Novel PDE-5 Inhibitory Activity of *Areca catechu* Extracts: Phytochemical Insights and Potential for Natural Erectile Dysfunction Therapy

Norkamilah Abdullah[1], Mohd Kamal Nik Hasan[2], Abu-Baker M. Abdel-Aal[1,3], Yuhanis Mhd Bakri[1], Rozita Yahaya[1], Chee Fah Wong[4], Mastura Ibrahim[1] and Saripah Salbiah Syed Abdul Azziz[1]*

[1]Department of Chemistry, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, 35900, Tanjong Malim, Perak, Malaysia

[2]Cell Signaling and in vivo Research Laboratory, Forest Research Institute Malaysia, 52109 Kepong, Selangor, Malaysia

[3]Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt

[4]Department of Biology, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, 35900, Tanjong Malim, Perak, Malaysia

*Corresponding author (e-mail: saripah@fsmt.upsi.edu.my)

The *Areca catechu* (AC) plant has traditionally been used as an aphrodisiac. In Indonesia, *Areca* nut extracts are found in beverages marketed to enhance vitality. This study focused on investigating the preliminary inhibitory potential of phosphodiesterase type-5 (PDE-5), the phytochemical composition, and the isolation of major bioactive constituents from AC extracts. Extraction was performed first by cold maceration using a polarity gradient of solvents (hexane, dichloromethane, ethyl acetate, and methanol), followed by aqueous Soxhlet extraction of the remaining residue. PDE-5 inhibitory activity was evaluated using a colorimetric assay kit. Statistical analysis was performed using one-way ANOVA and Dunnett's multiple comparison test. Phytochemical profiling of the active extracts was conducted using GC–MS. The nut extracts presented the most promising PDE-5 inhibitory activity, exhibiting a higher percentage of inhibition than the husk. Among the solvent fractions, the hexane (63 %), dichloromethane (60 %), and ethyl acetate (64 %) extracts displayed modest PDE-5 inhibition percentages compared with the reference inhibitor 3-isobutyl-1-methylxanthine (IBMX). Subfraction Unripe nut DCM 3 (UND 3) exhibited 42 % PDE-5 inhibition, which was equivalent to IBMX. The phytochemical profiling of AC nut extracts identified five major compound groups: fatty acids, methyl esters, phenolics, alkaloids, and hydrocarbons. The major compounds trimyristin (1) and myristic acid (2) isolated from UND 3 did not inhibit PDE-5, suggesting that minor compounds or synergistic effects contributed to the inhibition. This is the first study to report PDE-5 inhibitory activity in AC, highlighting its potential for the development of natural, plant-based treatments for erectile dysfunction.

Keywords: *Areca catechu*, PDE-5 inhibitor, erectile dysfunction, GC-MS

Received: August 2025; Accepted: December 2025

Infertility is an immense problem at present, as around 8–12 % of couples experience infertility worldwide. Statistical data suggests that all over the world, 40 % of infertility is due to male factors. More than 90 % of male infertility problems occur due to poor sperm count, poor sperm quality, or both. The remaining cases of male infertility may be caused by a variety of conditions involving anatomical problems, hormonal imbalances, and genetic defects [1]. Research by Laumann et al (1999) conducted in the United States found that 14.8 % of men aged 18-59 years experienced a lack of sexual desire [2]. Whereas 30.6 % experienced a climax that was too fast, which may be caused by premature ejaculation. As many as 10.2 % had difficulty in achieving or maintaining an erection, commonly known as erectile dysfunction (ED), or male impotence.

ED represents an increasing health concern, causing a significant impact on the quality of life (QOL) of men globally. It was estimated that 322 million men worldwide would be affected by ED by 2025, a dramatic increase compared with 152 million men who suffered from ED in 1995 [3]. ED is commonly caused by chronic diseases such as hypertension [4], diabetes [5], neurological disorders [6], lifestyle practices including alcoholism and smoking [7], and age-related hormonal disturbances [8]

In Malaysia, ED is believed to be the leading cause of male infertility. Malaysia's male fertility rate is at an alarming level, dropping from 6.376 to 1.924 between 1959 and 2023 [9]. A previous study of outpatient clinic attendees in Johor found that the prevalence of ED, categorized by severity, was as follows: mild (17.0 %), mild to moderate (23.8 %), moderate (11.3 %), and severe (29.5 %) [10].

The PDE-5 inhibition assay is used to detect ED rapidly. The drugs that are currently used to treat ED are called PDE-5 inhibitors. However, these drugs are incompatible with some chronic diseases. The most well-known PDE5 inhibitor is Sildenafil, also known as Viagra. If patients with high blood pressure take an alpha blocker and Viagra at the same time, the concentration of drugs in their blood will be four times higher.

In contrast, patients with heart problems taking nitrates or nitric oxide donors together with Viagra may also experience a sudden drop in blood pressure. Because of this, Viagra cannot be used in conjunction with alpha blockers and nitrates. Other common side effects of using Viagra include headache, flushing, dyspepsia, nasal congestion, and impaired vision [11].

To mitigate the side effects of modern drugs, many researchers are now exploring alternative medicines derived from natural sources. Substances that enhance sexual desire or libido are known as aphrodisiacs [12]. Many studies have been conducted on various plants and herbs, including *Areca catechu* (AC). AC has traditionally been used as an aphrodisiac, with the root being the most widely used part of the plant [13]. In previous studies, alcohol extracts of AC increased rats' sperm counts and sexual activity [14]. On the other hand, Rahman (2020) reported that the mounting frequency decreased after feeding mice with the ethanol areca nut extract [15, 16].

Despite numerous traditional claims, the scientific evidence supporting the pro-erectile effects of *Areca catechu* (AC) remains inconsistent. Critically, no published study has evaluated the PDE-5 inhibitory activity of AC extracts or demonstrated their ability to elevate cGMP levels and promote vasodilation, which represents a clear gap in current knowledge. To address this, the present study will investigate the PDE-5 inhibitory potential of AC extracts, perform comprehensive phytochemical profiling using GC–MS, and isolate the major bioactive constituents from the most active subfraction. These objectives aim to generate robust evidence to substantiate the therapeutic claims of AC and support its development as a natural PDE-5 inhibitor.

EXPERIMENTAL

Plant Material Collection

Ripe and unripe AC fruits were collected from Hulu Selangor, Selangor, Malaysia in 2020. The species was identified by Mrs. Tan Ai Lee, a research officer at the Department of Natural Products in the Forest Research Institute Malaysia (FRIM). A Voucher specimen (No: SBID 010/21) was deposited in the herbarium of the Chemistry Department, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris (UPSI), Tanjong Malim, Perak, Malaysia.

Chemicals and Instruments

The study was conducted using the following chemicals and solvents: hexane (Hex), dichloromethane (DCM), ethyl acetate (EA), methanol (MeOH), silica gel (230-400 mesh, 38-63 mm), and a PDE-5 inhibition activity assay kit (ab139460, Abcam). All organic solvents were purchased from *ChemAR*. The instruments utilized included a rotary evaporator, a Soxhlet apparatus, a GC-MS (Shimadzu GC-2010 Plus) and a microplate reader (Tecan, Switzerland).

Preparation of Powder and Extracts

The ripe nut, ripe husk, unripe nut and unripe husk were classified and extracted using the same following protocol. All samples were dried and ground into a fine powder (mesh size 0.7 mm) using an electric grinder. Ground ripe (700 g) and unripe AC nuts (993 g) were extracted with 2.5 L of hexane (Hex) at room temperature over three cycles for three days. The extraction process was then repeated sequentially using 2.5 L of fresh dichloromethane (DCM), ethyl acetate (EA), and methanol (MeOH), respectively. Finally, the remaining AC residue was subjected to Soxhlet extraction with water to produce an aqueous extract. Organic solvents were evaporated using a rotary evaporator under reduced pressure at 40 °C to give the respective crude extracts. This extraction procedure was also applied to the husks of both ripe (359 g) and unripe (1063 g) AC fruits.

PDE-5 Inhibition Assay for Crude Extracts, Subfractions and Isolated Compounds

A preliminary screening of 20 crude extracts of *Areca catechu* including unripe and ripe husk and nut samples was conducted to evaluate PDE-5 inhibitory activity. Subsequently, eight targeted subfractions and two major purified compounds isolated from the nut were assessed using a colorimetric PDE-5 inhibition assay kit (ab139460). All assays were performed in triplicate according to the manufacturer’s protocol. Samples were dissolved in DMSO and tested at a standardised concentration of 5 mg/mL. A blank was included in every assay run.

The subfraction exhibiting the strongest PDE-5 inhibitory effect was selected for further isolation and purification of potential bioactive constituents. Statistical analysis of PDE-5 inhibition was carried out by comparing sample activity to the positive control, isobutylmethylxanthine (IBMX). Data were analysed using GraphPad Prism, employing one-way ANOVA followed by Dunnett's multiple comparison test to determine statistical significance ($p < 0.05$).

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Crude Extracts

The AC nut extracts were prioritized over the husk due to their promising preliminary findings. One microlitre of crude extracts from the ripe and unripe nuts were subjected to GC-MS phytochemical profiling utilizing a Shimadzu GC-2010 Plus instrument equipped with an HP-5 column (30 m length, 0.25 μm ID, and 2.50 μm film thickness) and a flow rate of 1.0 mL/min. The initial temperature was set at 80 °C for 1 min, followed by a first temperature ramp to 200 °C over 12 min, then a second temperature ramp to 255 °C over 10 min. The total time of the run was 24.5 min. All samples were dissolved in 100 % dichloromethane with a concentration of 5 mg/ml. Compounds with a quality score above 90, as identified in the National Institute of Standards and Technology (NIST) library, were selected, listed, and the group of identified compounds were reported [17].

Isolation and Elucidation of Major Compounds from Targeted Fractions

For bioassay-guided PDE-5 inhibitory activity, the crude DCM extract of unripe AC nuts (UND, 20.0 g) was subjected to column chromatography (CC) using silica gel (230-400 mesh, 38-63 mm) and a gradient elution of Hex, DCM and MeOH to give eight sub-fractions (UND 1-8) as shown in **Figure 1**. Subfraction UND 3 (10.0 g) was selected to isolate active compounds. **Compound 1** was purified by recrystallizing with cold methanol, resulting in white crystals of trimyristin (6.37 g, 31.9%). **Compound 2** was isolated from the residue of the UND 3 filtrate (190 mg) by further silica gel column chromatography, using gradient elution with Hex, DCM and MeOH, to afford myristic acid (5.0 mg, 2.6 %)

RESULTS AND DISCUSSION

Extraction Yields

Twenty crude extracts were obtained from the husk and nut of both ripe and unripe *Areca catechu*. Notably, the yields of extracts from the nut were higher compared to those from the husk. The methanolic (MeOH) extracts of both ripe and unripe nuts exhibited the highest yields, followed by the nonpolar hexane (Hex) extract, the intermediate polar dichloromethane (DCM) extract, and the ethyl acetate (EA) extract. **Table 1** presents the yields of neutral extracts of *Areca catechu.*

For the ripe nut (700 g), MeOH extraction produced the highest yield at 9.40 % (65.4 g), followed by EA at 4.40 % (30.7 g), DCM at 2.70 % (19.4 g), and Hex at 2.32 % (16.2 g). Similarly, for the ripe husk (359 g), MeOH yielded the most extract at 1.40 % (5.0 g), EA produced 0.84 % (3.0 g), while both Hex and DCM gave identical yields of 0.42 % (1.5 g).

For the unripe nut (993 g), MeOH produced a substantial yield of 7.86 % (78.0 g), followed by EA with 4.84 % (48.0 g), Hex at 2.32 % (23.0 g), and DCM at 2.22 % (22.0 g). Lastly, for the unripe husk (1063 g), MeOH once again provided the highest yield at 0.78 % (8.3 g), with EA yielding 0.47 % (5.0 g), Hex 0.30 % (3.1 g), and DCM 0.28% (2.9 g).

Overall, MeOH extraction consistently gave the highest yields across all samples. The yields of the aqueous (AQ) extracts were not determined since AQ extracts were used only for PDE-5 studies and not for isolation purposes.

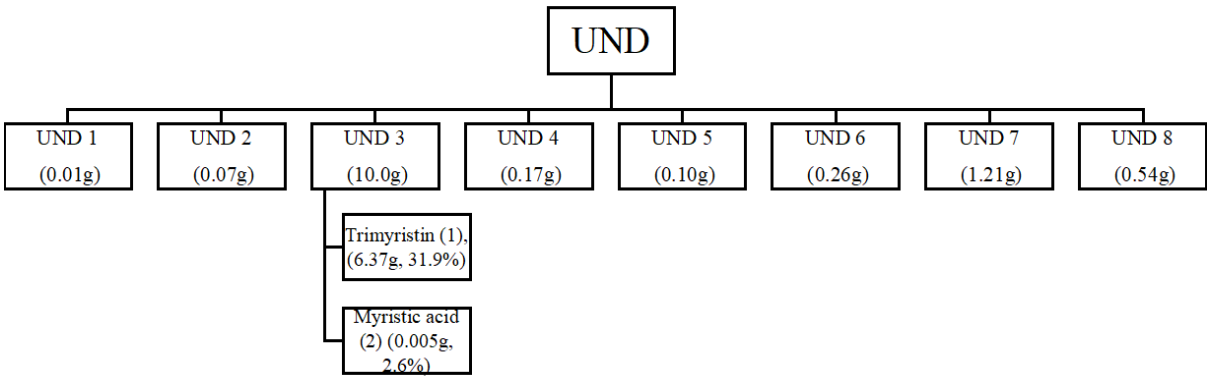


Figure 1. Bioassay guided fractionation and isolation of major compounds from unripe nut DCM (UND).

Table 1. Yields of neutral extracts of *Areca catechu.*

No	Extraction solvent	Code	Yield (g)	Yield Percentage (%)
Ripe nut (700.0 g)				
1	Hex	RNH	16.2	2.32
2	DCM	RND	19.4	2.70
3	EA	RNE	30.7	4.40
4	MeOH	RNM	65.4	9.40
5	AQ	RNA	-	-
Unripe nut (993.0 g)				
6	Hex	UNH	23.0	2.32
7	DCM	UND	22.0	2.22
8	EA	UNE	48.0	4.84
9	MeOH	UNM	78.0	7.86
10	AQ	UNA	-	-
Ripe husk (359.0 g)				
11	Hex	RHH	1.5	0.42
12	DCM	RHD	1.5	0.42
13	EA	RHE	3.0	0.84
14	MeOH	RHM	5.0	1.40
15	AQ	RHA	-	-
Unripe husk (1063.0 g)				
16	Hex	UHH	3.1	0.30
17	DCM	UHD	2.9	0.28
18	EA	UHE	5.0	0.47
19	MeOH	UHM	8.3	0.78
20	AQ	UHA	-	-

RNH: Ripe nut hexane; RND: Ripe nut dichloromethane; RNE: Ripe nut ethyl acetate; RNM: Ripe nut methanol; RNA: Ripe nut aqueous; RHH: Ripe husk hexane; RHD: Ripe husk dichloromethane; RHE: Ripe husk ethyl acetate; RHM: Ripe husk methanol; RHA: Ripe husk aqueous; UNH: Unripe nut hexane; UND: Unripe nut dichloromethane; UNE: Unripe nut ethyl acetate; UNM: Unripe nut methanol; UNA: Unripe nut aqueous; UHH: Unripe husk hexane; UHD: Unripe husk dichloromethane; UHE: Unripe husk ethyl acetate; UHM: Unripe husk methanol; UHA: Unripe husk aqueous.

GC-MS Analysis of Crude Extracts

Five classes of phytochemicals were identified from the GC-MS analysis of AC nut extracts, namely fatty acids, methyl esters, phenolic compounds, alkaloids and hydrocarbons, as summarized in **Table 2** (unripe nuts) and **Table 3** (ripe nuts). A previous study by Hugar et al. (2024) investigated the phenolic profile of only unripe nuts using UHPLC MS/MS [18]. Our results revealed significant differences in the phytochemicals identified between unripe and ripe AC nuts. Fatty acids were found to be more abundant in the hexane extracts of ripe nuts compared to the unripe nuts and other solvent extracts. For instance, tetradecanoic acid and dodecanoic acid were identified as the major fatty acids, with peak areas of 59.29 % and 27.53 %, respectively, in the RNH extract, compared to 20.95 % and 49.42 %, respectively, in the UNH extract. A study by Olubiyi (2022) reported that dodecanoic acid improved the relaxation of corpus cavernosum in streptozotocin-induced diabetic male Wistar rats [19], and possibly inhibited PDE-5 activity.

The alkaloid arecoline displayed a higher concentration in both methanolic and aqueous extracts of unripe and ripe nuts, respectively. It has been previously reported that arecoline may have beneficial effects on the reproductive system in male rats with type 1 diabetes, particularly by mitigating the negative impacts of insulin deficiency [20]. Further, eugenol in the UND extract was reported to improve diabetes-induced ED in rats [21]. These findings indicate that the PDE-5 inhibitory activity of *Areca catechu* nut extracts is likely mediated by the synergistic effects of fatty acids, phenolics, and alkaloids, which collectively facilitate smooth-muscle relaxation, oxidative balance, and modulation of the NO–cGMP pathway relevant to erectile function.

Table 2. Phytochemicals identified using GC-MS in unripe *Areca catechu* nuts.

No.	Compound Name	Group of compounds	RT	Peak Area (%)
Unripe nut hexane extract (UNH)				
1	Dodecanoic acid	Fatty acid	10.274	49.42
2	Methyl tetradecanoate	Fatty acid ester	11.406	1.68
3	*n*-Hexadecanoic acid	Fatty acid	14.256	17.05
4	Nonanoic acid	Fatty acid	6.938	3.42
5	Octanoic acid	Fatty acid	5.845	2.83
6	Tetradecanoic acid	Fatty acid	12.105	20.95
Unripe nut dichloromethane extract (UND)				
1	Dodecanoic acid	Fatty acid	9.907	24.21
2	Dodecanoic acid, methyl ester	Fatty acid ester	9.45	3.47
3	Eugenol	Phenolic compound	7.83	5.84
4	Hexadecanoic acid, methyl ester	Fatty acid ester	13.587	6.26
5	*n*-Hexadecanoic acid	Fatty acid	14.033	1.34
6	Phenol, 2-methoxy-3-(2-propenyl)-	Phenolic compound	7.83	5.84
7	Tetradecanoic acid	Fatty acid	11.87	28.26
8	Tridecanoic acid, 12-methyl-, methyl ester	Fatty acid ester	11.401	6.88
9	Undecanoic acid, 10-methyl-, methyl ester	Fatty acid ester	9.45	3.47
Unripe nut ethyl acetate extract (UNE)				
1	11-Octadecenoic acid, methyl ester	Fatty acid ester	15.435	13.48
2	2-Decenal, (E)-	Hydrocarbon and others	6.692	0.99
3	9,11-Octadecadienoic acid, methyl ester, (E,E)-	Fatty acid ester	15.372	6.66
4	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Fatty acid ester	15.372	6.66
5	9,12-Octadecadienoic acid, methyl ester, (E,E)-	Fatty acid ester	15.372	6.66
6	9-Octadecenoic acid, methyl ester, (E)-	Fatty acid ester	15.435	13.48
7	cis-13-Octadecenoic acid, methyl ester	Fatty acid ester	15.435	13.48
8	Dodecanoic acid	Fatty acid	10.016	9.67
9	Dodecanoic acid, methyl ester	Fatty acid ester	9.467	13.35
10	Hexadecanoic acid, methyl ester	Fatty acid ester	13.609	11.72
11	Methyl stearate	Fatty acid ester	15.652	1.55
12	Methyl tetradecanoate	Fatty acid ester	11.435	21.79
13	n-Hexadecanoic acid	Fatty acid	14.096	2
14	Tetradecanoic acid	Fatty acid	12.002	12.47
15	Tridecanoic acid, 12-methyl-, methyl ester	Fatty acid ester	11.435	21.79
16	Undecanoic acid, 10-methyl-, methyl ester	Fatty acid ester	9.467	13.35
Unripe nut methanolic (MeOH) extract (UNM)				
1	10-Octadecenoic acid, methyl ester	Fatty acid ester	15.412	9.04

2	11-Octadecenoic acid, methyl ester	Fatty acid ester	15.412	9.04
3	8-Octadecenoic acid, methyl ester	Fatty acid ester	15.412	9.04
4	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Fatty acid ester	15.355	5.1
5	9,12-Octadecadienoic acid, methyl ester,	Fatty acid ester	15.355	5.1
6	9,15-Octadecadienoic acid, methyl ester, (Z,Z)-	Fatty acid ester	15.355	5.1
7	Arecoline	Alkaloids	6.325	13.63
8	Dodecanoic acid, methyl ester	Fatty acid ester	9.45	9.89
9	Heptadecanoic acid, 16-methyl-, methyl ester	Fatty acid ester	15.646	1.04
10	Hexadecanoic acid, methyl ester	Fatty acid ester	13.592	8.64
11	Methyl tetradecanoate	Fatty acid ester	11.407	18.52
12	Tetradecanoic acid	Fatty acid	11.859	1.68
Unripe nut aqueous extract (UNA)				
1	Arecoline	Alkaloids	6.33	59.69
2	Azulene	Hydrocarbon and others	5.972	26.58
3	Naphthalene	Hydrocarbon and others	5.972	26.58

Table 3. Phytochemicals identified using GC-MS in ripe *Areca catechu* nuts.

No.	Compound Name	Group of compounds	RT	Peak Area (%)
Ripe nut hexane extract (RNH)				
1	n-Hexadecanoic acid	Fatty acid	14.159	9.13
2	1-Tetradecene	Hydrocarbon	8.076	4.79
3	3-Tetradecene, (E)-	Hydrocarbon	8.076	4.79
4	3-Tetradecene, (Z)-	Hydrocarbon	8.076	4.79
5	Azulene	Hydrocarbon	5.988	15.09
6	Cetene	Hydrocarbon	10.136	3.25
7	Dodecanoic acid	Fatty acid	10.102	27.53
8	Dodecanoic acid, methyl ester	Fatty acid ester	9.472	9.48
9	Hexadecanoic acid, methyl ester	Fatty acid ester	13.615	5.52
10	Methyl tetradecanoate	Fatty acid ester	11.424	18.31
11	Naphthalene	Hydrocarbon	5.988	15.09
12	Tetradecane	Hydrocarbon	8.151	1.73
13	Tetradecanoic acid	Fatty acid	12.139	59.29
14	Undecane	Hydrocarbon	4.809	2.18
15	Undecanoic acid, 10-methyl-, methyl ester	Fatty acid ester	9.472	9.48
Ripe nut dichloromethane (DCM) extract (RND)				
1	n-Hexadecanoic acid	Fatty acid	12.385	0.83
2	Tetradecanoic acid	Fatty acid	14.016	1.26
Ripe nut ethyl acetate (EA) extract (RNE)				
1	3- Tetradecene, (Z)	Hydrocarbon	8.076	3.65

2	3-Tetradecene, (E)-	Hydrocarbon	8.076	3.65
3	Azulene	Hydrocarbon	5.988	10.7
4	Carbonic acid, hexadecyl 2,2,2-tri	Fatty acid ester	12.145	1.12
5	Cetene	Hydrocarbon	10.136	3.62
6	Chloroacetic acid, pentadecyl ester	Fatty acid ester	10.136	3.62
7	Cyclodecane	Hydrocarbon	5.851	1.11
8	Cyclohexadecane	Hydrocarbon	10.136	3.62
9	Decanoic acid, methyl ester	Fatty acid ester	9.467	14.7
10	Dodecanoic acid, methyl ester	Fatty acid ester	9.467	14.7
11	Hexadecanoic acid, methyl ester	Fatty acid ester	13.61	7.1
12	Methyl tetradecanoate	Fatty acid ester	11.424	28.98
13	Naphthalene	Hydrocarbon	5.988	10.7
14	n-Hexadecanoic acid	Fatty acid	14.062	2.46
15	Tetradecane	Hydrocarbon	8.151	1.36
16	Tetradecanoic acid/myristic acid	Fatty acid	11.882	6.51
17	Tridecanoic acid, 12-methyl-, methyl ester	Fatty acid ester	11.424	28.98
18	Undecanoic acid, 10-methyl-, methyl ester	Fatty acid ester	9.467	14.7
Ripe nut methanolic (MeOH) extract (RNM)				
1	6-Octadecenoic acid, methyl ester, (Z)	Fatty acid ester	15.412	8.59
2	7-Octadecenoic acid, methyl ester	Fatty acid ester	15.412	8.59
3	8-Octadecenoic acid, methyl ester,(E)	Fatty acid ester	15.412	8.59
4	9,12-Octadecadienoic acid, methyl ester	Fatty acid ester	15.36	4.58
5	9,12-Octadecadienoic acid, methyl ester, (E,E)-	Fatty acid ester	15.36	4.58
6	9,15-Octadecadienoic acid, methyl ester, (Z,Z)-	Fatty acid ester	15.36	4.58
7	Arecoline		6.32	69.58
8	Dodecanoic acid, methyl ester	Fatty acid ester	9.45	2.81
9	Hexadecanoic acid, methyl ester	Fatty acid ester	13.587	4.25
10	Methyl 1-methyl-1,2,3,6-tetrahydro	Fatty acid ester	5.604	3.01
11	Methyl nicotinate	Fatty acid ester	5.376	2.55
12	Methyl tetradecanoate	Fatty acid ester	11.401	4.62
13	Pentadecanoic acid, 14-methyl-, methyl ester	Fatty acid ester	13.587	4.25
14	Trigonelline	Alkaloids	5.376	2.55
Ripe nut aqueous extract (RNA)				
1	2,4-Di-*tert-butyl*phenol	Phenolic compound	9.435	13.59
2	Arecoline	Alkaloids	6.331	42.55
3	Naphthalene	Hydrocarbon	5.975	26.54
4	Phenol, 2,5-bis(1,1-dimethylethyl)	Phenolic compound	9.435	13.59

Isolation and Elucidation of Major Compounds from the Promising Fraction

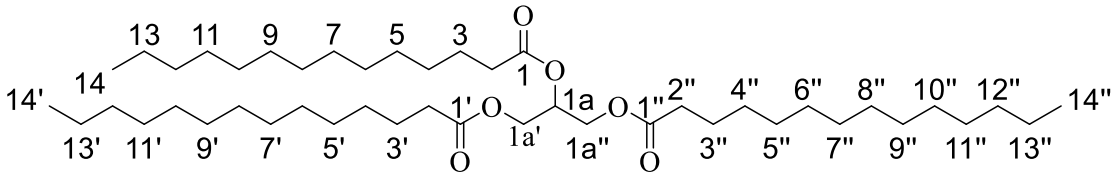
Two major compounds were isolated from fraction UND 3, namely trimyristin (**1**) and myristic acid (**2**) (Figure 2), and their structures were characterized by IR, NMR and mass spectral analysis.

Trimyristin (**1**) (6.37 g, 31.9 %) was the major secondary metabolite recrystallized from the UND 3 fraction. The TOF-MS measurement **(SI1)** of the molecular mass was indicated by the molecular peak at *m/z* 745.6750 $[M+Na]^+$, corresponding to the molecular formula of $C_{45}H_{86}O_6$. The FTIR spectrum **(SI2)** showed absorptions at 2956, 2916, and 2850 cm^{-1} corresponding to the C-H stretching vibrations of the methyl ($-CH_3$) and methylene ($-CH_2-$) groups of long aliphatic hydrocarbon chains. A strong peak at 1738 cm^{-1} was attributed to the carbonyl (C=O) stretching of ester groups [22], confirming the ester linkage between glycerol and myristic acid. The peak at 1172 cm^{-1} represented C-O stretching vibrations, further supporting the presence of ester bonds. Additionally, the absorption at 718 cm^{-1} was associated with the rocking vibration of methylene groups, a characteristic of long, saturated fatty acid chains.

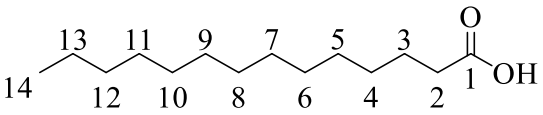
The ^{1}H-NMR spectrum **(SI3)** of **1** revealed a signal corresponding to its molecular structure as a triglyceride composed of glycerol esterified with three myristic acid chains. The multiplet at 5.33 ppm corresponded to the proton on the oxygenated carbon (CH-O) in the glycerol backbone. Signals at 4.26 and 4.11 ppm (doublet of doublets) represented the methylene protons (CH_2) of glycerol. The signal at 2.29 ppm corresponded to the methylene protons adjacent to the carbonyl groups ($CO-CH_2$), confirming the ester linkages. The multiplet at 1.58 ppm represented the methylene protons beta to the carbonyl group ($COCH_2-CH_2$), while the broad multiplet at 1.27 to 1.23 ppm indicated the presence of saturated methylene chains in the myristic acid residues. Finally, the triplet at 0.85 ppm corresponded to the terminal methyl groups (CH_3) of the fatty acid chains.

The ^{13}C-NMR spectrum **(SI4)** of compound **1** revealed key carbon environments. The ester carbonyl carbons (C=O) resonated far downfield at 173.1 (C1) and 173.5 ppm (C1'/C1''), confirming the presence of triglyceride ester linkages [23]. The glycerol backbone methylene carbons were seen at 62.3 and 69.0 ppm, with the central carbon being more deshielded. The myristic acid chains showed signals for the terminal methyl groups at 14.3 ppm. The methylene carbons were assigned as follows: those adjacent to the carbonyl at 34.4 ppm while those next to β methylene at 32.1 ppm. Then, the bulk of the interior methylene groups (CH_2) appeared as a clustered series of signals between 29.9 and 22.9 ppm.

Myristic acid (2) (5.0 mg, 2.6%) was isolated from the DCM extract of AC nut as a white solid. The GC-MS analysis **(SI5)** revealed a molecular ion peak at *m/z* 228.2, corresponding to the molecular formula of $C_{14}H_{28}O_2$. The FTIR spectrum **(SI6)** revealed strong signals at 2916 and 2850 cm^{-1} corresponding to C-H stretching of the methylene groups in the aliphatic chain. The sharp peak at 1707 cm^{-1} represented the C=O stretching vibration of the carboxylic acid group. Other characteristic signals included CH_2 bending at 1465 cm-1 and CH_2 rocking at 720 cm^{-1}.



Trimyristin **(1)**



Myristic acid **(2)**

Figure 2. Major compounds isolated from the AC DCM extract.

The ^{1}H-NMR spectrum **(SI7)** of 2 revealed a triplet at 2.32 ppm corresponding to the methylene protons adjacent to the carbonyl group (-$COCH_2$-). The multiplet at 1.61 ppm arose from the beta methylene group protons (-CH_2-CH_2-CO-), while the broad signal at 1.28 ppm (20H) corresponded to the repeating CH_2 units in the long hydrocarbon chain. Finally, a triplet at 0.87 ppm indicated the terminal methyl group (-CH_3). The ^{13}C-NMR spectrum **(SI8)** of 2 showed a key resonance at 180.85 ppm, corresponding to the carbonyl carbon (C=O) of the carboxylic acid. The peak at 34.57 ppm was associated with the methylene carbon directly adjacent to the carbonyl group (-$COCH_2$-). The peaks at 32.39, 30.14-29.53, and 25.13-23.13 ppm represented the methylene carbons along the hydrocarbon chain, while the signal at 14.57 ppm corresponded to the methyl carbon (-CH_3) signal. These proton and carbon signals collectively confirmed the saturated fatty acid structure of myristic acid [24]. The NMR data for both compounds are summarized in **Tables 4** and **5**.

Table 4. ^{1}H and ^{13}C NMR Data for Trimyristin (**1**).

Position	**δ^{13}C (ppm)**	**δ^{1}H (ppm)* (Int. mult. *J* in Hz) (CDCl3, 500MHz)**	**δ^{13}C (ppm)**	**δ^{1}H (ppm)Ω (Int. mult. *J* in Hz) (CDCl3, 500MHz)**
1	173.1	-	173.2	-
1'/1''	173.5	-	173.6	-
2/2'/2''	34.4	2.29 (6H, *m*)	34.5	2.29 (6H, *m*)
3/3'/3''	34.3	1.58 (6H, *m*)	34.3	1.57 (6H, *m*)
4/4'/4''	32.1		32.2	
5/5'/5''	29.9		29.9	
6/6'/6''	29.9		29.9	
7/7'/7''	29.8		29.7	
8/8'/8''	29.7	1.27-1.23 (60H, *m*)	29.6	1.29-1.20 (60H, *m*)
9/9'/9''	29.6		29.5	
10/10'/10''	29.5		29.3	
11/11'/11''	29.3		29.3	
12/12'/12''	25.1		25.1	
13/13'/13''	22.9		22.9	
14/14'/14''	14.3	0.85 (9H, *t*, 6.9)	14.4	0.85 (9H, *t*, 6.9)
CH_2-O	62.3	4.26 (2H, *dd*, 4.6, 12.0)	62.3	4.27(2H, *dd*, 4.0, 12.0)
		4.11 (2H, *dd*, 6.3, 12.1)		4.12 (2H, *dd*, 6.3, 12.0)
CH-O	69.0	5.33 (1H, *m*)	68.7	5.24(1H, *m*)

Note: * trimyristin (**1**); Ω data extracted from Standard trimyristin

Table 5. ^{1}H and ^{13}C NMR Data for Myristic acid (**2**).

Position	$\delta^{13}C$ (ppm)	$\delta^{1}H$ (ppm) * (Int. mult. *J* in Hz) ($CDCl_3$, 500MHz)	$\delta^{13}C$ (ppm)	$\delta^{1}H$ (ppm)Ω (Int. mult. *J* in Hz) ($CDCl_3$, 500MHz)
1	180.4	-	180.4	-
2	34.1	2.32 (2H, *t*, 7.4)	34.2	2.34 (2H, *t*, 7.4)
3	24.6	1.61 (2H, *m*)	24.7	1.68 (2H, m)
4	29.7		29.7	
5	29.6		29.7	
6	29.6		29.5	
7	29.5		29.5	
8	29.4	1.28 (20H, *m*)	29.4	1.2-1.4 (20H,*m*)
9	29.3		29.4	
10	29.3		29.3	
11	29.2		29.1	
12	31.9		32.0	
13	22.7		22.7	
14	14.1	0.87 (3H, *t*, 6.9)	14.2	0.88 (3H, *t*, 7.0)

Note: *Myristic acid (**2**), Ω data extracted from Wimalasena et al., 1994 [24]

PDE-5 Inhibition Activity

PDE-5 inhibitory activity was evaluated for the extracts of unripe and ripe AC husks and nuts, the DCM subfraction of unripe nuts (UND), and two major isolated compounds. The assay quantified the amount of 5'-GMP released, measured in triplicate, as an indicator of PDE-5 enzymatic activity. A lower 5'-GMP concentration reflects stronger PDE-5 inhibition, which corresponds to higher intracellular cGMP levels and, consequently, improved vasodilatory response.

Isobutyl methylxanthine (IBMX), a well-established PDE-5 inhibitor, served as the positive control to benchmark the activity of all test samples. Statistical comparisons were interpreted using standard significance levels (*** - highly significant, ** - moderate, * - mild, ns - not significant).

The extract exhibited no significant difference in activity compared to IBMX ($p > 0.05$), indicating that its response closely mirrored the positive control throughout the experiment and suggesting that the extract may possess comparable bioactive potential under the tested conditions.

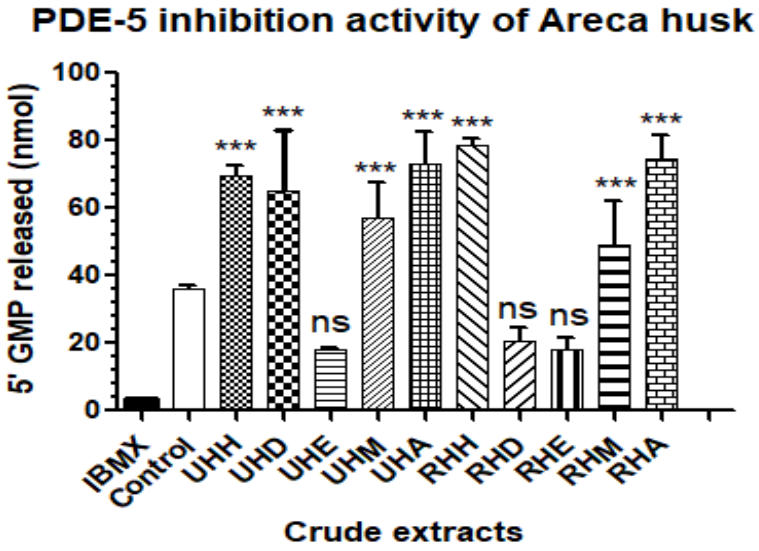
PDE-5 Inhibition Activity of Crude Husk and Nut Extracts

The crude extracts of the *Areca catechu* (AC) husk and nut exhibited statistically significant variations in PDE-5 inhibitory activity (Figure 3). Among the husk extracts**, UHE, RHD,** and **RHE** showed no significant difference relative to the reference inhibitor IBMX, although their percentage inhibitions were relatively low (**5 %**, **8 %,** and **−8.9 %**, respectively); thus these were excluded from further investigation. In contrast, other husk extracts displayed highly significant differences (***) compared with IBMX, indicating negligible PDE-5 inhibitory potential. The **UNH, UNM, UNA,** and **RNA** nut extracts (Figure 3b), also showed highly significant differences (***) relative to IBMX, reflecting limited inhibitory activity. Notably, the **RNH, RND**, and **RNE** extracts demonstrated PDE-5 inhibition comparable to IBMX, with inhibition rates of **63 %, 60 %,** and **64 %**, respectively **(Table 6)**. Overall, the screening results revealed that nut extracts possessed stronger PDE-5 inhibitory activity than husk extracts in both ripe and unripe samples, with the ripe nut extracts showing the highest inhibition. These findings highlight distinct bioactivity profiles influenced by solvent polarity and the maturity stage of the plant material. Based on its intermediate polarity and ethnomedicinal relevance, the unripe nut dichloromethane (UND) extract was selected for further fractionation due to its intermediate polarity, which enables efficient extraction of semi-polar bioactive constituents commonly associated with enzyme inhibition. In addition, the use of unripe *Areca catechu* nuts is supported by ethnomedicinal practices that link this plant part to vitality enhancement and aphrodisiac effects, suggesting the presence of potential PDE-5 inhibitory compounds.

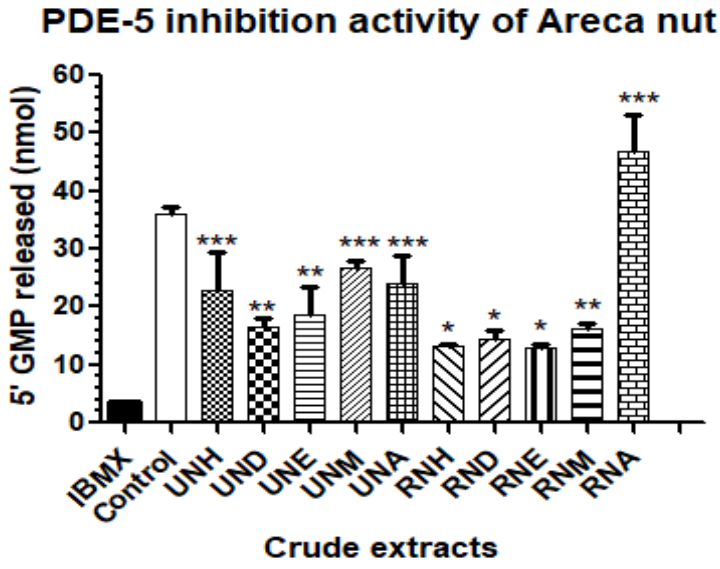
Table 6. Percentage inhibition and amount of 5' GMP released from crude extracts of AC.

No.	Crude extract	Code	Amount (nmol)	Percentage of inhibition (%)
Control			36.080	0
IBMX			3.690	90
Unripe husk				
1	Hex	UHH	54.601	-51
2	DCM	UHD	65.294	-81
3	EA	UHE	34.070	5
4	MeOH	UHM	57.018	-58
5	AQ	UHA	72.904	-117
Ripe husk				
6	Hex	RHH	59.763	-66
7	DCM	RHD	32.895	8
8	EA	RHE	39.289	-8.9
9	MeOH	RHM	48.886	-35
10	AQ	RHA	74.820	-107
Unripe nut				
11	Hex	UNH	38.947	-8
12	DCM	UND	22.140	39
13	EA	UNE	30.360	16
14	MeOH	UNM	26.654	26
15	AQ	UNA	24.158	33
Ripe nut				
16	Hex	RNH	13.105	63
17	DCM	RND	14.557	60
18	EA	RNE	12.961	64
19	MeOH	RNM	16.132	55
20	AQ	RNA	46.706	-29

RNH: Ripe nut hexane; **RND**: Ripe nut dichloromethane; **RNE**: Ripe nut ethyl acetate; **RNM**: Ripe nut methanol; **RNA**: Ripe nut aqueous; **RHH**: Ripe husk hexane; **RHD**: Ripe husk dichloromethane; **RHE**: Ripe husk ethyl acetate; **RHM**: Ripe husk methanolic; **RHA**: Ripe husk aqueous; **UNH**: Unripe nut hexane; **UND**: Unripe nut dichloromethane; **UNE**: Unripe nut ethyl acetate; **UNM**: Unripe nut methanolic; **UNA**: Unripe nut aqueous; **UHH**: Unripe husk hexane; **UHD**: Unripe husk ethyl acetate; **UHE**: Unripe husk ethyl acetate; U**HM**: Unripe husk methanolic; **UHA**: Unripe husk aqueous.



(a) Husk



(b) Nut

Figure 3. PDE-5 inhibition activity of AC crude extracts: (a) husk, B) nut. Statistical analysis compared to isobutyl methylxanthine (IBMX): *** - highly significant, ** - moderate, * - mild significance, ns - non-significant.

PDE-5 Inhibition Activity of Subfractions and Isolated Compounds

The results of PDE-5 inhibitory activity screening of subfractions UND 1-8 are shown in **Table 7** and **Figure 4.** These indicate that UND 3 was the most active, showing no significant difference compared to the positive control, IBMX. UND 3 released 9.105 nmol (41 % inhibition) of 5' GMP, while IBMX released 3.690 nmol (79 %). Inhibition of PDE-5 increases the production of cGMP, which promotes tissue relaxation and vasodilation, particularly in the corpus cavernosum of the penis, thereby improving blood flow and enhancing erectile function [25]. Subfractions UND 1, UND 2, UND 5, and UND 7 showed highly significant differences from IBMX, suggesting limited potential for PDE-5 inhibition.

Based on its relatively strong PDE-5 inhibitory activity (41 %), UND 3 was subjected to further isolation and characterization. Subsequent purification using recrystallization and column chromatography (CC) yielded two major compounds: trimyristin (1) and myristic acid (2). Crucially, neither trimyristin (1) nor myristic acid (2) demonstrated PDE-5 inhibitory activity; they showed highly significant differences compared to IBMX. This finding suggests that other minor compounds may be responsible for the observed inhibitory effects. While trimyristin (1) is not directly known to treat ED, it may offer indirect benefits

related to cardiovascular health due to its fatty acid composition. For example, a study by Astley et al. (2021) demonstrated that resveratrol (RV), when delivered via nanostructured lipid carriers (NLCs) composed of trimyristin, can be utilised in antihypertensive treatments [26]. Additionally, research by Okorie (2022) revealed that myristic acid and lauric acid, both present in virgin coconut oil, could alleviate benign prostatic hyperplasia (BPH) by inhibiting testosterone-induced BPH development [27]. These findings suggest the potential of the isolated major compounds to exert synergistic effects, warranting further investigation to explore this hypothesis.

Table 7. Percentage inhibition of UND subfractions and compounds and amount of 5' GMP released.

No	Crude extract	5' GMP Released (nmol)	Percentage inhibition (%)
Control		33.618	0
IBMX		3.690	90
Subfraction Unripe nut Dichloromethane (UND)			
1	UND 1	28.020	-79
2	UND 2	18.082	-16
3	UND 3	9.105	42
4	UND 4	14.958	4
5	UND 5	17.874	-14
6	UND 6	13.058	16
7	UND 7	15.846	-1
8	UND 8	10.930	30
Trimyristin (1)		17.76	-13
Myristic acid (2)		18.22	-16

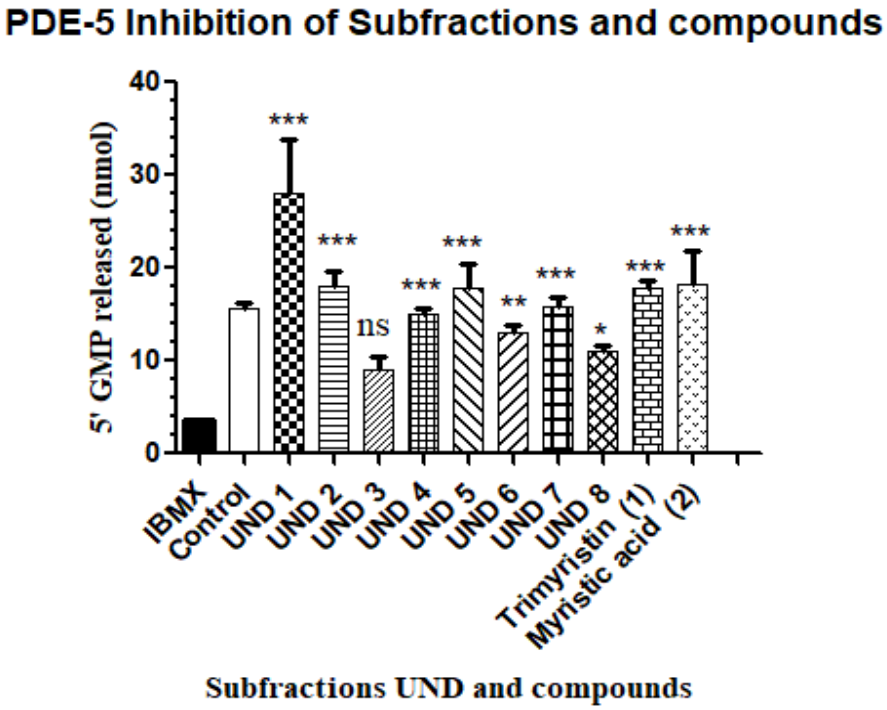


Figure 4. PDE-5 inhibition activity of subfractions and compounds. Statistical analysis was compared to isobutyl methylxanthine (IBMX): *** - highly significant, ** - moderate, * - mild significance, ns - non-significant.

CONCLUSIONS AND RECOMMENDATIONS

The PDE-5 inhibitory activity of twenty crude extracts, derived from both unripe and ripe AC husks and nuts, was evaluated. Nonpolar and intermediate-polar extracts of the ripe nut were found to exhibit significant PDE-5 inhibitory activity compared to the reference inhibitor, IBMX. These results underscore the influence of both solvent polarity and nut maturity on the bioactivity of the extracts. Although the major compounds isolated from subfraction UND 3, trimyristin (1) and myristic acid (2), were inactive, the subfraction itself demonstrated no significant PDE-5 inhibition compared to IBMX. This suggests that minor bioactive compounds within the crude extract were responsible for the observed effect, highlighting the complex phytochemical composition of the plant. These findings characterise *Areca catechu* as a potential natural source of a PDE-5 inhibitor, as an alternative treatment against erectile dysfunction. However, one limitation of this study was that the nonpolar and intermediate-polar extracts or compounds exhibited poor solubility, which restricted further evaluation. Due to synergistic effects, future studies should focus on bioassay-guided fractionation, isolating the minor constituents and conducting *in vivo* investigations.

ACKNOWLEDGEMENTS

The authors would like to thank the Fundamental Research Grant Scheme (FRGS) for its financial support from the Government of Malaysia, Ministry of Higher Education (FRGS/1/2020/WAB13/UPSI/02/1). The authors are also deeply grateful to the Department of Chemistry, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris and the Cell Signalling and *in vivo* Research Laboratory, Forest Research Institute Malaysia (FRIM) for providing all necessary facilities to carry out this research.

REFERENCES

1. Sharma, M., Arya, D., Bhagour, K. & Gupta, R. S. (2017) Natural aphrodisiac and fertility enhancement measures in males: A review. *Current Medicine Research and Practice*, **7(2)**, 51–58. https://doi.org/10.1016/j.cmrp.2017.02.007.

2. Laumann, E. O., Paik, A. & Rosen, R. C. (1999) Sexual dysfunction in the United States: Prevalence and predictors. *Journal of the American Medical Association*, **281(6)**, 537–544. https://doi.org/10.1001/jama.281.6.537

3. Aytaç, I. A., Mckinlay, J. B. & Krane, R. J. (1999) The likely worldwide increase in erectile dysfunction between 1995 and 2025 and some possible policy consequences. In *BJU International*, **84**, 50–56.

4. Lou, I. X., Chen, J., Ali, K. and Chen, Q. (2023) Relationship between hypertension, anti-hypertensive drugs, and sexual dysfunction in men and women: A literature review. *Vascular Health and Risk Management*, **19**, 691–705.

5. Defeudis, G., Mazzilli, R., Tenuta, M., Rossini, G., Zamponi, V., Olana, S., Faggiano, A., Pozzilli, P., Isidori, A. M. and Gianfrilli, D. (2022) Erectile dysfunction and diabetes: A melting pot of circumstances and treatments. *Diabetes/Metabolism Research and Reviews*, **38(2)**, 1–25.

6. Milam, D. F. (2023) Male erectile dysfunction. In D. Robertson, I. Biaggioni, G. Burnstock, P. A. Low, and J. F. R. Paton (Eds.). *Primer on the autonomic nervous system 4th ed., Academic Press*, 691–695.

7. Sansone, A., Di Dato, C., de Angelis, C., Menafra, D., Pozza, C., Pivonello, R., Isidori, A. and Gianfrilli, D. (2018) Smoke, alcohol, and drug addiction and male fertility. *Reproductive Biology and Endocrinology*, **16(3)**, 1–13.

8. Gokce, M. I. and Yaman, O. (2017) Erectile dysfunction in the elderly male. *Turkish Journal of Urology*, **43(3)**, 247–251.

9. The Star (2023) Fertility problems among men in Malaysia alarming, says deputy minister, April 5, 2023. https://www.thestar.com.my/news/nation/2023/04/05/fertility-problems-among-men-in-malaysia-alarming-says-deputy-minister.

10. Nordin, R., Soni, T., Kaur, A., Loh, K. P. & Miranda, S. (2019) Prevalence, and predictors of ED in adult male outpatient clinic attendees in Johor, Malaysia. *Singapore Medical Journal*, **60(1)**, 40.

11. Medicines and Healthcare products Regulatory Agency (2017).

12. Singh, R., Kumar Gupta, A., Jainand, A. & Kakar, S. (2018) Review Article Traditional medicinal plants as scientifically proven Aphrodisiacs. *International Journal of Health and Biological Sciences*, **1(1)**, 29–36. https://doi.org/10.30750/IJHBS.1.1.6.

13. Fauzi., Widodo, H. & Haryanti, S. (2018) Kajian Tumbuhan Obat yang Banyak Digunakan untuk Aprodisiaka oleh Beberapa Etnis Indonesia. *Media Litbangkes*, **(29)1**. DOI: https://doi.org/10.22435/mpk.v29i1.466.

14. Anthikat, R. R. N., Micheal, N. & Ignacimuthu, S. (2013) Aphrodisiac effect of *Areca catechu* L.

and Pedalium murex in Rat. *Journal of Mens Health*, **10(2)**, 65–70.

15. Rahman, A. O., Dewi, H., Geniro, B. B. & Amdely, E. V. (2022) Evaluation of the Antifertility Effect and Toxicity of Areca nut as Oral Contraceptive: Study on Male Rats. *Jurnal Kedokteran Brawijaya*. https://doi.org/10.21776/ub.jkb.2022.032.01.3

16. Rahman, A. O., Purwakanthi, A. & Dewi, H. (2020) Antifertility effect of betel nut (Areca catechu L) in male rat. *MEDISAINS*, **18(2)**, 52. https://doi.org/10.30595/medisains.v18i2.7588.

17. Setyowati, H., Rahayu, E., Wijayanti, K. & Dianita, P. S. (2019) *International Journal of Research In*, **10(4)**, 3755–3760.

18. Hugar, P., Dutta, A., Srilakshmi, S., Belur, P. D., Raval, K. and Iyyaswami, R. (2024) Phenolic profile of unripe *areca* nuts cultivated in various districts of Karnataka, India. *JSFA Reports*, **4(2)**, 102–113.

19. Olubiyi, M. V., Kawu, M. U., Magaji, M. G., Salahdeen, H. M. and Magaji, R. A. (2022) Influence of lauric acid on the relaxation of corpus cavernosum in streptozotocin-induced diabetic male Wistar rats. *Future Journal of Pharmaceutical Sciences*, **8**, 60.

20. Saha, I., Das, J., Maiti, B. and Chatterji, U. (2015) A protective role of arecoline hydrobromide in experimentally induced male diabetic rats. *Biomed Research International*, 1–12.

21. Alev, D. Y., Serap, O., Kaya-sezginer, Á. A. C. E., Volkan, C., Murat, O., Eugenia, S., Caryophyllus, L. & Syzygium, L. (2020). The beneficial effect of clove essential oil and its major component, eugenol, on erectile function in diabetic rats. *March*, 1–8. https://doi.org/10.1111/and.13606.

22. Kapelle, I. B. and Laratmase, M. S. (2014) Trimyristin isolation from nutmeg and synthesis of methylester using heterogen catalyst. *Indonesian Journal of Chemical Research*, **2(1)**, 160–165.

23. Hakim, A., Jamaluddin and Al Idrus, S. W. (2021) Isolation of trymiristin from *Myristica fragrans* for natural product chemistry laboratory. *IOP Conference Series: Earth and Environmental Science*, **913**, 1–4.

24. Wimalasena, S. & Karunawansha, E. (1994) Characterization of A New Aryl Alkanone and Other Compounds Present In Horsfieldia Irya Seeds. In *J. Natn. Sci. Coun. Sri Lanka*, **22(3)**.

25. Burnett, A. L. (2006) The role of nitric oxide in erectile dysfunction: Implications for medical therapy. *Journal of Clinical Hypertension (Greenwich)*, **8(12 Suppl 4)**, 53–62.

26. Astley, C., Houacine, C., Zaabalawi, A., Wilkinson, F., Lightfoot, A. P., Alexander, Y., Whitehead, D., Singh, K. K. and Azzawi, M. (2021) Nanostructured lipid carriers deliver resveratrol, restoring attenuated dilation in small coronary arteries via the AMPK pathway. *Biomedicines*, **9(12)**, 1852–1869.

27. Okorie, C. C., Onyekwelu, K. C., Okorie, C. O., Ikekpeazu J. E. and Ezeagu, E. I. (2022) Effect of virgin coconut oil, lauric acid, and myristic acid on serum and prostatic markers of benign prostatic hyperplasia. *Tropical Journal of Pharmaceutical Research*, **21(4)**, 809–815.