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ORIGINAL RESEARCH

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Counterfeiting honey with phosphodiesterase inhibitors for sexual activity enhancement: detection using high-performance liquid chromatography coupled with a photodiode array detector (HPLC-PAD)

Abdullah M. Alkhalaf[1], Fouza K. Alenazi[1], Mubarak M. Abudahash[1], Reem Alfadhli[1], Ghadah H. Altoum[1], Naif D. Alenzi[1,*]

[1]Research and Laboratories Sector, National Drug and Cosmetic Control Laboratories (NDCCL), Saudi Food and Drug Authority, P.O Box 7148, 13513 Riyadh, Saudi Arabia

***Correspondence**
NDenzi@sfda.gov.sa
(Naif D. Alenzi)

Abstract

Globally, there have been several reports of natural products contaminated with illegal adulterants that threaten consumer health because of their adverse pharmacological effects. To treat erectile dysfunction in men, herbal medicine is often adulterated with synthetic phosphodiesterase type 5 (PDE-5) inhibitors. Honey, which is popularly used for its health benefits, is subjected to adulteration with PDE-5 inhibitors. In this study, a rapid and reliable analytical method was proposed to determine the level of adulteration in honey samples. A total of 18 honey samples were tested for the presence of PDE-5 inhibitors (sildenafil, tadalafil and vardenafil) using a high-performance liquid chromatographic method coupled with a photodiode array detector (HPLC-PAD). Chromatography was performed using the Agilent ZORBAX SB-C18 column, with acetonitrile and buffer in the ratio of 1:4 as the mobile phase. Sildenafil, tadalafil and vardenafil reference standards were used for calibration. The retention times of the standards were 21.67, 18.15 and 21.30 min respectively. Of the total samples tested, 55% were positive for PDE-5 inhibitors. Six samples contained sildenafil, three samples contained tadalafil and one sample contained vardenafil. The findings from this study demonstrate that the HPLC-PAD method is suitable and economical for screening PDE-5 inhibitors as adulterants in honey samples marketed for use as sexual activity enhancers. Regular inspections of adulterated products are thus warranted to ensure the safety and health of consumers.

Keywords

Honey; Adulteration; Erectile dysfunction; Phosphodiesterase type 5 inhibitors; Sildenafil; Tadalafil; Vardenafil

1. Introduction

Honey, a precious food commodity, is regarded as nature's best and powerful superfood owing to its high nutritive and therapeutic value besides its deliciousness [1]. Honey, which is a supersaturated solution of sugars and comprises mainly fructose (38%) and glucose (31%), is a treasure trove of various pharmacologically active compounds [2, 3]. The type of floral source, seasonal and environmental conditions, processing and preservation of honey are a few of the factors that augment its flavor. Honey, a natural product that heals various health problems, has been used widely since ancient times. Historically, honey has been used to treat cataracts, ulcers, and wounds owing to its antibacterial action [4]. The high sugar content in honey exerts a bactericidal effect on the growth of various microorganisms, especially methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *enterococci* [5, 6]. Several researchers have reported the beneficial effect of honey in the treatment of chronic diseases, such as cancer and inflammatory diseases, and also in ameliorating the risk of cardiac failure [7, 8]. Moreover, honey's antiallergic, antithrombotic and vasodilatory properties have been proven [9]. The therapeutic potential of honey is attributed to the presence of various antioxidant molecules, including phenolic compounds, flavonoids, and phenolic acids [10].

In the reproductive field, honey has been known to enhance fertility. In the past 30 years, a significant decline in reproductive health and fertility rates has been observed worldwide owing to the exposure to food and environmental toxins in our modern lifestyle [11]. Research indicates that the proportion of men suffering from various forms of erectile dysfunction (ED) is expected to double by 2025, with men in developing countries of Africa and Asia mostly affected [12]. The decreased testosterone levels in men with aging are linked to ED [13]. ED is one of the growing medical concerns in men, ranging from 2% in men <40 years of age to 86% in those

≥80 years [14]. During sexual activity, an inefficiency in maintaining the erection is termed ED [15]. PDE-5 inhibitors are recommended as the first-line treatment. Other methods include the administration of testosterone [15].

In the treatment of fertility-related issues, the field of complementary and alternative medicine has been revolutionized with the advent of natural or herbal products. Importantly, honey has been recognized for improving infertility issues in men by maintaining sperm quality [16, 17] and restoring testosterone levels [18]. Honey has also been reported to eliminate toxins from the body [19]. Currently, honey is an attractive target for commercial fraudsters owing to its economic value in offering health benefits. As a result, honey is adulterated with low-quality sweeteners and mislabeled. Food adulteration is a common practice worldwide, except for a few countries. Honey is frequently adulterated with PDE-5 inhibitors illegally to enhance sexual performance as a treatment for ED [20].

Honey is regarded as one of the most prominent indigenous medicines. According to international food standards by Codex Alimentarius and other standards, including Saudi Food and Drug Authority's standards (SFDA.FD/GSO 147: 2021), honey is a pure product to which other substances should not be added [1]. However, the U.S. Food and Drug Administration (FDA) has identified the presence of tadalafil and sildenafil in certain honey-based products from different companies and has warned them against the sale of such adulterated products as this could be detrimental to human health [21].

Current medications for ED include oral medications such as PDE-5 inhibitors. Sildenafil, the first PDE-5 inhibitor, was introduced in 1998. More than 20 million men were treated with sildenafil in its first 6 years in the market [19]. The PDE5 inhibitors widely used to treat sexual dysfunction include sildenafil citrate (Viagra), vardenafil hydrochloride (Levitra), and tadalafil (Cialis) [22, 23]. Sildenafil has been licensed for clinical use in treating ED as it can improve penile erectile function. This drug can enhance the relaxation of the corpus cavernosum. Chemically, it is (1-{[3-(6,7-Dihydro-1-methyl-7-oxo-3-isobutyl-1H-pyrazolo[4,3-d] pyrimidin-5-yl)-4-ethoxyphenyl] sulfonyl}-4-methylpiperazine) [24]. In 2003, the FDA approved two other PDE-5 inhibitors for treating ED, namely, vardenafil hydrochloride (Levitra) and tadalafil. Vardenafil is chemically 2-[2-ethoxy-5-(4-ethyl-piperazine-1-sulfonyl)-phenyl]-5-methyl-7-propyl-3H-imidazo[5,1-f]-[1,2,4]triazin-4-onemonohydrochloride, trihydrate and tadalafil is chemically pyrazino [1′,2′:1,6]pyrido[3,4-b]indole-1,4-dione,6-(1,3-benzodioxol-5-yl)-2,3,6,7,12,12a-hexahydro-2-methyl-,(6R-trans)-(6R-,12aR)-2,3,6,7,12,12a-hexahydro-2-methyl-6-[3,4-(methylenedioxy)phenyl]-pyrazino[1′,2′:1,6]pyrido[3,4-b]indole-1,4-dione [24]. During sexual arousal, the formation and accumulation of cyclic guanosine monophosphate (cGMP) leads to smooth muscle relaxation in the corpus cavernosum, thereby increasing the blood flow to the penis. cGMP is degraded by the enzyme PDE-5 in the smooth muscle of the corpus cavernosum. Intriguingly, an increased influx of cGMP prolongs penile erection [25]. PDE-5 inhibitors that are structurally similar to cGMP block its hydrolysis *via* competitive inhibition and thereby maintain cGMP levels required for prolonged erection [25].

Currently, several commercial products, such as herbal preparations, health foods, and dietary supplements, are available in the market to manage this medical disorder. Honey-based products are sold commercially as sexual enhancers to treat ED. Sildenafil citrate marketed as Viagra, a medicine for the treatment of ED in men, is quite often added to honey-based products and marketed to the public to increase their popularity [16]. Adulteration of drugs is an illegal practice; such products may be harmful to the health of consumers as the ingredients and the dosage of drugs are unknown [23]. Most of these preparations seem to be popular as people believe that these products are sourced from nature and are therefore safe and associated with better health outcomes than modern pharmaceutical preparations [26]. Adulterated products marketed as "natural" or "herbal" remedies for ED often contain sildenafil or other PDE-5 inhibitors beyond the permissible limit. Although PDE-5 inhibitors are accepted for the treatment of ED, their incorrect use can result in side effects such as headache, flushing, dyspepsia, nasal congestion, and rhinitis [27]. Therefore, monitoring the distribution of PDE-5 inhibitor drugs should be intensified. Furthermore, patients on other medications should take additional care because severe hypotension and syncope have been reported in patients taking nitrate medications and PDE-5 inhibitors [28, 29]. Fatal cases caused by adulterated dietary supplements have also been reported [30, 31]. Aphrodisiac drugs added to honey by the manufacturers are sometimes undetected using routine testing methods, such as the examination of honey density in water and detection of hydroxymethylfurfural, a commonly used honey adulterant. Several analytical methods, such as high-performance liquid chromatography (HPLC), gas chromatography (GC), and mass spectroscopy (MS), are used in the screening of PDE-5 inhibitors to assess the authenticity of honey. However, each method has its own advantages and disadvantages [24, 32–38]. GC-MS is suitable for the analysis of volatile compounds with high sensitivity. Nonetheless, its disadvantage is that it has limited applicability as some of the non-volatile and thermally unstable compounds are not well-suited for GC-MS analysis. Nuclear magnetic resonance (NMR) is a non-destructive technique that allows sample recovery after analysis. One of the prominent applications of this technique is that it provides structural information about the compounds in honey. However, the instruments used in NMR are often expensive, large, and require specialized facilities. Moreover, its sensitivity is lower than that of MS. HPLC is a versatile technique with wide applicability. This technique permits the separation and quantification of a wide range of compounds in honey with high sensitivity and selectivity. The method can be adapted for various detectors, making it versatile for several analytes. HPLC is suitable for the analysis of sugars, organic acids, phenolic compounds, and other chemical markers relevant to the authenticity of honey. The method applies to both polar and non-polar compounds, which makes it suitable for analyzing a diverse range of chemical components present in honey. However, a certain level of expertise is required to operate and optimize the system for specific analyses, which constitutes its limitation. Moreover, the matrix used

may interfere with the analysis, and it is also expensive and difficult to maintain. Although HPLC has its own challenges, its versatility, quantitative capabilities, and wide applicability make it a suitable method for the authentication of honey. Based on the above perspectives, more studies are required to effectively regulate herbal medicines and protect consumers from the emerging threat of adulteration. The Saudi Food and Drug Authority aims to ascertain the quality and safety of food products and monitor adulteration in these products. Hence, this study was undertaken to screen the presence of PDE-5 inhibitors in different honey-based products marketed for use in the treatment of ED.

2. Materials and methods

2.1 Reference standards

The reference standards for sildenafil citrate (Y0001578, Sigma, Jordan), tadalafil (LRAB8867, Sigma, Jordan) and vardenafil (SML2103, Sigma, Jordan). Other chemicals used were monobasic potassium phosphate (17338401, Scharlau, Spain), dibasic sodium phosphate dihydrate (0000703155, Panreac, Italy) and acetonitrile; HPLC grade (1672630 306, Sigma, Jordan).

2.2 Instrumentation

Equipment used was HPLC with a photodiode array (PN: 7995218-585, HPLC PROMINENCE-i 2030C 3D, Shimadzu, Japan) with PAD, sonicator (Elma Elmasonic P300H) and column (ZORBAX SB-C18 250 × 4.6 mm, Agilent, USA) was used.

2.3 Standard solutions

Standard solutions were prepared by dissolving each reference standard in methanol to obtain a concentration of 1 mg/mL and stored at −20 °C. Intermediate standard solutions were prepared by dissolving each standard solution in a mixture of 20% acetonitrile and buffer to obtain the desired concentrations. Sildenafil citrate 1 and 2 were prepared at concentrations of 0.395 mg/mL and 0.266 mg/mL, respectively. Similarly, tadalafil 1 and 2 at concentrations of 0.183 mg/mL and 0.160 mg/mL, respectively, and vardenafil 1 and 2 at concentrations of 0.153 mg/mL and 0.194 mg/mL, respectively, were prepared.

2.4 Sample collection

At alpha 0.05 with effect size 0.75 and power 0.85, the total sample size determined was 18. Eighteen honey samples, which were manufactured in different countries and marketed as aphrodisiacs containing natural ingredients, were collected. Samples were randomly obtained from medicine and spice shops and on social media. Both locally manufactured and imported herbal products were considered, and different dosage forms were sampled, including powders, liquids, capsules, and tablets.

2.5 Sample preparation

Approximately 1.0–1.25 g of the sample was dissolved in 20 mL of 20% acetonitrile, sonicated for 45 min at 37 °C, followed by the addition of 40 mL of buffer (80% NaH_2PO_4 buffer), and sonicated further for 45 min at 37 °C. The separation of the two phases was achieved *via* centrifugation (6000 rpm, 20 min), after which the supernatant was filtered through a 0.2 μm nylon syringe filter to be placed in HPLC autosampler vials.

2.6 Analysis method

For chromatographic separation, the Agilent ZORBAX SB-C18 column (250 × 4.6 mm) was used. The system was equipped with an autosampler, degasser, quaternary solvent delivery unit and PAD. The chromatograms were analysed and integrated using LabSolutions software (Version 5.3, Shimadzu Corporation, Japan). The mobile phase comprised a binary system that included two components: phase A and phase B. Mobile phase A comprised sodium dihydrogen phosphate buffer and mobile phase B comprised acetonitrile. The flow rate was adjusted to 1 mL/min. The column temperature was set at 45 °C during the analysis. The injection volume of the sample solution was 10 μL. Gradient elution was performed as follows: At time 0 min, mobile phase A was pumped at the rate of 80%, the system was kept constant as stated for the next 2 min, which was then decreased linearly to 20% for 22 min, and the system remind stable as stated for 27 min. The detection wavelength was set at 290 nm for sildenafil, 285 nm for tadalafil and 242 nm for vardenafil. The PDE-5 inhibitors were detected by comparing the retention time (RT) of the spiked sample solution with that of the standard solutions in the chromatograms. On the contrary, quantitative analysis was performed by calculating the peak areas of the spiked sample and standard solutions [39].

2.7 Method validation

The linearity and dynamic range of the method were assessed using a series of standard solutions (Table 1). A wide range of concentrations of vardenafil, sildenafil and tadalafil were injected multiple times to construct calibration curves, and the correlation coefficient, y-intercept and slope were calculated. Furthermore, average RT was determined. Method precision was measured using various dosage forms and determined by comparing the assay values with those obtained using the manufacturer's methods [39].

3. Results and discussion

The hallmark finding of this report was that the 18 honey samples purchased online or from specialized stores were reported to contain PDE-5 inhibitors. For quantification, the RT of reference standard PDE-5 inhibitors was compared with that of the analysed honey samples. The precision value (relative standard deviation), RT and recovery value of the standards are presented in Table 1. The recoveries ranged from 98.39% to 99.70%. The RT of the sildenafil standard was 21.67 min, tadalafil was 18.15 min and vardenafil was

TABLE 1. Precision (RSD) values of standards.

Name of the standard	Retention time (Average of five injections)	Relative standard deviation (RSD) %	Tailing factor (Average of five injections)	Efficiency (Average of five injections)	Recovery %	Drift %
Sildenafil	21.676	0.030	1.378	111,399	98.39	98.64
Tadalafil	18.156	0.537	1.346	68,825	99.70	99.93
Vardenafil	21.307	0.050	1.341	73,178	99.23	100.90

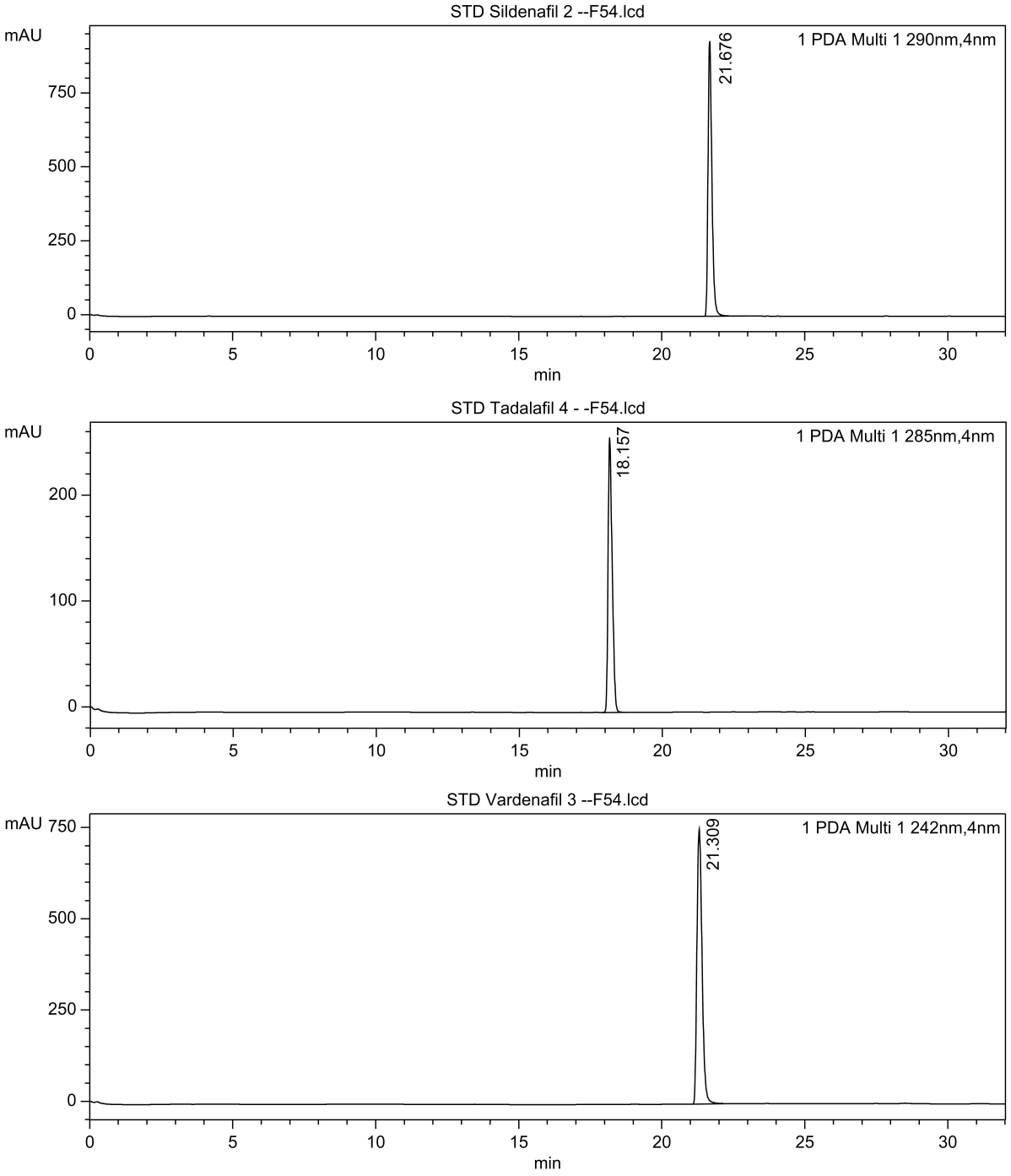


FIGURE 1. Chromatogram profiles of the reference standards sildenafil, tadalafil and vardenafil with their corresponding retention times. STD: Standard; PDA: Photodiode Array.

21.30 min. Fig. 1 depicts the chromatogram of the reference standards. The chromatograms of the honey samples that tested positive for PDE-5 inhibitors are shown in Figs. 2,3. Ten samples were positive for PDE-5 inhibitors, and sildenafil citrate was the most common compound found in the samples. While six samples were adulterated with sildenafil, tadalafil was detected in three samples and vardenafil in one sample. The remaining eight samples were negative. The sample characteristics analysed using HPLC-PAD and the quantity of the fortified sample are shown in Table 2. Based on data from this investigation, the HPLC-PAD method was effective in determining the concentrations of aphrodisiacs in honey products.

The results obtained from this study agree with those from previous studies [24, 40–42]. Adulteration of several herbal medicines and other herbal products with PDE-5 inhibitors has been reported. However, identifying adulteration in honey during aphrodisiac testing is rare. In a study by Sirhan *et al.* [40] (2023), honey products were observed to be adulterated with PDE-5 inhibitors, which were observed in the range of 93.0%–103.3%. The most frequently encountered adulterant in our study was sildenafil, a result supported by the outcome analysis of other studies on aphrodisiac compounds present in honey used to treat ED [24, 41, 42]. Similarly, in a French study by AbdelShakour *et al.* 2021 [24], 47% of the products from China, Taiwan, Estonia and Spain were positive for PDE-5 inhibitors, and 29.41% of the samples contained sildenafil as the major adulterant.

Literature review revealed that several studies had examined the chemical composition of honey (sucrose and hydroxymethylfurfural levels) to determine adulteration. Furthermore, several studies have investigated the biological properties of honey directly or as nanoparticles [43–46]. Adulteration of several herbal medicines and other herbal products with PDE-5 inhibitors has been reported, but reports on the adulteration of honey with aphrodisiacs are scarce [24, 42]. Manufacturers take all possible efforts to mask the presence of PDE-5 inhibitors in sex-enhancement dietary supplements as they advertise and sell these products as natural and safe. Therefore, this study was conducted to determine the presence of aphrodisiacs in honey samples using a simple and economical method.

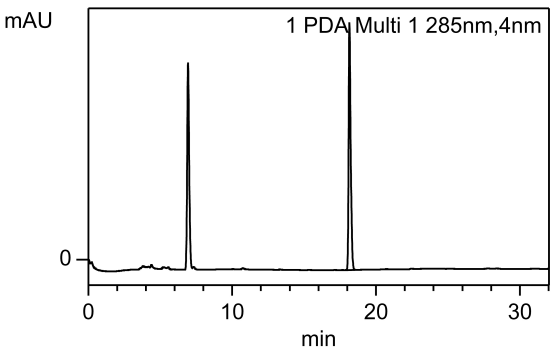
Honey products analysed in this study were those marketed as natural sexual enhancers and sold unofficially on social media or illegally in local markets. Honey samples obtained were from manufacturers from different countries such as Saudi Arabia, Malaysia, India, Turkey, Korea, Jordan and Australia. The findings indicated that most of the adulterated samples were from Malaysia (4 of 10 samples tested positive for PDE-5 inhibitors). Sildenafil was the most common PDE-5 inhibitor added to honey as an aphrodisiac. None of the samples tested from Saudi Arabia were positive for aphrodisiac drugs. These results are in line with those from previous studies [47, 48]. In Saudi Arabia, Haider *et al.* [48] (2017) investigated adulteration in herbal medicine and food supplements. Sildenafil was found to be the most commonly used adulterant, and tadalafil was detected at the highest concentration (39 wt%) in Malaysian honey [48]. A validated HPLC method to detect sildenafil in herbal dietary supplements was reported by Dural (2020) [49]. Other studies from Malaysia and Kenya have also reported the use of PDE-5 inhibitors for sexual enhancement [50, 51]. In a Kenyan study, herbal medicines used as sex enhancers were evaluated for adulteration with PDE-5 inhibitors, which revealed that approximately 60% of the samples contained sildenafil [51].

Adulteration of honey meant for use as a sex enhancer is intensively practiced worldwide. In a Hong Kong-based study, approximately 58% of the honey samples were found to be adulterated [52]. Adulterating honey with PDE-5 inhibitors and their analogues for sex enhancement has been reported in the Czech Republic too [53]. Similarly, ultrahigh-performance liquid chromatography with quadrupole time-of-flight MS has been shown to efficiently detect the presence of 23 illegal adulterated aphrodisiac chemical ingredients in food and traditional patent medicines by Wang *et al.* [54] (2018).

The maximum daily permissible dose of sildenafil recommended for the treatment of ED is 100 mg and that of tadalafil and vardenafil is 20 mg each administered orally 1 h before intercourse [55]. Honey products are adulterated way above the daily allowable dose of PDE-5 inhibitors, which poses a major health risk to the consumer. Honey, deliberately adulterated with aphrodisiac chemicals, is often packaged as natural and without any declaration of added adulterants. This deception of the consumer poses an immense health risk. The undeclared components threaten the health of high-risk patients who are on treatment for diabetes, hyperlipidaemia or heart disease and are even fatal in some cases. The popularity of the social media and the increase in the number of satellite channels that are now available in almost every home have provided platforms for drug counterfeiters to promote their unscrupulous products. People tend to be deceived that aphrodisiac drugs are natural products, have no side effects, are suitable for patients with heart problems and diabetes, and are effective in treating ED and premature ejaculation as they are advertised in such a manner. Thus, strict guidelines should be implemented to check the quality of such adulterated foods.

4. Conclusions

Adulteration of honey with PDE-5 inhibitors was successfully detected in this study using the HPLC-PAD method. It is a simple, accurate and rapid screening tool to test adulterated samples and can be applied successfully to commercial and suspiciously marketed brands too. A significant proportion of honey samples marketed for use as sex enhancers was observed to be adulterated with PDE-5 inhibitors. The maximum number of positive samples was from Malaysia. Distressingly, none of the products listed the adulterants on their labels, thereby putting consumers at risk and further raising health concerns. Compared with other sophisticated methods, such as GC-MS and NMR, the HPLC-PAD method could be applied as a routine quality check for detecting adulteration with PDE-5 inhibitors in various honey products intended to boost sexual activity in men. However, there are some limitations associated with the detection of adulterated honey using HPLC. Honey is a complex matrix containing various components, which may interfere with the chromatographic analysis. Furthermore, matrix effects can affect the accuracy

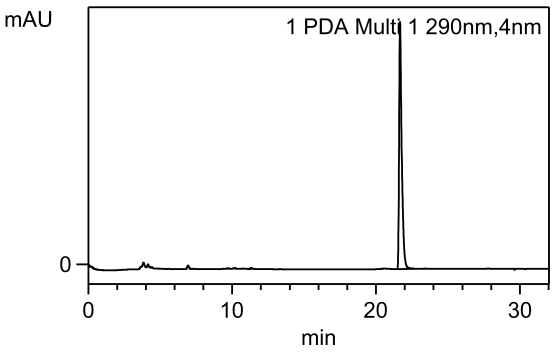


PDA Ch1 285nm

Peak#	Ret. Time	Name	Area	Height
1	18.153	Tadalafil	932248	95658
Total			932248	95658

PDA

Sample No.1 Adulterated with Tadalafil

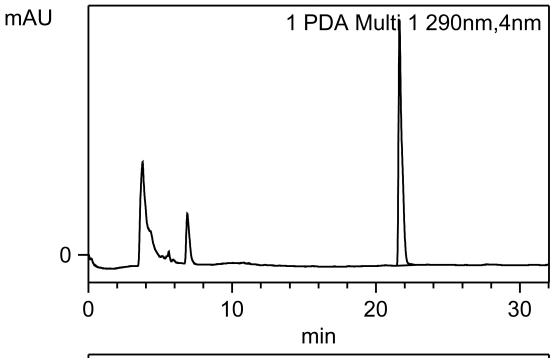


PDA Ch1 290nm

Peak#	Ret. Time	Name	Area	Height
1	21.669	Sildenafil	2084746	163608
Total			2084746	163608

PDA

Sample No.3 Adulterated with Sildenafil

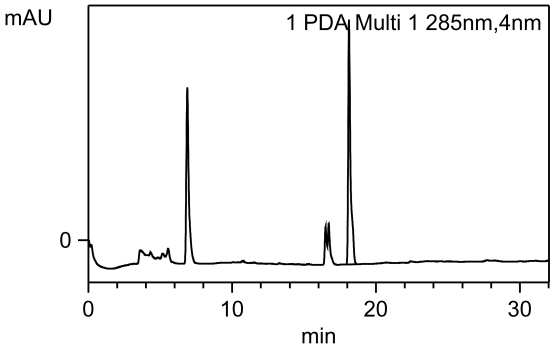


PDA Ch1 290nm

Peak#	Ret. Time	Name	Area	Height
1	21.632	Sildenafil	999179	62161
Total			999179	62161

PDA

Sample No.4 Adulterated with Sildenafil

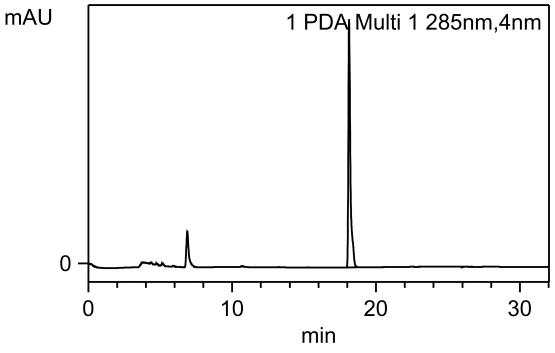


PDA Ch1 285nm

Peak#	Ret. Time	Name	Area	Height
1	18.123	Tadalafil	367233	33139
Total			367233	33139

PDA

Sample No.6 Adulterated with Tadalafil



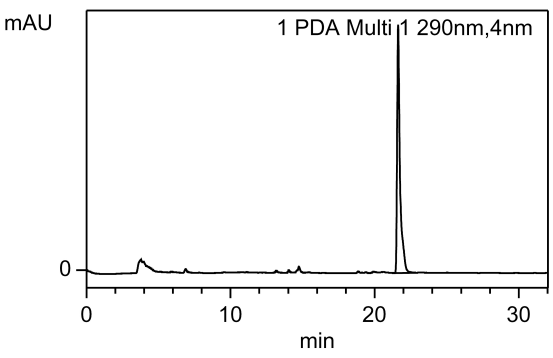
PDA Ch1 285nm

Peak#	Ret. Time	Name	Area	Height
1	18.130	Tadalafil	1828817	187066
Total			1828817	187066

PDA

Sample No.8 Adulterated with Tadalafil

FIGURE 2. Chromatograms of honey samples (samples 1, 3, 4, 6 and 8). The retention times of S1, S3, S4, S6 and S8 are 18.153, 21.669, 21.632, 18.123 and 18.130 corresponding to the presence of tadalafil, sildenafil, sildenafil, tadalafil and tadalafil, respectively. PDA: Photodiode Array.

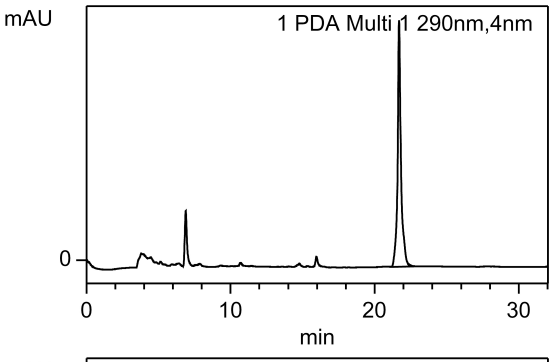


PDA Ch1 290nm

Peak#	Ret. Time	Name	Area	Height
1	21.626	Sildenafil	2716392	196107
Total			2716392	196107

PDA

S9

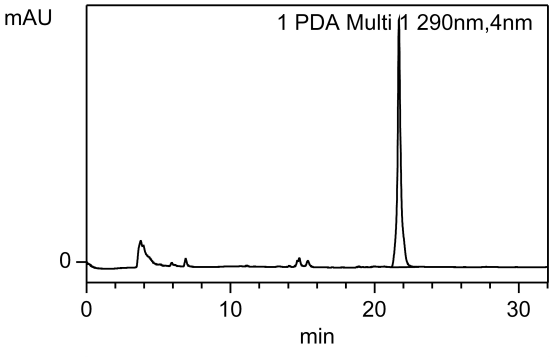


PDA Ch1 290nm

Peak#	Ret. Time	Name	Area	Height
1	21.693	Sildenafil	1229951	75195
Total			1229951	75195

PDA

S11

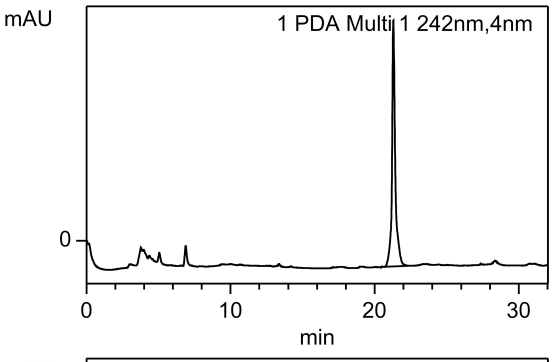


PDA Ch1 290nm

Peak#	Ret. Time	Name	Area	Height
1	21.683	Sildenafil	2011447	121274
Total			2011447	121274

PDA

S12

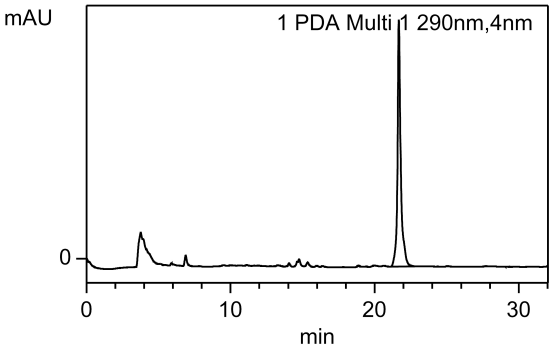


PDA Ch1 242nm

Peak#	Ret. Time	Name	Area	Height
1	21.297	Vardenafil	801460	50863
Total			801460	50863

PDA

S16



PDA Ch1 290nm

Peak#	Ret. Time	Name	Area	Height
1	21.675	Sildenafil	1269432	80420
Total			1269432	80420

PDA

S17

FIGURE 3. Chromatograms of honey samples (samples 9, 11, 12, 16 and 17) illustrating the retention times corresponding to the presence of sildenafil in samples 9, 11, 12 and 17 and vardenafil in sample 16. PDA: Photodiode Array.

TABLE 2. HPLC-PAD determination of adulterants in honey samples.

	Sample	Manufacturing country	Sample weight during preparation	Result	Quantity of adulterant (mg)	Final quantity of adulterant (mg)
S1	RHM1	Malaysia	1.13 g	Tadalafil	6.588 mg	116.60 mg/Sachet
S2	RHK	Korea	1.01 g	---	---	---
S3	RHVM1	Malaysia	1.201 g	Sildenafil	9.339 mg	116.64 mg/Sachet
S4	VHI	India	1.01 g	Sildenafil	15.241 mg	226.35 mg/Sachet
S5	HMT	Turkey	1.04 g	---	---	---
S6	VTM	Malaysia	1.07 g	Tadalafil	2.595 mg	36.38 mg/Sachet
S7	HAS	Saudi Arabia	1.16 g	---	---	---
S8	RHVM2	Malaysia	1.201 g	Tadalafil	12.924 mg	215.22 mg/Sachet
S9	BST	Turkey	1.02 g	Sildenafil	12.168 mg	11.93 mg/g
S10	HWSA	Saudi Arabia	1.05 g	---	---	---
S11	EM	Not given	1.19 g	Sildenafil	5.510 mg	4.63 mg/g
S12	EMT	Turkey	1.21 g	Sildenafil	14.215 mg	11.75 mg/g
S13	AZT	Turkey	1.24 g	---	---	---
S14	BMT	Turkey	1.15 g	---	---	---
S15	VLA	Australia	1.21 g	---	---	---
S16	GHJ	Jordan	1.23 g	Vardenafil	1.308 mg	10.64 mg/Sachet
S17	BMT	Turkey	1.13 g	Sildenafil	5.687 mg	5.03 mg/g
S18	RHM2	Malaysia	1.24 g	---	---	---

and precision of the results. In addition, variability in sample preparation techniques can impact the reproducibility of the results. Differences in extraction methods and conditions can also lead to variations in the detected compounds. Moreover, disparities in regulatory standards and methods for honey analysis may impact the consistency of results across different regions or countries.

5. Future prospects

The future prospects for detecting adulterated honey and ensuring product authenticity using advanced analytical techniques are promising. With further advancements, this method would provide more comprehensive profiling of honey components, making it easier to identify authentic honey and detect potential adulterants. Strengthening and enforcing regulatory measures related to the authenticity of honey is likely to play a pertinent role in mitigating the circulation of adulterated products in the market. Implementing stringent penalties for adulteration and fraud can act as a deterrent. Furthermore, consumers should be empowered with information about the authenticity of the product *via* proper labeling, certifications, and traceability initiatives, which can create a demand for genuine products.

6. Future requirements

The future requirements are rapid on-site detection technologies with improved sensitivity and specificity. International standards for honey authentication methods should be established and harmonised. The presence of consistent and recognized standards will facilitate cross-border trade and cooperation in combating honey fraud. Interdisciplinary research can lead to innovative approaches and comprehensive solutions for honey authentication. Moreover, establishing a global database of authentic honey profiles can serve as a reference for comparison during analytical testing. In addition, regulatory bodies should actively enforce penalties for fraud and adulteration.

AVAILABILITY OF DATA AND MATERIALS

Data is available with the corresponding author and can be obtained on request.

AUTHOR CONTRIBUTIONS

NDA—designed the research study, performed research experiments, and wrote the manuscript. AMA, FKA and MMA—performed the research and analyzed the data. RA and GHA—assisted in experimental work and reviewing of the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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