 ± 0.30	1.45 ± 0.05	-
AS3	Dried seeds	bright brown	pungent	2.25 ± 0.22	1.57 ± 0.06	-
Commercial samples						
PS1	Dried seeds	bright brown	pungent	1.90 ± 0.37	1.57 ± 0.06	-
PS2	Dried seeds	bright brown	pungent	2.05 ± 0.57	1.60 ± 0.10	-
PS3	Dried seeds	brown	pungent	3.78 ± 0.19	1.78 ± 0.14	-
PS4	Dried seeds	bright brown	pungent	2.03 ± 0.25	1.47 ± 0.03	-
PS5	Dried seeds	brown	pungent	3.07 ± 0.25	1.60 ± 0.10	-
PS6	Seed Powders	bright brown	pungent	-	-	-
PS7	Seed Powders	brown	pungent	-	-	-
PS8	Seed Powders	bright brown	pungent	-	-	-
PS9	Dried seeds	brown	pungent	2.38 ± 0.28	1.65 ± 0.05	-
PS10	Dried seeds	brown	pungent	3.03 ± 0.25	1.57 ± 0.15	-
PS11	Dried seeds	brown	pungent	2.20 ± 0.10	1.23 ± 0.06	-
PS12	Dried seeds	brown	pungent	2.92 ± 0.14	1.65 ± 0.05	-

Table S2 Primers used in this study

Regions	Primers	Sequence (5' > 3')	Reference
ITS	ITS1	CCT TAT CAT TTA GAG GAA GGA G	[1]
	ITS4	TCC TCC GCT TAT TGA TAT GC	[2]
<i>matK</i>	<i>matK</i> -1RKIM-f	ACC CAG TCC ATC TGG AAA TCT TGG TTC	[3]
	<i>matK</i> -3FKIM-r	CGT ACA GTA CTT TTG TGT TTA CGA G	[3]
<i>rbcL</i>	<i>rbcL</i> 1F	ATG TCA CCA CAA ACA GAG ACT AAA GC	[4]
	<i>rbcL</i> 724R	GTA AAA TCA AGT CCA CCG CG	[4]
<i>trnH-psbA</i>	<i>trnH</i> (GUG)	CGT AAC AAG GTT TCC GTA GGT GAA	[5]
	<i>psbA</i>	GTT ATG CAT GAA CGT AAT GCTC	[5]
<i>trnL-F</i>	c	CGA AAT CGG TAG ACG CTA CG	[6]
	f	ATT TGA ACT GGT GAC ACG AG	[6]

Reference:

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Table S3 The PCR conditions for DNA amplification of each DNA barcoding regions

PCR conditions	DNA barcoding regions				
	ITS	<i>matK</i>	<i>rbcL</i>	<i>trnH-psbA</i>	<i>trnL-F</i>
Pre-heating	94°C, 2 min	94°C, 2 min	94°C, 2 min	94°C, 2 min	94°C, 2 min
Denaturation	94°C, 15 s	94°C, 15 s	94°C, 15 s	94°C, 15 s	94°C, 15 s
Annealing	54°C, 15 s	52°C, 15 s	52°C, 15 s	56°C, 15 s	52°C, 15 s
Extension	68°C, 45 s	68°C, 45 s	68°C, 45 s	68°C, 45 s	68°C, 45 s
Final extension	68°C, 20 min	68°C, 10 min	68°C, 10 min	68°C, 20 min	68°C, 10 min
Cycle (loops)	40	40	35	40	35

Table S4 %AUC of the major phytochemicals found in the volatile oil extracted from the representative aril and seed of globose-shaped and oval-shaped nutmegs

No.	RT	components	%Area Under the Curve (%AUC)			
			AA1	AS1	PA4	PS3
1	6.7	pinene, and terpinen-4-ol	15.68	11.74	3.04	3.41
2	7.9	β -phellandrene	33.94	30.98	18.99	27.00
3	9.7	(5R)-1-methyl-5-(1-methylethenyl)cyclohexene	7.43	5.89	20.29	18.71
4		terpinene	3.64	2.91	2.81	2.72
5	15.7	terpinen-4-ol	6.48	4.22	4.34	6.93
6	20.4	safrole	0.32	6.17	33.35	19.61
7	30.1	myristicin	5.23	3.39	0.04	1.48