PROBABLE NEURO SEXUAL MODE OF ACTION OF CASIMIROA EDULIS SEED EXTRACT VERSES SILDENAFIL CITRATE (VIAGRA^{TM}) ON MATING BEHAVIOR IN NORMAL MALE RATS

SYED TABREZ ALI AND NABEEH I. RAKKAH

Department of Physiology, Faculty of Medicine
Umm-Al-Qura University, P.O. Box 7607, Makkah, Saudi Arabia

ABSTRACT

The present study deals with the aphrodisiac actions of the aqueous extract of the seeds of the hypotensive plant Casimiroa edulis on the sexual behavior of normal male rats.

In this investigation 30 healthy male Wister strain white albino rats showing the brisk sexual activity age 15 weeks, weighing 400-450 grams were included. Female rats were artificially brought into estrous by hormonal treatment. Receptivity was checked by exposing them to the male rats and the most receptive females were selected for the stud The mating responses including Mounting Frequency (MF), Intromission Frequency (IF), Mounting Latency (ML), Intromission Latency (IL), Ejaculatory Latency in first and second series (EL1 and EL2) and Post Ejaculatory Interval (PEI) were recorded after treating the animals with 250 mg/kg casimiroa edulis extract (test reference) and 5 mg/kg sildenafil citrate (standard reference) respectively orally per day for 7 days.

Both the groups exhibited a significant increase in Mounting Frequency, Intromission Frequency, and first and second ejaculatory latencies, where as Mounting and Intromission latencies and the Post Ejaculatory Interval showed a significant reduction than the controls.

Although a similar pattern of mating behavior was observed among the test and the standard groups, however in all the cases as expected, sildenafil produced greater activity than the casimiroa edulis extract. These results suggest the possibility of a similar mode of action of casimiroa edulis and sildenafil citrate on mating behavior in these animals. Our work reported in this research thus provide preliminary evidence that the aqueous seed extract of casimiroa edulis possesses alphrodisiac activity and may be used as an alternative drug therapy to restore sexual functions probably via a neurogenic mode of action.

Keywords: *Casimiroa edulis;* sildenafil citrate; mating behavior; rat.

INTRODUCTION

In the present study we have tried to investigate the reproductive profiles of the aqueous extracts of the seeds of casimiroa-edulis Lia Llave et Lex, the "Zapote blano" (white Zapote) a tropical plant used by Mexican herb medicine since remote times (Power *et al*., 1911) as the "sleep-producer-fruit" when infusions of leaves or seeds were administered.

This pioneer phytochemical work was later followed by studies on casimiroa edulis (Kinkle and Romo, 1956; Merzels *et al*., 1957, Djerassi *et al*., 1958; Lozoya *et al*., 1978; 1987, Gil *et al*., 1991; 1995), trying to corroborate the hypnotic properties attributed to this sweet fruit.

A number of chemical investigations of the seeds of casimiroa edulis have been reported causing a lowering of blood pressure in animals suggesting that pharmacological activity of casimiroa edulis seeds may be attributable to the presence of N^{α}, N^{α} dimethylhistamine (Major, 1968., Murphy, 1968).

Corresponding author: e-mail: shazali_2004@hotmail.com

In an initial report (Lozoya *et al*., 1977) have confirmed the vigorous hypotensive effect produced by the aqueous and alcoholic extract from casimiroa edulis seeds on cats, rabbits and dogs, together with the constrictor effect produced on the uterus by *in vitro* experiments on several animal species including human. More recently, the hypotensive principle has been isolated and its structure elucidated, corroborating the original and consistent use of this plant (Ortega, *et al*., 1978). The main use among the population is always related to cardiac or cardiovascular diseases (Magos *et al.*, 1999).

From the literature cited above, it is now clear that the seeds and leaves of the plant casimiroa edulis have been used in Folk medicine as an hypotonic and sedative and more recently as an antihypertensive.

This compound illicit effect, including hypotension, indistinguishable from those of histamine (Lozoya *et al*., 1978; Garcia *et al.*, 1994). The fall in blood pressure induced by histamine is characteristically transient (Gil *et al*., 1995; Godlewski *et al*., 1997), where as that caused by casimiroa edulis is remarkably long lasting (Lozoya *et al*., 1978; Garcia *et al*., 1994; Gil *et al*., 1995; Baisch *et al*., 2004; Maiti *et al*., 2007), and could thus be attributed instead of any of the various imidazoles derivatives found in

the plant. The pharmacology of these constituents has not been determined in detail, but their histamine like activity is not improbable, in view of their structural similarity with the biogenic amine.

Similar to the hypotensive nature of casimiroa edulis, is the compound sildenafil citrate (Viagra) a selective vasodilator that prolongs the action of cyclic guanosine monophosphate (cGMP), the primary mediator of vasodilatation in the corpus cavernosum of penis by selectively inhibiting cGMP-specific phosphodiesterase type-5 (PDE5) facilitating smooth muscle relaxation and the flow of blood (Goldstein, 1998). These effects occur to some extent in other portions of the cardiovascular system as well (Weishaar, 1987).

Sildenafil was originally designed to take advantage of the arterial dilation of PDE-5 in the coronary arteries, allaying the symptoms of angina. Indeed, coronary artery dilation was identified in dogs, rabbits and hypertensive rats (Kloner *et al*., 1999). In a similar manner to casimiroa edulis (Baish *et al*., 2004, Maiti *et al.*, 2007), sildenafil citrate shows transient decrease in blood pressure followed by a change in pulse rate or amplitude in all the dose ranges (ACC/AHA, 1999).

In an unpublished preliminary study, we noted a marked relaxation of the rat corpus cavernosum necessary for penile erection and sexual stimulation at the standardized dose of 250 mg/kg of the seed extract of casimiroa edulis. Similar effects have been reported in the literature with sildenafil citrate as well (Moralend *et al*., 1998).

Keeping in view the similarity between the mode of action of both the compounds as a potent peripheral vasodilator and as a positive inotropic agent both in vivo and vitro, present study has been designed to determine the mechanisms involved on the sexual responses of the aqueous extract of the seeds, the part of the plant casimiroa edulis reported to be more active (Flores, 1907; Gil *et al*., 1991; 1995), which may be helpful for the identification of active compounds of the fraction of the crude extract for further pharmacological evaluation to determine its influence in diverse reproductive parameters. This would benefit exploring the tremendous untapped potential of new drug development from plants for complementary medical practice.

MATERIALS AND METHODS

Preparation of the Casimiroa edulis extract

The aqueous extract of casimiroa edulis seeds was prepared as described previously (Gil and Horacio, 1991; Baish *et al*., 2004). Briefly, ripe fruits of casimiroa edulis were obtained and after being deflated, the dry powdered kernels were extracted successfully by maceration at room temperature with hexane, dichloromethane, 4:1 and 1:1 mixtures of dichloromethane-methanol, methanol and finally water. Organic solvents were eliminated in a rotatory evaporator; water was removed by lyophilization. For the pharmacological tests, the solid aqueous extract thus obtained (approximately yield: 5.5%) was dissolved in isotonic $NaCl_2$ solution to a concentration of 100 mg/ml.

Aphrodisiac studies

Wister strain white albino male and female rats, age 15 weeks, weighing 400-450 grams and 250-300 grams respectively, were obtained from the institutional animal house facility and were hosted singly in separate standard cages at standard laboratory environment (22-24C^o room temperature, 60-70% humidity, 12 hour light-dark cycle, free access to solid pellet diet).

30 healthy and sexually experienced male rats showing the brisk sexual activity were selected and divided in 3 groups as Control, Test, and Standard. In each group, 10 animals were selected for the experimental purposes receiving 10 ml/kg Millipore water, 250 mg/kg casimiroa edulis extract and 5 mg/kg sildenafil citrate (Pfizer-USA) respectively orally per day for 7 days. Since the sildenafil citrate has a maximum mode of action for 4 hours only after the administration, an additional oral suspension dose of 5mg/kg was given to sildinafil group, 1 hour before the mating behavior study.

Female rats were artificially brought into estrous by administrating oral suspension of ethyl estradiol (Schering-Germany) 48 hours before the mating by the established methods (Szechtman *et al*., 1981). An additional 1 mg/ml subcutaneous injection of progesterone (Schering-Germany) was given to all female rat 6 hours before the mating. Receptivity was checked by exposing them to the male rats and the respective females were selected for the study. The recipient female rats were introduced into the male cages of all 3 groups and the mating responses including mounting frequency (MF), intromission frequency (IF), mounting latency (ML), intromission latency (IL), ejaculatory latency in first and second series (EL1 and EL2) and post ejaculatory interval (PEI) were recorded by established methods (Dewsbury *et al*., 1970; Rutna *et al*., 2000). Statistical analysis for all the parameters was done using one-way analysis of variance (ANOVA) method.

RESULTS

The data for the mounting frequency (MF) after the oral administration of 250 mg/kg. casimiroa edulis extract and 5 mg/kg. sildenafil citrate dose in 10 male rats and in 10 age-matched controls is shown in (fig. 1). The values of the mounting frequency obtained by the casimiroa edulis extract/sildenafil citrate treated rats compared with the values obtained by the control group showed a highly significant increase ($p<0.0001$). A significant increase

was again noted in intromission frequency in both the casimiroa edulis extract ($p<0.001$) and sildenafil citrate ($p<0.0001$) groups compared with the control group (fig. 2). Similarly values for the ejaculatory latency tests both in first and second series further indicated a highly significant increase ($p<0.0001$) in the casimiroa edulis/sildenafil treated groups compared with the controls (figs. 3 and 4 respectively).

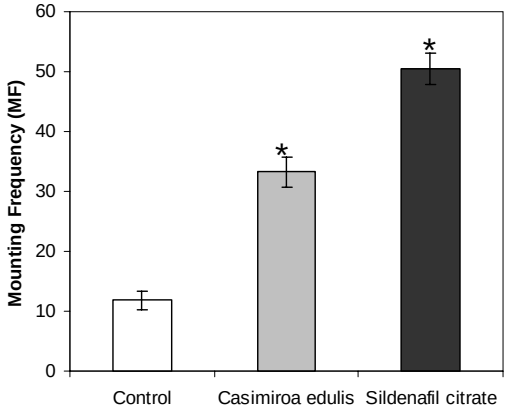

Fig. 1: Effect of the seed extract of casimiroa edulis and sildenafil citrate on mounting frequency in normal male rats. Values are Mean ± SE (n=10).

Note: n = Total number of the rats examined. Casimiroa edulis/sildinafil citrate treated values are compared with age matched control rats.

* $p<0.0001$

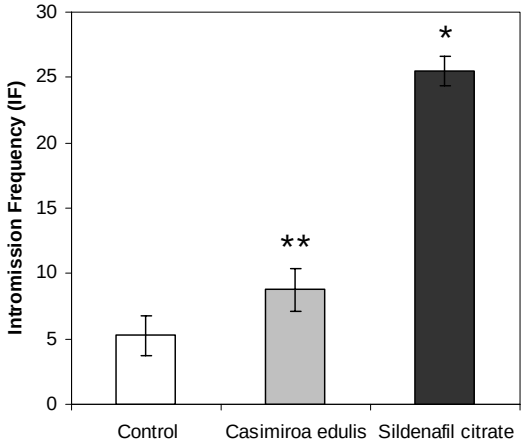

Fig. 2: Effect of the seed extract of casimiroa edulis and sildenafil citrate on intromission frequency in normal male rats. Values are Mean ± SE (n=10).

Note: n = Total number of the rats examined. Casimiroa edulis/sildinafil citrate treated values are compared with age matched control rats.
* $p<0.0001$, ** $p<0.001$

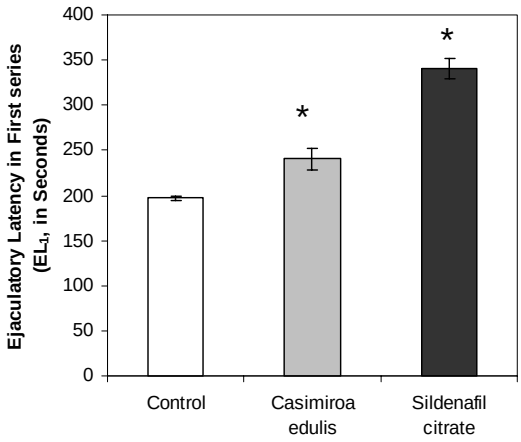

Fig. 3: Effect of the seed extract of casimiroa edulis and sildenafil citrate on ejaculatory latency in first series in normal male rats.
Values are Mean ± SE (n=10).
Note: n = Total number of the rats examined. Casimiroa edulis/sildinafil citrate treated values are compared with age matched control rats.
* $p<0.0001$

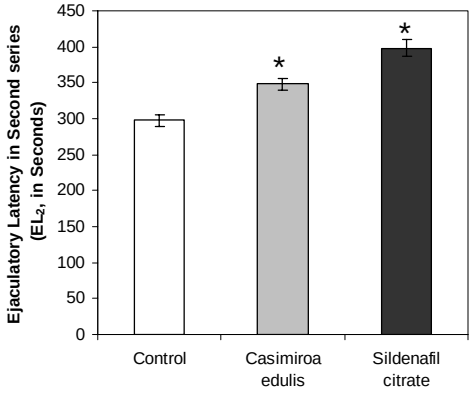

Fig. 4: Effect of the seed extract of casimiroa edulis and sildenafil citrate on ejaculatory latency in second series in normal male rats.
Values are Mean ± SE (n=10).
Note: n = Total number of the rats examined. Casimiroa edulis/sildinafil citrate treated values are compared with age matched control rats.
* $p<0.0001$

In contrast an inverse relationship was noted when the mounting latency, intromission latency, and post-ejaculatory interval were studied in the casimiroa edulis and sildenafil citrate treated rats (figs. 5, 6 and 7 respectively). In comparison with control group, the test group (casimiroa edulis) showed a significant decrease in mounting latency and intromission latency ($p<0.001$), where as this decrease was found to be highly significant in the standard group (sildenafil citrate) ($p< 0.0001$). In a similar manner, values of the post ejaculatory interval

showed a highly significant decrease ($p<0.0001$) both in the test as well as in the standard group, when compared with the controls.

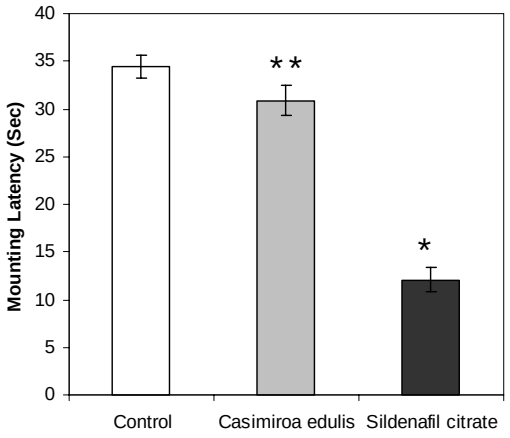

Fig. 5: Effect of the seed extract of casimiroa edulis and sildenafil citrate on mounting latency in normal male rats. Values are Mean ± SE (n=10).
Note: n = Total number of the rats examined. Casimiroa edulis/sildinafil citrate treated values are compared with age matched control rats.
* $p<0.0001$, ** $p<0.001$

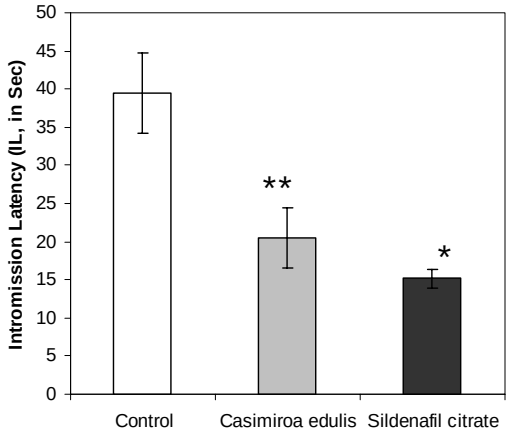

Fig. 6: Effect of the seed extract of casimiroa edulis and sildenafil citrate on intromission latency in normal male rats. Values: Mean ± SE (n=10).
Note: n = Total number of the rats examined. Casimiroa edulis/sildinafil citrate treated values are compared with age matched control rats.
*$p<0.0001$, ** $p<0.01$

Although a similar pattern of mating behavior was observed between the test and the standard group of animals, however in all the cases as expected, sildenafil produced greater activity than the casimiroa edulis extract. These results suggest the possibility of a similar mode of action of casimiroa edulis and sildenafil citrate on mating behavior in these rats.

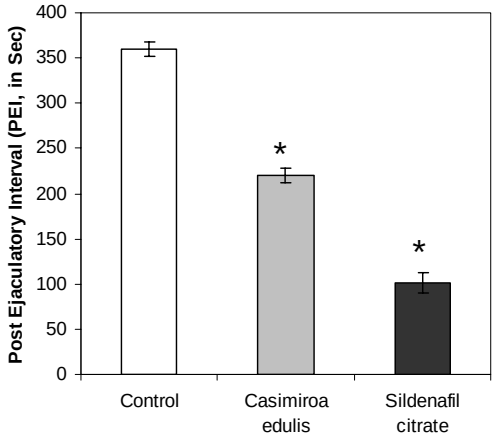

Fig. 7: Effect of the seed extract of casimiroa edulis and sildenafil citrate on post ejaculatory interval in normal male rats. Values are Mean ± SE (n=10).
Note: n = Total number of the rats examined. Casimiroa edulis/sildinafil citrate treated values are compared with age matched control rats.
* $p<0.0001$

DISCUSSION

Plants are described as the major alternate source of medicines, not only as isolated active principle to be dispensed in standardized dosage forms but also as crude drugs for the population (Bannerman *et al*., 1983). On the other hand, the revival of interest in herbal medicine as a system of natural cure has emerged as a new trend worldwide (Phillipson, 1981., Aftab *et al*., 1996). It aims at reporting and rationalizing the traditional uses of pharmacologically active natural products to explore the tremendous untapped potential of new drug development from plants. In the present study perhaps for the first time we have tried to establish the reproductive profile of chemical constituents of the plant *casimiroa edulis* which has established smooth muscle relaxation, hypotensive and hypnotic effects with extract of the seeds in comparison with sildenafil citrate (viagra) as the standard reference.

Our results showed that 250 mg/kg aqueous seed extract of casimiroa edulis produced significant enhancing sexual activity in male rats as noted by their mating behavior tests.

In all the experiments, a significant increase was observed in mounting and intromission frequency as well as ejaculatory latencies both in the first and second series in both the test and standard groups when compared with the controls of the same aged rats, probably resulting due to an increase in potency and libido, an effect exerted by the casimiroa edulis extract comparable with the standard drug (sildenafil citrate) as expected with much greater tendency.

On the other hand, a significant reduction was noted in the mounting and intromission latencies in both the test and

standard groups than the controls, again indicating a significant progression in the sexual behavior of these animals, an effect exerted by the test as well as the standard drug. Post ejaculatory interval observations revealed the similar results.

Since post ejaculatory interval generally indicates the rate of recovery from exhaustion after first series of mating, as well as an index of sexual drive, a significant decrease in this parameter in our test and standard experiments indicates lesser exhaustion in the first series of mating, a sign of increased frequency of sex drive in these animals. These results are in conformity with the previous findings (Ottani *et al.,* 2002, Pattij *et al.,* 2005).

Effect of the hydro alcoholic extract of casimiroa edulis on nervous system activity in rats and mice has been reported previously (Mora *et al.,* 2005). Oral administration of the casimiroa edulis extract in mice showed prolongation of pentobarbital-induced hypnosis, increased exploration of elevated plus-maze (EMP) open arms and partially protected from the pentylenetetrazol-induced convulsions. These results provide evidence that hydro alcoholic extract of casimiroa edulis may contain sedative principles of anxiolytic and antidepressant properties affecting the nervous system activity. Further evidence for a neurogenic effect of casimiroa edulis comes from the anticonvulsive studies of the aqueous extract of the seeds and leaves of this compound in rats. Garzon *et al.,* (1999) noted a maximum protection of 70% at the 2^{nd} and 4^{th} hour with 100 mg/kg. casimiroa edulis seed extract in pentylenetetrazole induced seizure in rat. Similar observations have been made in a previous study by using the leave extract of this compound (Navarro *et al*., 1995).

Sildenafil enhanced neurogenesis in young rats has been reported by augmenting neurogenesis in aged rats after focal cerebral ischemia (Zhanq *et al*., 2006). In these experiment treatments with sildenafil citrate, improved functional recovery thus suggesting that inhibition of PDE5 activity by sildenafil augments neurogenesis in the sub ventricular zone of the aged ischemic rats although these rats had reduced number of neural progenitor and stem cells in sub ventricular zone. Casimiroa edulis has also been reported to have its hypotensive and vaso relaxing activity through the activation of adrenergic mechanism via mediating nitric oxide release (Baisch *et al*., 2004).

Significant improvement in sexual activity in our study may be interpreted as an effect of casimiroa edulis that stimulates the neural mechanism. The extract may induce changes at the neurotransmitter levels or at the neuronal levels in a similar manner like sildenafil citrate by enhancing the relaxant effects of nitric oxide in response to sexual stimulation. Casimiroa may promotes penile erection in these rats by blocking the activity of type 5-PDE, which causes cGMP to accumulate in the corpus cavernosum of the penis leading to an increased sexual drive. Similar suggestions have been made previously with other compounds (Suresh *et al.,* 2000).

Our work reported in this research thus provide preliminary evidence that the aqueous seed extract of casimiroa edulis possesses alphrodisiac activity and may be used as an alternative drug therapy to restore sexual functions probably via a neurogenic mode of action. Further studies are however recommended to determine the exact dose and the mode of action of this compound to enhance the sexual performance in both vivo and vitro.

REFERENCES

ACC/AHA Expert Consensus Document (1999). Use of sildenafil (Viagra) in patients with cardiovascular disease. *J. Am. College Cardiol.,* **33:** 273-282.

Aftab K, Ahmed SI and Usmanghani K (1996). Toladitional medicine cassia Absus L (Chaksu) Pharmacological evaluation. *Phytomedicine*, **2**(3): 213-219.

Baisch AL, Urban H and Ruiz AN (2004). Endothelium-dependent vasorelaxing activity of aqueous extract of lyophilized seeds of casimiroa edulis (AECe) on rat mesenteric arterial bed. *J. Ethnopharmacol.,* **95**(2-3): 163-167.

Bannerman RH, Burtan J and Lwen-Chiehc (Eds) (1983). *In*: Traditional Medicine in Health Care Coverage.

Dewsbury DA and Davis HN (1970). Effect of reserpine on the copulatory behavior of male rats. *Physiol. Behav***.,** **5**(1): 1331-1333.

Djerassi CC (1958). Partial structure of Casimiroa edine. *Int. J. Org. Chem.*, **168**(2): 170.

Djerassi CC, Bankiewicz A, Kapoor L and Riniker B (1958). Partial structure of Casimiroedine. *Tetrahedron*, **2**: 168a. 172.

Flores L (1907). Encuesta Sobre el uso actual de plantas en la medicina traditional mexicana. *Ann. Int. Med. Nac.,* **9**: 297-391.

Garcia GM, Freer BE and Morales MO (1994). Effects of casimiroa edulis (Rutacea) on blood pressure and heart rate in albino rats. *Rev. Biol. Trop.,* **42**(1-2): 115-119.

Garzon-De la Mora P, Garcia LP, Garcia EJ, Navarro RA, Villanueva MT, Villarrea LM and Casillase OJ (1999). Casimiroa edulis seed extracts show anticonvulsive properties in rats. *J. Ethnopharmacol.,* **68**(1-3): 275-282.

Gil AM and Horacio V (1991). Pharmacology of Casimiroa edulis; Part I. Blood Pressure and heart rate effects in anesthetized rat. *Planta. Med.,* **57**: 20-24.

Gil AM, Horacio V and Raul E (1995). Pharmacology of Casimiroa edulis; III. Relaxation and contractile force in rat aortic ring. *J. Ethnopharm.,* **47**: 1-8.

Godlewski G, Malinowska B and Buezko W (1997). Inhibitory H_3-receptors on sympathetic nerves of pithed rat: Activation of endogenous histamine and operation in

spontaneously hypertensive rats. *Naunyn-Schmiedeberg's Arch. Pharm.*, **355**: 261-266.

Goldstein I, Lue T, Padma-Nathan H, Rosen RC, Steers WD and Wicker PA (1998). Oral sildenafil in the treatment of erectile dysfunction. *N. Engl. J. Med.*, **338**: 1397-1404.

Huebner JC, Marr S and Hu T (1951). Detection of new 7-0-acyl derivatives of dimethyl histamine detected by liquid chromatography-mass spectrometry. *J. Am. Chem. Soc.*, **80**: 201-210.

Kinkle FA and Romo J (1956). The constituents of Casimiroa-edulis Llava et Lex. Part 1, The seed. *J. Am. Chem. Soc.*, **76**: 4163-4173.

Kloner RA and Zusman RM (1999). Cardiovascular effects of sildenafil citrate and recommendations for its use. *Am. J. Cardiol.*, **84**: 11N-17N.

Lozoya X, Romero G and Olmedo (1977). Farmacodinamia de los extractos alcoholicos YH acuosos de la semilla de casimiroa edulis. *Arch Invest. Med (Mex).*, **8**: 145-152.

Lozoya X, Rodriguez D, Ortega J and Enriquez R (1978). Isolation of a hypotensive substance from seeds of casimiroa edulis. *Arch Invest Med (Mex).*, **9:** 565-573.

Lozoya X (1980). Mexican Medicinal Plants used for the treatment of cardio vascular diseases. *Am. J. Chinese. Med.*, VIII. **1**: 86-95.

Lozoya X, Auglar A and Camacho JR (1987). Encuesta Sobrle el uso actual de plantas enla medicina traditional maxicana. *Revista Medica del Instituto Mexicano del seguro social.* **25**: 283-291.

Magos GA, Vidrio H and Enriquez R (1999). Pharmacology of Casimiroa edulis; III. Relaxant and contractile effects in rat aortic rings. *J. Ethnopharmacol.*, **47**(1): 1-8.

Maiti A, Cuendet M, Kondratyuk T, Croy VL, Pezzuto JM and Cushman M (2007). Synthesis and cancer chemopreventive activity of zapotin, a natural product from casimiroa edulis. *J. Med. Chem.*, **50**(2): 350-355.

Major RT (1968). Na - Na, dimethyl histamine, a hypotensive possible principal in casimiroa edulis. *J. Org. Chem.*, **23**: 1569.

Merzels A (1958). The constituents of Casimiroa edulis IV. *J. Org. Chem.*, **23**: 762-764.

Mora S, Diaz-veliz G, Lungenstrass H, Garcia-Gonzalez M, Coto-Morales T, Poletti G, De Lima TC and Herrera-Ruiz M Tortoriello J (2005). Central nervous system activity of the hydro alcoholic extract of casimiroa edulis in rats and mice. *J. Ethnopharmacol.*, **97**(2): 191-197.

Moralend RB, Goldstein I and Traish A (1998). Sildenafil a novel inhibitor of phosphodiesterse type 5 in human corpus cavernosum smooth muscle cells. *Life Sci.*, **62**: PL309-PL318.

Murphy W (1968). The structure of Zapoterin. *Tetrahedron letters*, **49**: 5153.

Navarro RA, Bastidas R, Garcia EJ, Garcia LP and Garzon P (1995). Anticonvulsant activity of casimiroa edulis in comparison to phenytoin and Phenobarbital. *J. Ethnopharmacol.*, **45**(3): 199-206.

Ottani A, Giuliani D and Ferrari F (2002). Modulatory activity of sildenafil on copulatory behavior of both intact and castrated rat. *Pharmacol. Biochem. Behav.*,**72**(3): 717-722.

Ortega J, Lozoya X, Enriquez R and Aislamiento del (1978). Principio Hipotensor de la semilla de casimiroa edulis. *Arch. Invest. Med.*, 119.

Pattij T, de Jong TR, Uitterdijk A, Waldinger MD, Cools AR, Van der Graaf PH and Olivier B (2005). Individual differences in male rat ejaculatory behavior: searching for models to study ejaculation disorders. *Eur. J. Neuro Sci.*, **22**(3): 724-734.

Phillipson JD and Anderson LA (1981). Economic and Medicinal Plants in research. *Pharm. J.*, **227**: 387-392.

Power FB and Callan T (1911). The constituents of the seeds of casimiroa edulis. *Trans. Chem. Soc. (London).*, **99**: 183-193.

Ratna SWD and Dharmasiri MG (2000). Effect of Terminalia catappa seeds on sexual behavior and fertility in male rats. *Asian J. Androl.*, **20**: 213-219.

Suresh K, Subramoniam A and Pushpangadan P (2000). Aphrodisiac activity of Vanda tessellate (ROXB). *J. Pharmacol.*, **32**: 300-304.

Weishaar RE, Kobylarz-Singer DC, Steffen NP *et al* (1987). Subclasses of cyclic cAMP-specific phosphodiesterase in left ventricular muscle and their involvement in regulating cardiac contractility. *Circ Res.*,61 **(4):** 539-547.

Zhanq RL, Zhanq Z, Zhanq L, Zhanq C, Wang Y and Choop M (2006). Delayed treatment with sildenafil enhances neurogenisis and improves functional recovery in aged rats after focal cerebral ischemia. *J. Neuro. Sci. Res.*, 15; **83**(7): 1213-1219.