Reducing sexual males dysfunction using natural foods.

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Abstract

Objective: Lack of important vitamins and mineral in food lead to a disturbance in testosterone level, also lead to an increase in the activity of each enzyme monoamine oxidase (MAO-B) which responsible for depression, and Phosphodiesterase (PDE-5) which enzyme responsible for low blood flow to peripheral vascular. Despite the prevalence of drugs to this problem did not reach the level of satisfaction of the efficacy and safety effects. In this study, male sexual dysfunction has required the agent that is effective, cheap and easy. Though natural food is reputed for aphrodisiac activity in traditional folklore, no scientific evidence is available.

Method: Therefore, we aimed to determine the effect of date paste with *Angelica sinensis* and *Calycotome villosa* as dietary supplement on male dysfunction in animal model. Our data showed a significant increase in serum testosterone level. However, also suppress MAOB and PDE-5 activities.

Results: Natural dietary supplement to improve male sexual behaviour in rats via the increased of testosterone level suppression of MAOB and PDE-5 activities.

Conclusion: Enhance male sexual desire and performance. This enhancement can be ascribed to the suppression of MAOB and phosphodiesterase activities and improved testosterone level. Therefore, date paste with *Angelica sinensis* and *Calycotome villosa* may be served as the natural resource for developing functional food and food supplement to reduce male dysfunction. The dietary supplement was produced in this study is a potential agent to manage sexual dysfunction especially for acute and short term application.

Keywords: *Angelica sinensis*, *Calycotome villosa*, Date paste, Dietary supplement, Male dysfunction, Monoamine oxidase (MAO-B), Phosphodiesterase (PDE-5), Testosterone.

Introduction

Increasing sexual dysfunction in males with age, which is considered as one of the Important in human life and social relationship, it effects of Millions of men all over the world [1,2]. Despite the prevalence of drugs to this problem, but did not reach the level of satisfaction of the efficacy and safety also had many side effects. Therefore, the sexual dysfunction consists of two main problems the first problem is Erectile Dysfunction (ED) is the most commonly found after the age of forty. The second problem, which is short ejaculation latency: The time interval between the primary intromission and ejaculation, However, it refers to a problem during any phase of the sexual response [3].

Causes of sexual dysfunction too many, but the ones primarily within these categories: Issues blood circulation, hormone issues, side-effects of certain medicines, nerve issues and psychological issues [4]. An aphrodisiac is a type of food or drink that makes those who eat or drink more, rose in a sexual manner. Aphrodisiacs can be classified according to the mode of action into three groups: Substances that increase sexual desire and arousal and materials increase the sexual potency of any effectiveness of Erection and materials that increase sexual pleasure.

Phosphodiesterase (PDE) (EC 3.1.4.-) enzymes catalyze the degradation of cyclic adenosine monophosphate (cAMP) or cyclic guanosine monophosphate (cGMP) to the corresponding AMP or GMP. To date, at least 11 different families of PDE isozymes have been identified [5]. The PDE isozymes described as being expressed in distinct types of vascular smooth muscle are PDE1, PDE3, PDE4 and PDE5 [6]. In humans, three PDE5 isoforms (PDE5A1, A2 and A3) have been identified. Unexpectedly, during clinical studies, sildenafil ameliorated erectile dysfunction pointed out PDE5 inhibition as a new target for treatment of erectile dysfunction and increasing the development of PDE5 inhibitors [7].

Monoamine oxidases, especially monoamine oxidase B (MAO-B), also play an important role in neurodegenerative disorders. MAO-B is the main enzyme responsible for the oxidative deamination of dopamine in the substantia nigra of the brain.

By inhibiting MAO-B, dopamine is increased in the brain providing symptomatic relief in Parkinson's disease [8].

Dates palm the most important foods that are working on regulating the hormones activity of into the body as it is rich of important vitamins and minerals, date palm contains estradiol and flavonoid components that have positive effects on the sperm quality, enhance fertility in the male adult rat. Therefore, it may be useful to solve infertility problems [9].

Angelica sinensis (Dong quai) root contains (0.4-0.7) percent volatile oil, the key components of which are nbutylidenephthalide, ligustilide, n-butylphthalide, ferulic acid, nicotinic acid, and succinic acid [10-12]. Significant amounts of vitamin A and carotenoids (0.675%), vitamin B12 (0.25-0.40 mcg/100 g), vitamin E, ascorbic acid, folinic acid, biotin; various phytosterols (e.g. beta-sitosterol), calcium, magnesium and other essential macrominerals are also found in dong quai root [13]. Other constituents include n valerophenone-O-carboxylic acid, delta-2,4-dihydrophthalic anhydride, uracil, adenine, carvacrol, safrole, isosafrole, sesquiterpenes, beta-cadinene, n-dodecanol, n tetradecanol, palmitic acid, angelic acid, myristic acid, sucrose (40%), and a polysaccharide with a molecular weight of approximately 3,000 [14]. Due to its varied constituents, several pharmacological actions may be attributed to dong quai. Such characteristics include anticoagulation and antiplatelet activity, as well as hematopoiesis, immune support and males dysfunction [15-18].

Calycotome villosa (Kandol) a common pioneer Leguminosae, with yellow flowers during the spring season, very familiar in the Mediterranean area, where it grows near the sea (1.2 m altitude). The aerial parts of the plant were collected during the flowering season, which is used as a drink to overcome the low blood pressure and increase the flow to the peripheral parts [19].

Materials and Methods

Plant material preparation

The roots of *Angelica sinensis* were collected from Elmaghara in Middle Sinai and stored at 20°C. The leaves and flowers of *Calycotome villosa* were collected from Elmaghara in Middle Sinai and stored at 20°C. *Angelica sinensis* root (500 g) and *Calycotome villosa* leaves and flowers (500 g) were dried and at 60°C. Dried samples were ground to pass through a 60-mesh sieve using an analytical mill to fine powder. Date paste was obtained from El-Nour for food industry in North Sinai.

Preparation of dietary supplement

Dietary supplements were prepared as follows:

Concentration (A): Mixing of 30 g of date paste+0.25 g of *Angelica sinensis* root powder+0.25 g of *Calycotome villosa* leaves and flower's powder.

Concentration (B): Mixing of 30 g of date paste+0.50 g of *Angelica sinensis* root powder+0.50 g of *Calycotome villosa* leaves and flower's powder.

Concentration (C): Mixing of 30 g of date paste+0.75 g of *Angelica sinensis* root powder+0.75 g of *Calycotome villosa* leaves and flower's powder.

Concentration (D): Mixing of 30 g of date paste+1 g of *Angelica sinensis* root powder+1 g of *Calycotome villosa* leaves and flower's powder.

Animal and diet protocol

Ninety (90) Healthy male rats (250 to 300 g) were selected for the experiment. And were housed in standard metal cages at 22 ± 2°C on 10:14 h light-dark cycle. All animals were given access to food and water. The rats were divided into six groups each group consists of fifteen rats.

Date paste (dietary supplement) with different concentrations processing in the water doses formed Mixing date paste (dietary supplement) with 100 ml water and divided into 3 doses. Each dose 30 ml gives it to the mice at three times per day as a full dose to ensure mice eating a full sample.

Fed mice groups for 30 days explained below:

Group 1 was fed by basic meals only which was used as negative control.

Group 2 was fed by dietary supplement concentration (A).

Group 3 was fed by dietary supplement concentration (B).

Group 4 was fed by dietary supplement concentration (C).

Group 5 was fed by dietary supplement concentration (D).

Group 6 was fed by Sildenafil citrate 50 mg which was used as positive control.

Determination of Vitamins in Plant Sample

Determination of fat-soluble vitamins

In 10 g plant samples and dietary supplement powder, 1 g of pyrogallic acid, 70 mL ethanol and 30 mL (50%) KOH were added, stirred and refluxed for 40 min using a water bath at 50 ± 2°C [20,21]. Extracts were obtained three times using various ether concentrations (50 mL, 30 mL and 20 mL). Double-distilled water was used to neutralize the extract, which was dehydrated using anhydrous sodium sulfate. Further, the extract was concentrated to approximately 5 mL by using a water bath (50 ± 2°C), diluted to 10mL by using methanol, filtered using a 0.45 µm membrane, and finally subjected to HPLC analysis. RP-HPLC analysis was performed with the Agilent 1100 series HPLC system (Agilent; USA), including a diode array detector. The column was made of stainless steel. For fat-soluble vitamins, the Agilent Eclipse XDB-C18 column was used (5 µm, 4. 6 × 150 mm), V (B12), the solvent was methanol and UV detection was recorded at 325 nm for vitamin A, 265 nm for vitamin D3, 290 nm for vitamin E and 244 nm for vitamin K3. Separation of all vitamins was based on isocratic elution and the solvent flow rate was maintained at 1 mL/min.

20 µL of sample's oil were directly injected into the HPLC column. Fat-soluble vitamins were identified by comparing their retention times with those of authentic standards. All procedures were carried out under subdued light conditions. Standard solutions of vitamins were prepared by serial dilution to concentrations of 0.1, 1, 2, 5 and 10 mg/L of vitamins D3, E, K3 and A, respectively. Standard solutions were prepared daily from a stock solution, which was stored in the dark at –20°C. Twenty microliters of standard solution were injected, and peak areas were determined to generate standard curves.

Determination of Water-Soluble Vitamins

Determination of vitamin B group

The vitamin B group was extracted according to a previously described method [20]. Plant samples and dietary supplement powder (2 g) was placed in 25 mL of H_2SO_4 (0.1 N) solution and incubated for 30 min at 121°C. Then, the contents were cooled and adjusted to pH 4.5 with 2.5 M sodium acetate and 50 mg enzymatic hydrolysis was added. The preparation was stored at 35°C overnight. The mixture was then filtered through a Whatman No. 4 filter and the filtrate was diluted with 50 mL of pure water and filtered again through a micropore filter (0.45 µm). Twenty microliters of the filtrate was injected into the HPLC system. Quantification of vitamin B content was accomplished by comparison to vitamin B standards. Standard stock solutions for thiamine (B1), riboflavin (B2), niacin (B3), pyridoxine (B6) and cobalamin (B12) were prepared as reported previously [22,23].

Chromatographic separation was achieved on a reversed phase-(RP-) HPLC column (Agilent ZORBAX Eclipse Plus C18; 250 × 4.6 mm i.d., 5 µm) through the isocratic delivery mobile phase (A/B 33/67; A: MeOH, B: 0.023 M H_3PO_4, pH=3.54) at a flow rate of 0.5 mL/min. Ultraviolet (UV) absorbance was recorded at 270 nm at room temperature [24].

Vitamin C (ascorbic acid)

Vitamin C was extracted according to a modification of a published method [25]. The sample powder (10 g) was blended and homogenized with an extracting solution containing metaphosphoric acid (0.3 M) and acetic acid (1.4 M). The mixture was placed in a conical flask and agitated at 10,000 rpm for 15 min. The mixture was the filtered through a Whatman No. 4 filter, and samples were extracted in triplicate. The ascorbic acid standard was prepared by dissolving 100 mg of l-ascorbic acid in a metaphosphoric acid (0.3 M)/acetic acid (1.4 M) solution at a final concentration of 0.1 mg/mL. The calibration line was converted to a linear range based on four measured concentration levels.

Quantification of ascorbic acid content was performed on an Agilent HPLC system. Chromatographic separation was achieved on an RP-HPLC column through isocratic delivery of a mobile phase (A/B 33/67; A: 0.1 M potassium acetate, pH=4.9, B: acetonitrile: water [50:50]) at a flow rate of 1 mL/min. UV absorbance was recorded at 254 nm at room temperature.

Determination of Mineral Elements in Plant Sample

The mineral elements were determined using the analytical method of determining mineral constituents of food products [26]. Samples obtained through ashing were used for this procedure, which was the white fluffy mass. 5 mL of concentrated hydrochloric acid were used to digest each ash, content in a glass petridish. The mixture was transferred to 50 mL chemical flask using distilled water particles, which cannot dissolve and would cause contamination were filtered off using Whatman's no.1 filter paper in a funnel. The new filterate was made up to mark in readiness for mineral nutrient determination. The elements determined include Na, Ca, K, P, Mg, Mn, Fe, Cu and Zn. The determination was made using method described by [26] standard reagents for the various elements to be determined were prepared. The series spectrophotometer was first warmed up for 30 min. Then, the standard reagent of the elements to be determined and distilled water was used to standardize the equipment. The sample contained in 10 mL cuvette was then introduced into the sample chamber where the samples were read and recorded.

High Performance Liquid Chromatography (HPLC) for the Identification of *A. sinenses* and *C. villosa*. High-performance liquid chromatography (HPLC) has been widely used in the evaluation of the components of *A. sinenses* and *C. villosa*. HPLC with ultraviolet detection analyzed ligustilide and ferulic acid content in extracts of *A. sinenses* and chrysin 7-O-(β-D-glucopyranoside) content in extracts of *C. villosa*.

Sample preparation

Extract 5 g of samples powder in a soxhlet extractor with 50 mL n-hexane for 1 h. Evaporate the extract to dryness and redissolve in 2.5 mL ethanol and filter (0.45 µm Millipore or equivalent).

Standard preparation

For *A. sinenses* dissolve each of Z-ligustilide and ferulic acid individually in ethanol (1 mg/mL). Z-Ligustilide is unstable in air and requires refrigeration. It must flush the standard with nitrogen and store in a freezer. For *C. villosa* dissolved of chrysin in ethanol (1 mg/mL).

Chromatographic conditions

Apparatus: Hewlett Packard 1050 liquid chromatograph with photodiode array detector, auto sampler and gradient pump.

Column: LichroCART 125-4 with Lichrospher 100 C-18 (5 µm), Merck or equivalent.

Pre-column: LichroCART 4-4 with Lichrospher 100 C-18 (5 µm), Merck or equivalent.

Sensory evaluation of characteristics of dietary supplement

Sensory evaluation was carried out by a panel of six judges with experience in the field of food science and technology.

Sensory analysis of dietary supplement produced was conducted for various sensory parameters by assigning scores between 1 to 10 for color, flavor, sweetness, firmness and desirability [27].

Determining Testosterone Level and Enzyme Activity

Determination of testosterone level

Measurement of plasma testosterone level of rats were every five days, samples are taken about three hours after intake of the dietary supplement (date paste) all of experimental period, the rat blood samples were collected and kept on ice and then centrifuged immediately at 2000×g at 4°C for 15 min. The obtained plasma was kept at -80°C until analysis. Testosterone levels were measured by Egyptian National Cancer Institute [28].

Determination of monoamine oxidase type B inhibition

The inhibitory action of the plant extracts on monoamine oxidase type B was determined by incubating a series of concentrations of the test samples in the reaction mixture, including rat brain homogenates. In brief, 2.75 mL Tris buffer (0.1 M, pH 7.4) and 100 µL of 0.1 M benzylamine was mixed in a quartz cuvette which was placed in double beam spectrophotometer and followed by the addition of 150 µL solution of brain homogenate to initiate the enzymatic reaction. The change in absorbance was recorded at wavelength of 249.5 nm for 5 min against the blank containing Tris buffer and 5-hydroxytryptamine [29].

Determination of phosphodiesterase (PDE) activity

Testis was collected from healthy male rat in order to Determine Phosphodiesterase Enzyme (PDE) activity. The testis was washed with phosphate buffer saline (PBS) and weighted before cut to small pieces. Then, it was homogenized with 5 volumes of lysate RIPA buffer (50 mM Tris-HCl, pH=7.4). The testicular solution was centrifuged at 14,000× g for 15 min at 4°C and the supernatant was collected and used as PDE substrate. The standard curve was prepared from PDE (testicular lysate) at the various concentrations. Phosphodiesterase substrate or testicular lysate was incubated with cGMP. Then, PDE reaction solutions were added and incubated for 20 min at room temperature. The cGMP in the mixture then drives a kinase reaction leading to a reduction of ATP levels. Following the kinase reaction, a Kinase Glo reagent was added and reactions were mixed and incubated for 10 min at room temperature. Luminescence was measured using a SpectraMax L microplate luminometer. The luminescent signal produced is directly related to the amount of ATP remaining and correlates with phosphodiesterase activity use remaining unit (RLU Relative Light Unit) [28].

Nutritional Studies of Dietary Supplement Products

The dietary supplement produced was analyzed for the nutritional parameters, carbohydrate, fiber, crude protein, ash; fat and moisture were determined by the method of A.O.A.C [30].

Statistical Analysis

All data were expressed as mean ± SEM value. The significant differences among various groups were compared by ANOVA and followed by Duncan's test. The statistical difference was regarded at $p<0.05$.

Results and Discussion

Determination of vitamins in plant sample

Table 1 shows important variations of the fat-soluble vitamin compositions in the studied *A. sinensis* root, *C. villosa* (leaves and flowers) and Date paste samples. The *A. sinensis* root showed the highest content of vitamin E (7.2 µg/100 g); while the *C. villosa* had the lowest content (0.05 mg/100 g). No detectable quantities of vitamin K3 and D3 were observed in all samples. Date paste had significant amount of A (0.06 µg/100 g).

Table 1. Fat-soluble vitamin contents of A. sinensis root, C. villosa (leaves and flowers), date paste.

Vitamins	*A. sinensis*	*C. villosa*	Date paste
Tocopherol (vitamin E) (mg/100 g DW)	7.2[a]	0.03[b]	ND
Menaphthone (vitamin K3) (mg/100 g DW)	ND	ND	ND
Retinol (vitamin A) (g/100 g DW)	ND	ND	0.06[a]
Cholecalciferol (vitamin D3) (g/100 g DW)	ND	ND	ND

Dry weight (DW) was not detected (ND). Retinol (vitamin A) and Cholecalciferol (vitamin D3) are expressed in g per 100 g Tocopherol (vitamin E) and Menaphthone (vitamin K3) are expressed in mg per 100 g

Table 2 shows the water-soluble vitamin contents (B1, B2, B3, B6, B12 and C) for *A. sinensis* root, *C. villosa* (leaves and flowers), date paste samples. The vitamin C content of the *A. sinensis* root was approximately (29.3 mg/100 g), while it was rich in vitamin B2, B12 (0.31 µg/100 g, 0.38 µg/100 g) and had the highest content of vitamin B3 (5.7 µg/100 g).

C. villosa (leaves and flowers) showed the lowest content of vitamin C (17.4 mg/100 g) and a slightly high content of

vitamin B3 (2.6 µg/100 g). No detectable quantities of vitamin B2, B6 and B12 were observed. The *C. villosa* showed the highest vitamin B1 content (0.59 µg/100 g), whereas Date paste showed the lowest, in which value of vitamin B1 was detectable (0.09 µg/100 g). The date paste showed the lowest value of vitamin B3 (0.12 µg/100 g), but it had the highest content of vitamin C (68.9 mg/100 g), while it was had significant amount of vitamin B2, B6 (0.07 µg/100 g, 0.02 µg/100 g).

Table 2. Water-soluble vitamin contents of A. sinensis root, C. villosa (leaves and flowers), date paste.

Vitamins	*A. sinensis*	*C. villosa*	**Date paste**
Thiamine (Vitamin B1) (g/100 g DW)	ND	0.59[a]	0.09[b]
Riboflavin (vitamin B2) (g/100 g DW)	0.31[a]	ND	0.07[b]
Niacin (vitamin B3) (g/100 g DW)	5.7[a]	2.6[b]	0.12[c]
Pyridoxine (vitamin B6) (g/100 g DW)	ND	ND	0.02[a]
Cobalamin (vitamin B12) (g/100 g DW)	0.38[a]	ND	ND

Dry weight (DW) was not detected (ND). Thiamine (Vitamin B1) Riboflavin (B2), niacin (B3), pyridoxine (B6) and cobalamin (B12) are expressed in g per 100 g. Ascorbic acid (C) is expressed in mg per 100 g

Table 3. Mineral element composition on dry weight basis of A. sinensis root, C. villosa (leaves and flowers), date paste.

	K	**Na**	**Mn**	**Ca**	**Zn**	**Fe**	**Mg**	**Cu**	**P**
A. sinensis	1430[a]	-	0.02[c]	246[a]	-	82[a]	218[a]	0.05[c]	293[a]
C. villosa	0.29[c]	0.02[b]	0.37[b]	0.07[c]	0.78[b]	0.53[c]	0.04[c]	0.41[b]	0.05[c]
Date paste	627[b]	81[a]	0.63[a]	54[b]	1.9[a]	3[b]	64[b]	0.42[a]	106[b]

Determination of mineral elements in plant sample

Table 3 shows the values obtained for minerals (calcium, phosphorus, iron, zinc, magnesium, manganese, copper, potassium and sodium), In the *A. sinensis* root; sodium and zinc content of is no detectable quantities and it had the lowest measured amount of manganese and copper among the others sample (0.02 mg/100 g and 0.05 mg/100 g for manganese and copper, respectively) but *A. sinensis* is the richest regarding of iron, potassium, calcium, phosphorus and magnesium contents in compare with *C. villosa* (leaves and flowers) and date paste (82 mg/100 g, 1430 mg/100 g, 246 mg/100 g, 293 mg/100 g and 218 mg/100 g) for of iron, potassium, calcium, phosphorus and magnesium, respectively.

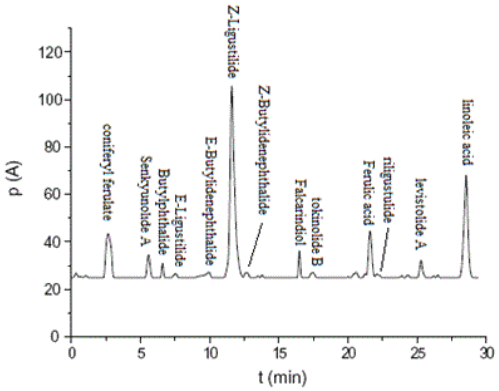

Figure 1. HPLC fingerprint chromatogram of (Angelica sinensis).

The *C. villosa* contains the significant amount of the zinc (0.78 mg/100 g) on the other hand it contains the lowest amount of minerals among the analyzed samples.

Date paste contains calcium 54 mg/100 mg, potassium 627 mg/100 mg, zinc 1.9 mg/100 g, iron, 3 mg/100 g sodium 81 mg /100 g, coper 0.42 mg/100 g, phosphorus 106 mg/100 g, magnesium 64 mg/100 g and manganese 0.63 mg/100 g. The date paste contains riche amount of several kinds of minerals, that human need every day with varying percentage.

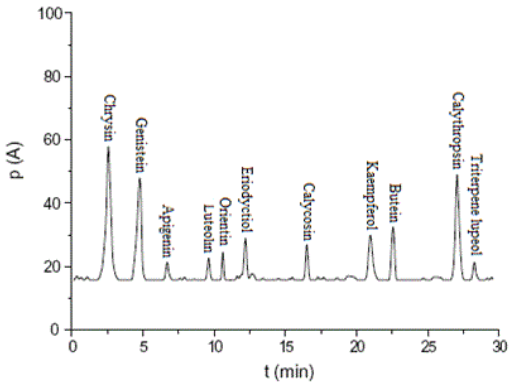

Figure 2. HPLC fingerprint chromatogram of Calycotome villosa.

High Performance Liquid Chromatography (HPLC) for the Identification of *A. sinenses* and *C. villosa*

Chromatography of HPLC extracts from Angelica root were shown in Figure 1. All the main components were separated completely, and 13 of them were identified by comparing the standard, peaks 1-13 were identified as coniferyl ferulate, Senkyunolide A, Butylphthalide, E-Ligustilide, E-But ylidenephthalide, Z-Ligustilide, Z-Butylidenephthalide, Falcarindiol, Tokinolide B, Ferulic acid, Riligustulide, levistolide A, linoleic acid. The structures are shown in Figure 1. The results are summarized in Table 4.

Chromatography of HPLC extracts from *Calycotome villosa* was shown in Figure 2. All the main components were separated completely, and 11 of them were identified by comparing the standard, peaks 1-11 were identified as Chrysin, Genistein, Apigenin, Luteolin, Orientin, Eriodyctiol, Calycosin, Kaempferol, Butein, Calythropsin, Triterpene lupeol. The structures are shown in Figure 2. The results are summarized in Table 5.

Table 4. The identification of peaks was determined using HPLC from Angelica sinensis.

Peak no.	Compound	Rt (min)
1	Coniferyl ferulate	2.9
2	Senkyunolide A	6.4
3	Butylphthalide	7.2
4	E-Ligustilide	8.1
5	E-Butylidenephthalide	10.7
6	Z-Ligustilide	12.3
7	Z-Butylidenephthalide	12.8
8	Falcarindiol	16.4
9	Tokinolide B	17.6
10	Ferulic acid	21.9
11	Riligustulide	22.6
12	Levistolide A	26.4
13	Linoleic acid	28.7

Table 5. The identification of peaks was determined using HPLC from Calycotome villosa.

Peak no.	Compound	Rt (min)
1	Chrysin	2.9
2	Genistein	6.4
3	Apigenin	7.2
4	Luteolin	8.1
5	Orientin	10.7
6	Eriodyctiol	12.3
7	Calycosin	12.8
8	Kaempferol	16.4
9	Butein	17.6
10	Calythropsin	21.9
11	Triterpene lupeol	22.6

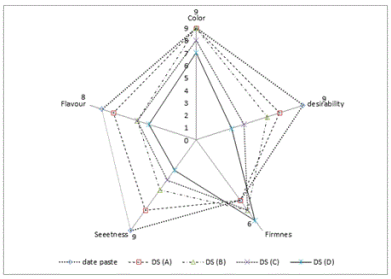

Figure 3. Sensory evaluation of characteristics of dietary supplement.

Sensory Evaluation of Characteristics of Dietary Supplement

Figure 3 present the results of dietary supplement (DS) from date paste with different concentration additives of *Angelica sinensis* and *Calycotome villosa* powders compared with date paste without additives. Evaluation of color, firmness, sweetness, flavor and desirability of dietary supplements were considered. Color is important sensory quality attribute of date paste because it's the first property consumers observes, and losses of color are a primary concern to consumers. Addition of plants powder to date paste caused a darker paste which disliked by panellist. This dislike was proportional to the % of plant additives used giving DS (D) the lowest score. Firmness of DS (A) was preferred than other attributes, whereas paste was the lowest score. This was due to the higher content of moisture as same control sample (date paste without additives).

Table 6. Chemical composition of dates and seeds powder.

	Moisture	Protein	Fat	Ash	Fiber	Carbohydrate
Date paste (control)	15.7^{a}	1.32^{a}	0.41^{a}	1.96^{a}	5.11^{a}	22.37^{a}
DS (A)	15.2^{a}	1.38^{b}	0.46^{a}	2.08^{b}	5.73^{a}	22.61^{a}
DS (B)	13.9^{b}	1.46^{b}	0.53^{b}	2.18^{c}	6.24^{b}	22.94^{b}
DS (C)	11.6^{c}	1.53^{b}	0.62^{c}	2.27^{c}	7.18^{c}	23.26^{c}
DS (D)	10.8^{c}	1.57^{b}	0.69^{c}	2.54^{d}	8.14^{d}	23.48^{c}

(DS) Dietary Supplement (date paste with *A. sinensis* and *C. villosa*, (A) concentration 0.25 g, (B) concentration 0.5 g, (C) concentration 0.75 g, (D) concentration 1 g Data were presented as mean ± SEM P-value<0.05

Table 7. Compounds derived from plants and their mechanisms of action [24,31].

Compound	Plant	PED5	MAOB	NO/CGMP
Coniferyl ferulate	*Angelica sinensis*	-	-	

Senkyunolide A			-	
Butylphthalide				+
E-Ligustilide		-	-	
E-Butylidenephthalide				+
Z-Ligustilide		-	-	
Z-Butylidenephthalide				+
Ferulic acid		-	-	
Riligustulide			-	
levistolide A			-	
Chrysin	*Calycotome villosa*		-	+
Genistein		-	-	
Apigenin			-	+
Luteolin				
Orientin				+
Eriodyctiol				+
Kaempferol		-	-	+
Butein			-	+

Nitric Oxide NO/cGMP pathway; PDE: Phosphodiesterase; MAO-B: Monoamine Oxidases type B; +: activation; -: inactivation

Sweetness of dietary supplement accepted more and gradually decreases with increasing of plant concentration % in date paste that it converted to bitter taste. Flavor also had a same pattern as sweetness; the increase of plants amount used reduces the sweetness and flavor of dietary supplement. The general desirability of the dietary supplement with the highest score followed by DS(A)>DS(B)>DS(C)>DS(D). Based on these results we can conclude that increasing amount of plants powder to paste gradually reduce the acceptance of color, sweetness and flavor, whereas their firmness was preferred. So we can conclude that the treatment DS (B) dietary supplement with concentration 0.5 g was the best result by panellist.

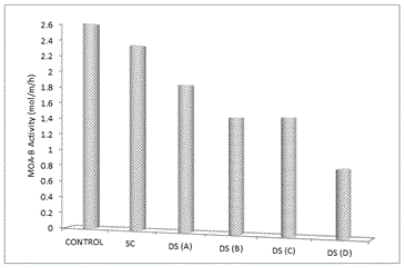

Figure 4. Determination of monoamine oxidase type B inhibition.

Evaluation of Sexual Behaviours

Determination of monoamine oxidase type B inhibition

The effect of dietary supplement (DS) on MAO-B activity was evaluated and the results were shown in Figure 4. Data clearly revealed that the effect of different concentration of *Angelica sinensis* and *Calycotome villosa* (0,25 g (A), 0.50 g (B), 0.75 g (C) and (D)) could significantly suppress MAO-B activity (p-value<0.05) respectively; compared to control. Sildenafil citrate which was used as positive control could non-significantly suppress MAO-B activity (P<0.05).

Determination of testosterone level

Figure 5 demonstrated the effect of dietary supplement (DS) on testosterone level. Dietary supplement caused significant increase in level of testosterone (P<0.05) as compared with the control. Dietary supplement with concentration 1g plant additives DS (D) significantly increased testosterone level as compared with the concentration A, B and C. However, the values still significantly higher than the control group. We have found the numbers without the significant change of testosterone level with Sildenafil citrate which was used as positive control.

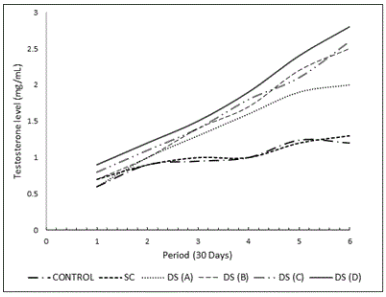

Figure 5. Determination of testosterone level.

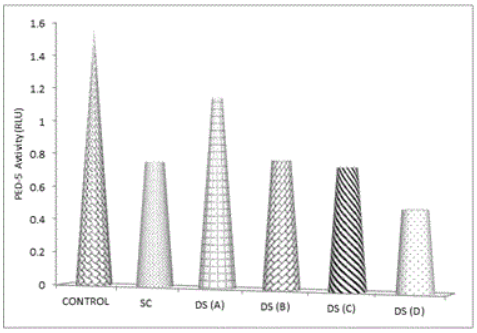

Figure 6. Determination of phosphodiesterase (PDE-5) activity.

Determination of phosphodiesterase (PDE-5) activity

We had determined the effect of the date paste with different concentration of *Angelica sinensis* and *Calycotome villosa* on the activity of PED-5 as the results were shown in Figure 6. It was found that Sildenafil citrate which was used as positive control in this study could significantly suppress PDE-5 activity. The dietary supplement both at concentrate of 0.5 g DS (B) and at concentrate of 0.75 g DS (C) have the similar significantly affect suppressed PDE activity (p-value <0.05 compared to control). Dietary supplement at dose of 1 g could suppress PDE activity as highest effect on PDE-5 activity.

Nutritional Studies of Dietary Supplement Products

Table 6 present the proximate analysis of date paste as control sample and different formulation of dietary supplement (DS). Moisture content of date paste (15.7%) decreased significantly after adding plants powder to range between 15.2% for DS (A) to 10.8% for DS (D). The decrease of moisture content was mainly due to high content of fiber and dry powder, which has absorbed amount of water content from paste. The lower content of moisture in DS will make it more solid. Protein content of DS significantly increased (1.38-1.57%) compare to paste (1.32%). The fat content of DS had the same pattern as protein content in seed, ranged between 0.46% for DS (A) to 0.69% for DS (D).

Discussion

The present results provide, for the first time, information concerning the ability of natural dietary supplement to improve male sexual behavior in rats. Accumulative lines of evidence have demonstrated that male sexual behavior is regulated mainly by neuroendocrine system and testosterone level [28]. Therefore, in this study we also determined the effect of the natural dietary supplement on testosterone level and MAO-B, PED5 activity.

Since monoamine oxidases type B (MAO-B), play an important role in neurodegenerative disorders. MAO-B is the main enzyme responsible for the oxidative deamination of dopamine in the brain. By inhibiting MAO-B, dopamine is increased in the brain [32]. MAO-B inhibitors are used in the treatment of depression and deficit in dopaminergic activity, Inhibition of this isoenzyme should raise the basal central level of dopamine [33].

Several flavonoids have been identified as inhibitors of MAO-B. Flavones may interact with the GABAA-receptor, producing sedation, anxiolytic. The flavonols and the flavones were isolated from a standardized plants extract by HPLC [34]. It could be possible that the natural dietary supplement suppresses MAOB and gave rise to the elevation of dopamine. Therefore the *Angelica sinensis* and *Calycotome villosa* content compounds can action as MOAB inhibitors as shown in Table 7.

Eroticism lead to activate an enzyme Nitric Oxide Synthase (NOS), Which converts the amino acid L-arginine to Nitric Oxide (NO) and thus increases pose NO, Which activates Guanelel cyclase enzyme which leads to form cGMP from GTP and increases the presence in the cavernous body of the penis .The cGMP activates the protein kinase enzyme leading to the occurrence of relaxation in smooth muscle surrounding arteries in the spongy cavernous body in penis that are in the normal case was cramped. Expansion of previous vessels leading to increased blood flow to the cavernous body and not to discharged again, which lead to increase the erection of the penis [35].

Cyclic nucleotide phospodiesterases (PDE5) are enzymes that regulate the cellular levels of cAMP by controlling their rates of degradation. The major PDE5 in arterial smooth muscle it has been found to be a major cGMP hydrolizing. The inhibition of PDE5 lead to increasing cGMP levels [35]. Several compounds, mostly flavonoids and chalcones have been described as PDE inhibitors and NO/cGMP activators [31].

In this study, we have found that natural dietary supplement also significantly suppressed PDE-5 activity. Therefore, the possible underlying mechanism of compounds in *Angelica sinensis* and *Calycotome villosa* might be due to its ability to suppress PDE-5 activity together with the suppression of MAOB activity as clearly in Table 7.

Vitamin E a strong anti-oxidant and has the ability to cross the blood-cerebral barrier and contributes to the creation of neurotransmitters and protects cells from the harmful effects of oxidative stress and also has the ability to inhibit the enzyme PED5 who works the degradation of cGMP [36]. All kinds of vitamin B, potassium and magnesium have increased the efficiency of Neurology, one of the most important anti-depressant substances which inhibit the of enzyme MAO-B activity, as it increases the level of testosterone in the body [37]. The presence of vitamins C, A and minerals such as phosphorus, zinc and potassium, have particular importance, it increases the male sex hormone testosterone levels in the blood, and also hinder the process of converting testosterone to estrogen they stop an enzyme aromatase, which converts testosterone to estrogen that helps increase testosterone levels, deficiency of potassium and zinc is always associated with low testosterone levels. Phosphorus helps build (Phospholipids) in the body, such as lecithin, which plays an important role in the production of sex hormones [37]. The results were shown in Tables 1-3. We have found that natural dietary supplement significantly improved testosterone level. Therefore, the possible underlying mechanism of vitamins and mineral in date paste, *Angelica sinensis* and *Calycotome villosa* might be due to its ability to increase testosterone level.

Conclusion

Enhance male sexual desire and performance. This enhancement can be ascribed to the suppression of MAOB and phosphodiesterase activities and improved testosterone level. Therefore, date paste with *Angelica sinensis* and *Calycotome villosa* may be served as the natural resource for developing functional food and food supplement to reduce male dysfunction. The dietary supplement was produced in this study is a potential agent to manage sexual dysfunction especially for acute and short term application.

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