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Original Research Article

In vitro Erectogenic Property of Aqueous Extract from Roasted and Raw Pepitas in Isolated Corpora cavernosa of Wistar Rats

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ABSTRACT

Pepitas have long been adopted in traditional medicine to to help with erectile dysfunction (ED) and cure impotence in males. This study investigated the inhibitory potentials of raw and roasted pepitas on some of the main enzymes associated with erectile dysfunction in the corpora cavernosa of rats. Arginase, Phosphodiesterase-5 (PDE-5), acetylcholinesterase (AChE), angiotensin I-conversion enzyme (ACE), and adenosine deaminase (ADA) were among the enzymes studied. The raw and roasted pepitas water extract (1:10 w/v) was employed. Gas chromatography-Pulsed Flame Photometric Detector (GC-PFPD) was also used to assess the amino acid content of roasted and raw pepitas. In the rat's corpora cavernosa, the roasted extract exhibited higher inhibitory activities for PDE-5 (IC_{50} = 15.76 g/mL), ACE (IC_{50} = 37.29 g/mL), AChE (IC_{50} = 28.34 g/mL), arginase (IC_{50} = 44.86 g/mL), and ADA (IC_{50} = 15.10 g/mL). The roasted extract had a lot of arginine, while the raw extract didn't have any. Pepitas' enzyme activity and erectogenic qualities might be linked to their amino acid contents. The pepitas that had been roasted seemed to be the most promising.

Keywords: Phosphodiesterase-5 (PDE-5), Acetylcholinesterase (AChE), Arginase, Adenosine deaminase, Pepitas, Erectile dysfunction.

Introduction

The inability of a man's sexual activity to develop or sustain a penile erection is known as erectile dysfunction (ED).[1] The prevalence and severity of ED are on the rise as people become older, and a number of pathogenetic variables may play a role in this.[2,3] In addition, it has been estimated that over 300 million men globally would be impacted by ED in the next six years, with Asia, Africa, and South America accounting for almost 2.5 times as many. This sexual illness is now believed to affect 150 million males globally.[4]

The corpus cavernosum (CC) is a vascular tissue made up of endothelial cells and smooth muscle that relax and contract in reaction to particular factors. Cyclic nucleotide phosphodiesterase (PDE) enzymes control the amounts of secondary messengers in the cytosol.[5,6] The most phosphodiesterase type-5 is found in the smooth muscle of the corporal cavernosum. As a result, phosphodiesterase-5 (PDE-5) inhibition might be employed as an ED treatment method. 6 Inhibition of acetylcholinesterase (AChE) is also an alternate approach for ED control since it modulates acetylcholine levels.[7] Furthermore, as acetylcholine is converted to its acetate and choline components by AChE, the quantity of acetylcholine is lowered.[8-10] AChE inhibition has been shown to increase acetylcholine levels in previous research.[11-12] Arginase also converts L-arginine to urea and ornithine, therefore increased arginase activity in the ED reduces the amount of L-arginine required for nitric oxide synthase to produce NO. The inhibitors of Arginase consequently conserve L-arginine in the cell, while preserving appropriate NO levels for smooth muscle cells and a healthy penile response.[13] Adenosine has also been demonstrated to modify penile erection, which is a known physiological vasorelaxant.[14,15] Reduced adenosine deaminase activity aids in the buildup of adenosine and subsequent activation of adenosine receptors in the cavernosum corpus, resulting in erection-friendly relaxation. Adenosine and adenosine receptors are considered to assist male erection induction.[14,16] The renin-angiotensin system (RAS) also plays a role in the development of erectile dysfunction. Angiotensin II is produced when the Angiotensin I-converting enzyme experiences an increase in erectile dysfunction.[13,17,18] Thus, inhibiting ACE activity improves erectile function while significantly lowering angiotensin II levels in ED patients.[19]

The pumpkin seed is one of the earliest cultivated plants known to man (Cucurbitapepo Linn, Family: Cucurbitaceae). Elegede is the Yoruba word for it, whereas Pumpkin is the English name. It's a common plant in Nigeria's southwest.[20] This plant may be found in northern Mexico, as well as the Southwest and Eastern United States. It can also be found throughout Europe and Asia in its native condition. In the region, the young leaf known as "Gboro" is usually consumed, and the pulp found in mature ripe fruits has been utilised as a vitamin A supplement as well as in the treatment of liver and stomach diseases as well as intestinal inflammation.[21] Pepita seeds, or pepita are small flat green edible seeds that are frequently advised as a nutritional supplement. The seeds can be crushed into a powder and used with bread-making grains or consumed as a snack after roasting. Pepitas are high in carotenoids, zinc, selenium, vitamin E, magnesium salts, linoleic, palmitic, oleic, and stearic acids.[22] In folkloric medicine, they're said to increase libido, improve sexual health, increase male fertility, and cure digestive problems including constipation and diarrhoea.[23] However, nothing is known about pepitas' potential methods of action in the management of ED. In the isolated corpus cavernosum of healthy albino Wistar rats, the inhibitory effects of water extracts of roasted and raw pepitas on enzymes such as angiotensin-1 converting enzyme (ACE), phosphodiesterase-5 (PDE-5), acetylcholinesterase (AChE), adenosine deaminase (ADA), and arginase were investigated. In addition, the amino acid content of roasted and raw pepitas was determined.

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Materials and Methods

Collection of plant Materials

A local market in Ado Ekiti, South Western Nigeria, sold matured fresh pumpkin fruits. The pumpkin fruits were purchased in May, 2020. The pumpkin fruits were verified by the Department of Plant Science and Biotechnology at Ekiti State University in Ado Ekiti, Nigeria, and were given the voucher number UHAE 433. The seeds were removed from the fruit using a table knife, cleaned with distilled water, and dried in the air at room temperature for three days. The seeds were broken and removed from their hulls by hand. After that, a part of the seed was roasted for 30 minutes at 100 degrees Celsius in an electric oven. At 40°C, the raw component was oven-dried to a consistent weight.[9] The samples were crushed individually, defatted in cold n-Hexane, and kept in an airtight container.

Preparation of the aqueous extracts

Ten (10 g) powdered samples (raw and roasted) were each steeped in 100 mL of water for 12 hours, mixed, and filtered. The filtrate was centrifuged for 10 minutes at 4000 rpm, and the supernatant was freeze dried to concentrate it. In distilled water, the yield was reconstituted and employed in several experiments.[24]

Animal

The Department of Anatomy, College of Medicine, Ekiti State University, Ado Ekiti, Nigeria, provided ten (10) mature Wistar male rats. The animals were kept at a constant temperature of 25°C with a 12-hour light/dark cycle and unrestricted access to food and drink. This investigation was authorised by the institutional ethics committee with the permission number ORD/AD/EAC/19/0057.

Tissue homogenates preparation

The penile tissues (corpora cavernosa) were quickly removed, stored on ice, and weighed after three rats were beheaded by cervical dislocation. In a cold saline solution containing phosphate buffer (pH 7.2; 1:5 w/v), penile tissues were washed and homogenised. In a Kenxin chilled centrifuge Model KX3400C, the matching homogenate was spun for 20 minutes at 4,000 rpm, and the resultant supernatant was employed as an enzyme source in each of the enzyme experiments.

Arginase assay

This test determines how much urea is present in a sample.[25] The seed extract (0-100 µg/mL) or standard drug L-2-amino-[4-(20-hydroxyguanidino)] butyric acid (L-NOHA) was diluted sufficiently in a reaction mixture of 1.0 mM Tris-HCl buffer (pH 9.5), 0.1M arginine, and 50 µL of the enzyme. The mixture was incubated for 10 minutes at 37°C, then 2.5 mL Erhlich was added, incubated for 20 minutes at room temperature, and the absorbance was measured at 450 nm. Arginase activity was calculated as a percentage inhibition.

Angiotensin-1-converting enzyme (ACE) assay

In the reaction mixture, sufficient dilutions of the seed extract (0-100 µg/mL) or standard drug, captopril and 50 uL of the enzyme were used, and it was incubated for 15 minutes at 37 °C. After that, 8.33 mM of substrate was added to 125 mM Tris-HCl (pH 8.3) and incubated at 37 °C for 30 minutes. 250 mL of 1 M HCl and 1.5 mL of ethyl acetate were added to the mixture. The separate layer was decanted, centrifuged, and the residue was re-suspended in 1 mL distilled water, with the absorbance measured at 228 nm.[26] ACE activity was measured as a percentage of inhibition in respect to control.

Acetylcholinesterase (AChE) assay

In the reaction mixture, 200 µL of enzyme, 0.1 M phosphate buffer (pH 8.0, 4 mM NaHCO3), 3.3 mM DTNB, and ample seed extract dilutions (0-100 µg/mL) or standard drug Prostigmine were used. The mixture was incubated at 25 °C for 20 minutes before adding 50 mM acetylthiocholine iodide. At 412 nm, the absorbance was measured at 30-second intervals for 30 minutes.[27] Since the seed extract was not used for control, AChE activity was expressed as a percentage inhibition.

Phosphodiesterase-5 (PDE-5) assay

The mixture contained sufficient dilutions of the extract (0-100 µg/mL) or standard drug sildenafil citrate and 100 µL of enzyme. After a 10-minute incubation period at 37°C, 5 mM p-nitrophenyl phenylephosphate was added to the reaction mixture. For 30 minutes, the absorbance was measured at 400 nm at 30 second intervals.[28] The seed extract was not used as a control, and PDE-5 activity was measured and expressed as a percentage inhibition.

Adenosine deaminase (ADA) activity

100 µL of enzyme, 21 mmol/L of adenosine, pH 6.5 and appropriate seed extract dilutions (0-100 µg/mL) or standard drug Deazaadenosine were present in the reaction mixture. For 60 mins, the reaction mixture was incubated at 37°C. 500 µL of phenolnitroprusside and hypochlorite solution were subsequently added and the mixture was incubated for 30 minutes at 37°C. At 620 nm, the absorbance was taken.[29] For control, the seed extract was not used and ADA activity was therefore expressed as a percentage inhibition.

Amino acid characterization using Gas chromatography - PFPD

This was done using the AOAC[30] method, with minor modifications.

Statistical analysis

The results are shown as Mean Standard Deviation (SD). The T test for the student (unpaired) was used to assess and compare mean values, with statistical significance set at $P \leq 0.05$. In addition, nonlinear regression analysis was used to establish IC50 values (effective extract concentration causing 50 percent inhibition). The Social Science Statistical Program was utilised (SPSS version 21.0, Armonk, NY: IBM Corp).

Results and Discussion

The inhibitory potentials of roasted and raw pepitas on the major enzymes linked with erectile dysfunction were examined in the corpora cavernosa of rats. Pepitas are a low-cost natural source of polyphenols and amino acids with functional significance[22] and a wide variety of health benefits.[22] Figure 1 depicts the arginase inhibitory activity of aqueous extracts of roasted and raw pepitas. The extracts reduced arginase activity in a concentration-dependent manner. The inhibitory activity of the aqueous extract of roasted pepitas on arginase was greater (44.86 g/mL) than that of raw pepitas (70.03 g/mL), according to the IC_{50} (Table 1). L-NOHA, on the other hand, exhibited the most inhibitory impact, as seen by the IC_{50} (23.88 g/mL) (Table 2). Arginase activity control has previously been connected to nitric oxide generation in several investigations.[31] Increased arginase synthesis and lower nitric oxide levels in penile tissues have been seen in the therapy of sexual disorders.[32,n33]

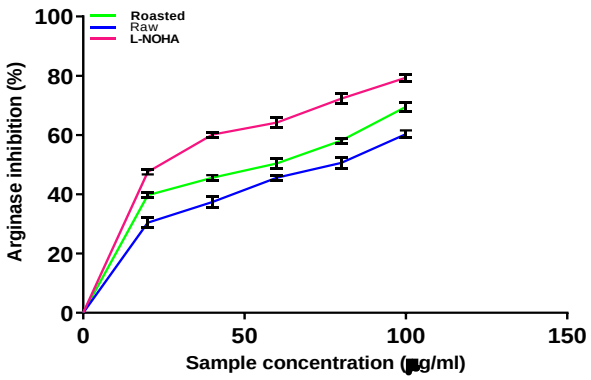

Figure 1: Effect of roasted and raw pumpkin seed on Arginase activity

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Reduced NO in the ED has been associated to increased arginase activity and altered endothelial NO synthase (eNOS) expression.[34] Nonetheless, boosting the presence and activity of eNOS by inhibiting arginase might be another strategy for the roasted and raw pepitas extracts to improve nitric oxide bioavailability.

Hypertension, which is a primary cause of erectile dysfunction, has been related to angiotensin-II.[8] As a result, blocking ACE and hence reducing angiotensin-II production might be a therapeutic target in the treatment of ED patients. According to Jin,[35] high angiotensin-II levels in the penis and testis can produce a large increase in the activity of NADPH oxidase, ROS generation, and nitric oxide synthase inhibititory activity. As a result, decreasing angiotensin II production may be applied in the treatment of ED. In the rat corpus cavernosum, aqueous extracts of roasted and raw pepitas inhibited ACE activity (Figure 2). ACE activity was suppressed by both the aqueous extract of pepitas and captopril. The IC_{50} values of the aqueous extract of roasted pepitas, on the other hand, were higher than those of raw pepitas, indicating that the aqueous extract of roasted pepitas exhibited stronger inhibitory effect on ACE. An aqueous extract from roasted and raw pepitas prevented the formation of ACE, reducing the quantity of angiotensin II in the erection organ, according to this study.

The cholinergic neurons produce acetylcholine, which is a key neurotransmitter in penile erection. Figure 3 depicts the aqueous extract of roasted and raw pepitas' inhibitory action on the acetylcholinesterase (AChE) enzyme. As the content of pepitas aqueous extract increased, it suppressed AChE activity. As a result, aqueous roasted pepitas extract demonstrated higher AChE inhibitory activity (IC50 = 28.34 g/mL) than raw pepitas (IC_{50} = 49.00 g/mL) (Table 1). Increased AChE activity has been shown in the literature to diminish acetylcholine availability, which causes smooth muscle constriction in the penis corpus cavernosum thereby leading to penile flaccidity.[36] In human endothelium,[37] dogs,[38] rabbits,[39,40] rats,[31] and mouse corpus cavernosum, bioavailability of acetylcholine has been shown to stimulate NO release. [41,42] Pepitas may be a promising seed with positive potential for ED therapy, based on the suppression of the enzyme in isolated corpus cavernosum. PDE-5 inhibitors are widely recognised as effective ED treatment options. Synthetic PDE-5 inhibitors, on the other hand, have been linked to a variety of adverse effects and experiences after their use.[43] The impact of aqueous extracts of roasted and raw pepitas on phosphodiesterase-5 (PDE-5) activity in the cavernosum corpus of rats was investigated (Figure 4). The results showed that the aqueous extract of pepitas suppressed PDE-5 enzymes. Phosphodiesterase-5 inhibitory activity was greater in the roasted extract (IC_{50} = 15.76 g/mL) than in the raw extract (IC_{50} = 44.14 g/mL). Sildenafil citrate showed the highest activity based on the IC_{50} (27.81 g/mL). Intriguingly, the observed suppression of PDE-5 activity in this study implies that both roasted and raw pepitas may produce endothelium-autonomous unwinding, resulting in a decrease in PDE-5 activities and therefore being advantageous for ED therapy. However, roasted pepitas are preferable to raw pepitas. The hemodynamic reaction to increased blood flow and penile tissue retention is penile erection which is mediated by smooth muscle cell relaxation.[15] Adenosine, identified as a popular vasorelaxant physiologically, has been found to modify penile erection.[45,15] Figure 5 shows the inhibitory action of aqueous extracts of roasted and raw pepitas on adenosine deaminase (ADA). As the quantity of pepitas extract increased, it decreased ADA action. Aqueous roasted pepitas extract (IC50 = 15.10 g/mL) demonstrated greater ADA inhibitory action than aqueous raw pepitas extract (IC50 = 23.73 g/mL) (Table 1). Thus, inhibition of ADA activity in isolated corpus cavernosum of rats induced by roasted and raw pepitas, of which roasted pepitas were found to be the best, indicated a modulatory effect on erectile function, because reducing ADA activity would encourage adenosine accumulation and subsequent adenosine receptor stimulation. In the induction of male erection, adenosine and adenosine receptor have been observed.[44,16]

It has been established that amino-acid supplementation is physiologically important in the treatment of ED.[45,46]

Table 1: Amino acid composition of roasted and raw pumpkin seeds

Amino acids	Concentration (mg/100g)	
	Roasted	Raw
Arginine	12.65	NF
Aspartate	17.03	5.82
Glutamate	19.04	436.34
Phenylalanine	48.2	NF
Serine	64.08	NF
Cysteine	28.02	NF
Tyrosine	8.95	NF
Methionine	4.29	NF
Alanine	1.38	132.34
Threonine	NF	92.12
Asparagine	NF	8.37
Proline	NF	1.39
Unidentified	29.01	49.44

NF = Not found

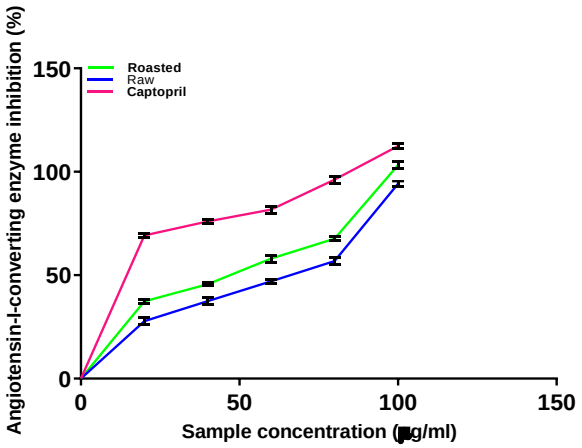

Figure 2: Effect of roasted and raw pumpkin seed on ACE activity

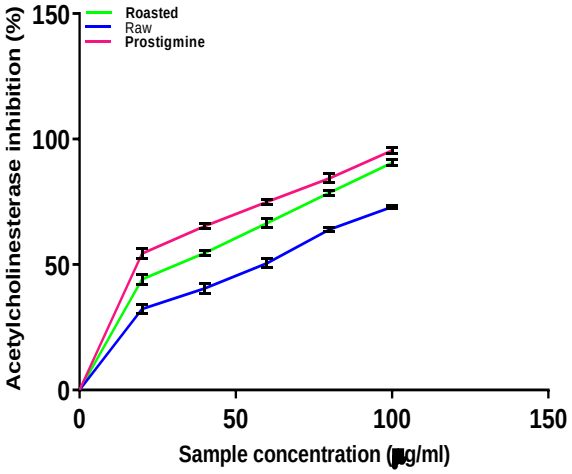

Figure 3: Effect of roasted and raw pumpkin seed on AChE activity

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Table 2: IC_{50} values (µg/mL) of arginase, ACE, AChE, PDE-5 and ADA inhibitory activities of roasted and raw pumpkin seed extracts and standard drugs/inhibitors

Sample	Arginase (µg/ml)	ACE (µg/ml)	AChE (µg/ml)	PDE-5 (µg/ml)	ADA (µg/ml)
Roasted	44.86 ± 1.65^a	37.29 ± 1.57^b	28.34 ± 1.45^b	15.76 ± 1.20^a	15.10 ± 1.19^a
Raw	70.03 ± 1.84^c	51.23 ± 1.71^c	49.00 ± 1.69^c	44.14 ± 1.65^b	27.73 ± 1.14^b
L-NOHA	23.88 ± 1.37^b	-	-	-	-
Captopril	-	12.75 ± 1.10^a	-	-	-
Prostigmine	-	-	19.26 ± 1.29^a	-	-
Sildenafil citrate	-	-	-	27.81 ± 1.45^c	-
Deazaadenosine	-	-	-	-	19.18 ± 1.47^c

Values represent mean ± standard deviation (n = 3). Values with the same superscript along the column are not significant ($p < 0.05$) different. Sildenafil citrate*: standard drug for phosphodiesterase-5 (PDE-5); L-NOHA*: standard inhibitor for arginase; Prostigmine*: standard drugs for AChE; Captopril*: standard drug for ACE; Deazaadenosine*: standard inhibitor for ADA.

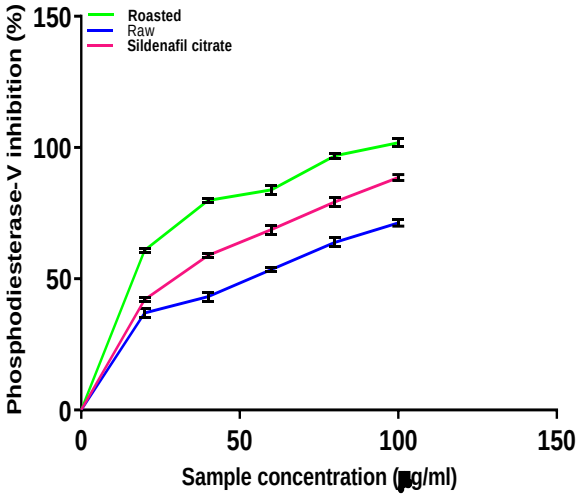

Figure 4: Effect of roasted and raw pumpkin seed on PDE-5 activity.

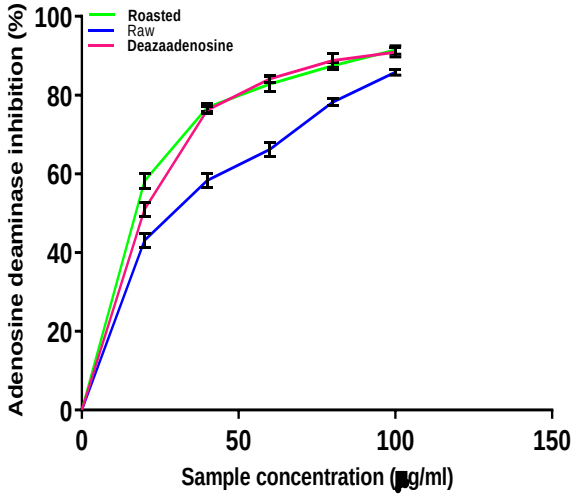

Figure 5: Effect of roasted and raw pumpkin seed on Adenosine deaminase (ADA) activity.

Table 1 shows the amino acid makeup of roasted and raw pepitas. Arginine, aspartate, glutamate, phenylalanine, serine, cysteine, tyrosine, methionine, and alanine are present in roasted pepitas, whereas aspartate, glutamate, alanine, threonine, asparagine, and proline are found in raw pepitas. The amino acid arginine was plentiful in roasted pepitas but not in raw pepitas, which is required for erection. In addition, roasted pepitas have more aspartate than raw pepitas. As a result, roasted pepitas can be a useful source of these necessary amino acids in the diet. Dietary arginine sources increase erectile function by boosting the production of synthetic nitric oxide (NOS), which improves NO synthesis, according to Boucher et al.[47] and Gur et al.[48]. As a result, roasted pepitas can be a rich source of arginine in the diet, which may aid with erectile function, according to folklore. Aspartate is an amino acid that helps regulate luteinizing hormone (LH) release and testosterone production at a homeostatic level.[49] Testosterone regulates eNOS corporeal expression and behaviour to maintain appropriate NO and erectile response levels.[50] According to Ademiluyi *et al*.[51], aspartate is also important in the stimulation of erectile functioning.

Conclusion

The ability of the roasted and raw pepitas extracts to inhibit the essential enzymes involved in erectile functions (Arginase, ACE, AChE, ADA, and PDE-5) may have triggered the erectogenic properties observed in pepitas. This may be due to the composition of amino acids.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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