



Endogenous proteins and lipids influence the phenolics profile, anti-nephrolithiatic and antioxidant activities of *Monodora myristica* seed extract



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INTRODUCTION

The bioactive compounds in plant foods matrix exist either in their free-state, or bound with the endogenous nutrients, such that they interact. Thus, investigating the influence of endogenous nutrients on the bioactive compounds and their bioactivities can enhance the understanding of their functionalities in real food systems and provide useful information on their health benefits (Zhang and Hamaker, 2003; Irondi *et al.*, 2022). Bioactive compounds of plant origin have been reported to possess anti-nephrolithiatic (kidney stone) (Patel *et al.*, 2012). Hence, this study evaluated the phenolics profile, anti-nephrolithiatic and antioxidant activities of *Monodora myristica* (African nutmeg) seed extract as influenced by endogenous proteins and lipids, *in vitro*.

RESEARCH METHODOLOGY

Sample (1 kg) of *Monodora myristica* seeds (Figure 1) was ground into flour and packed in an air-tight sample container. Endogenous proteins and/or lipids in the flour were removed from different portions (500 g each) of the flour by deproteinization and/or defatting, to obtain the protein-free, lipid-free, and protein- and lipid-free flours. The wholemeal flour, containing proteins and lipids, served as a control. The phenolics compounds in the flours were quantified using a reverse-phase HPLC. Nephrolithiasis-associated enzymes (xanthine oxidase and urease) inhibitory and antioxidant activities (DPPH[•] and ABTS^{•+} scavenging activities, reducing power and Fe²⁺ chelating capacity) and proximate bioactive constituents (total phenolics, tannins, total flavonoids, and total saponins) were determined using a spectrophotometer. Results of triplicate experiments were analyzed using one-way ANOVA, and the mean values of different treatments were compared by Duncan's multiple range test at a confidence level of 95%.



Figure 1. Sample of *Monodora myristica* seeds

RESULTS AND DISCUSSION

Table 1. Phenolic compounds in control, deproteinized, defatted, and defatted-deproteinized *Monodora myristica* flour

Phenolic compound (mg/g)/Sample	Control	Deproteinized	Defatted	Defatted-deproteinized	LOD µg/mL	LOQ µg/mL
Gallic acid	1.99 ± 0.02 ^a	1.27 ± 0.02 ^c	1.81 ± 0.01 ^b	0.4 ± 0.01 ^d	0.017	0.056
Catechin	0.85 ± 0.03 ^a	0.41 ± 0.01 ^c	0.72 ± 0.02 ^b	ND	0.010	0.034
Chlorogenic acid	4.36 ± 0.09 ^a	2.08 ± 0.07 ^b	4.02 ± 0.06 ^a	0.54 ± 0.01 ^c	0.024	0.079
Caffeic acid	4.29 ± 0.03 ^a	2.16 ± 0.04 ^c	3.09 ± 0.02 ^b	0.99 ± 0.01 ^d	0.008	0.025
<i>p</i> -Coumaric acid	1.85 ± 0.02 ^a	0.88 ± 0.01 ^c	1.66 ± 0.02 ^b	ND	0.023	0.076
Ellagic acid	4.19 ± 0.03 ^a	2.94 ± 0.02 ^c	3.66 ± 0.04 ^b	1.06 ± 0.01 ^d	0.025	0.083
Rutin	4.41 ± 0.06 ^a	2.16 ± 0.02 ^c	3.45 ± 0.05 ^b	1.18 ± 0.02 ^d	0.018	0.058
Quercetin	8.01 ± 0.05 ^a	2.28 ± 0.04 ^c	5.33 ± 0.04 ^b	1.34 ± 0.03 ^d	0.012	0.039
Luteolin	3.37 ± 0.04 ^a	1.71 ± 0.01 ^c	2.55 ± 0.02 ^b	0.82 ± 0.01 ^d	0.023	0.076

Values followed by different superscript letters along the same row differ significantly at $p < 0.05$. LOD, limit of detection; LOQ, limit of quantification

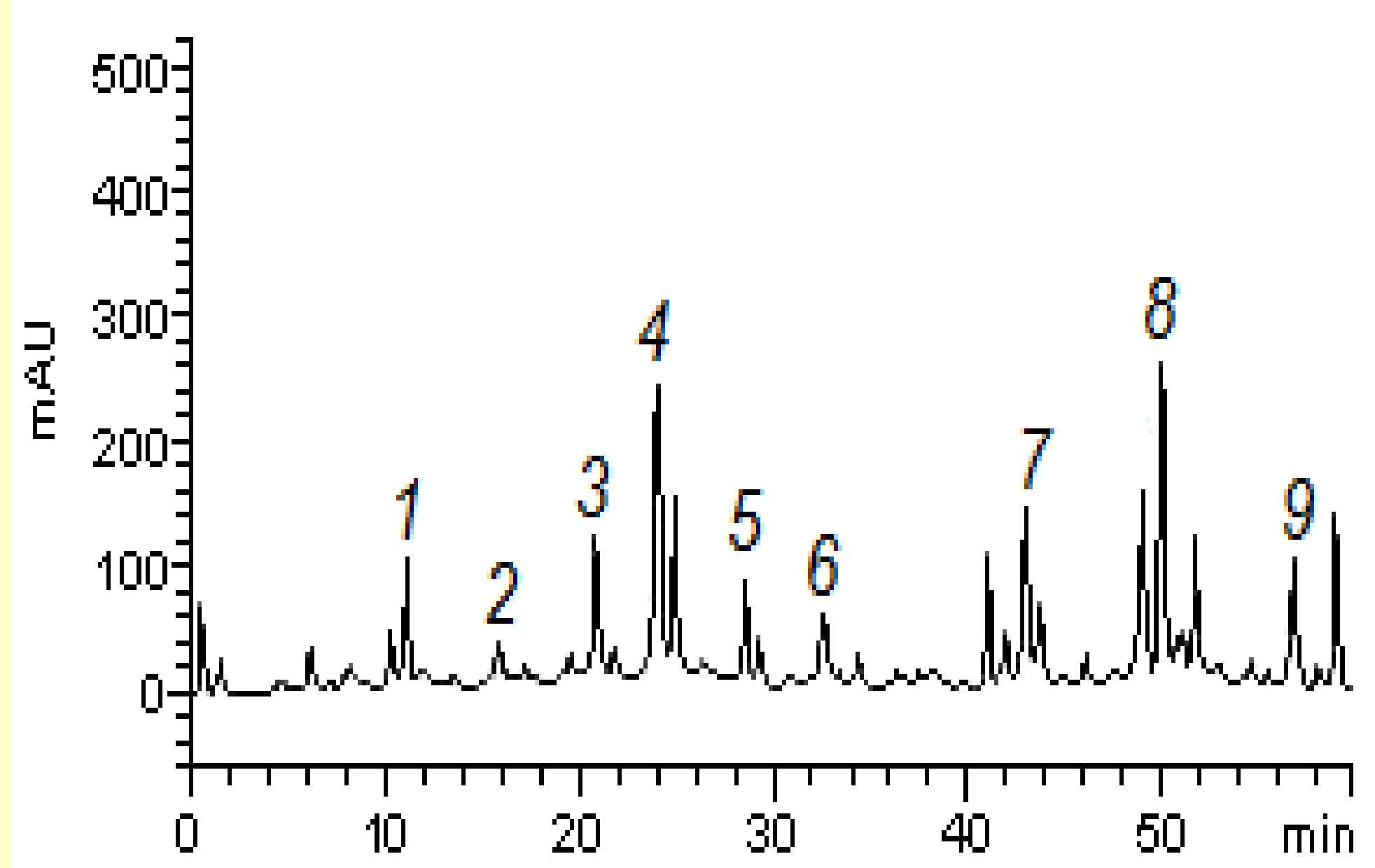


Figure 2 – Representative HPLC chromatogram of phenolics compounds in *M. myristica* flour (control). Peaks 1, 2, 3, 4, 5, 6, 7, 8 and 9 represent gallic acid, catechin, chlorogenic acid, caffeic acid, *p*-coumaric acid, ellagic acid, rutin, quercetin and luteolin, respectively.

Table 2. Nephrolithiasis-associated enzymes (xanthine oxidase and urease) inhibitory activity of control, deproteinized, defatted, and defatted-deproteinized *Monodora myristica* flour

Enzyme/ Sample	Control	Deproteinized	Defatted	Defatted-deproteinized	Allopurinol	Thiourea
Xanthine oxidase IC ₅₀ (µg/mL)	20.56 ± 1.72 ^c	23.32 ± 1.36 ^b	21.38 ± 1.04 ^c	25.92 ± 1.77 ^a	8.12 ± 0.61 ^d	-
Urease IC ₅₀ (µg/mL)	17.86 ± 1.13 ^d	20.84 ± 1.22 ^b	19.67 ± 0.95 ^c	22.49 ± 1.09 ^a	-	5.72 ± 0.07 ^e

Values followed by different superscript letters along the same row differ significantly at $p < 0.05$. IC₅₀, concentration of extract that inhibited enzyme activity by 50%

Table 3. Antioxidant activity of control, deproteinized, defatted, and defatted-deproteinized *M. myristica* flour

Parameter/Sample	Control	Deproteinized	Defatted	Defatted-deproteinized	Ascorbic acid
DPPH [•] SC ₅₀ (µg/mL)	9.39 ± 0.39 ^d	14.02 ± 0.81 ^b	11.87 ± 0.64 ^c	19.15 ± 1.03 ^a	5.58 ± 0.32 ^e
ABTS ^{•+} scavenging ability (mmol TEAC/g)	11.06 ± 0.96 ^a	9.16 ± 0.77 ^b	8.36 ± 0.44 ^c	6.07 ± 0.05 ^d	-
Reducing power (mg GAE/g)	71.02 ± 2.03 ^a	68.99 ± 2.07 ^b	67.84 ± 1.99 ^b	59.46 ± 2.63 ^c	-
Fe ²⁺ chelation CC ₅₀ (µg/mL)	14.91 ± 0.82 ^c	15.03 ± 0.67 ^c	18.16 ± 0.92 ^b	21.59 ± 1.01 ^a	7.96 ± 0.51 ^d

Values having different superscript letters along the same row vary significantly ($p < 0.05$). TEAC: Trolox equivalent antioxidant capacity; SC₅₀: extract conc. that scavenged 50% of DPPH[•]; GAE: Gallic acid equivalent. CC₅₀: extract conc. that chelated 50% of Fe²⁺

Table 4. Proximate bioactive constituents of control, deproteinized, defatted, and defatted-deproteinized *M. myristica* flour

Bioactive/Sample	Control	Deproteinized	Defatted	Defatted-deproteinized
Total Phenols (GAE mg/g)	32.07 ± 1.23 ^a	26.57 ± 1.34 ^c	28.83 ± 1.55 ^b	24.85 ± 1.19 ^d
Tannin (TAE mg/g)	30.17 ± 1.26 ^a	24.26 ± 1.64 ^c	27.50 ± 1.69 ^b	20.38 ± 1.26 ^d
Total Flavonoids (QE mg/g)	11.03 ± 0.88 ^a	7.84 ± 0.66 ^c	9.02 ± 0.81 ^b	4.51 ± 0.17 ^d
Total Saponins (DE mg/g)	8.39 ± 0.72 ^a	6.94 ± 0.21 ^b	7.33 ± 0.19 ^b	4.72 ± 0.23 ^c

Values having different superscript letters along the same row vary significantly ($p < 0.05$). TAE, tannic acid equivalent; QE, quercetin equivalent; DE, diosgenin equivalent

➤*M. myristica* flour contained flavonoids (catechin, rutin, quercetin and luteolin) and phenolic acids (gallic, chlorogenic, caffeic, *p*-coumaric, and ellagic acids).

➤Deproteinization and/or defatting led to a significant decrease in the phenolic compounds levels, xanthine oxidase and urease inhibitory and antioxidant activities, and proximate bioactive constituents level in *M. myristica* flour.

➤These parameters were in a consistent order of control (wholemeal) > Defatted > Deproteinized > Defatted-deproteinized.

CONCLUSIONS

The control (wholemeal) *M. myristica* seed flour contained the highest levels of the phenolic compounds and displayed the most potent nephrolithiasis-associated enzymes (xanthine oxidase and urease) inhibitory and antioxidant activities. Deproteinization, defatting, and defatting-deproteinization resulted in a decrease in these parameters, with defatting-deproteinization leading to the highest decrease. Therefore, endogenous proteins and lipids may enhance the phenolics profile, anti-nephrolithiatic, and antioxidant activities of *M. myristica* seed flour. These findings give credence to the use of wholemeal flours in making food products over refined flours, due to their health-promoting properties.

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