World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 3.347

GC-MS ANALYSIS AND APHRODISIAC ACTIVITY OF *TRIAMTHEMA DECANDRA* LINN.

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Article Received on 29/10/2016 Article Revised on 18/11/2016 Article Accepted on 08/12/2016

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ABSTRACT

Alcoholic extract of aerial parts of *Trianthema decandra* showed the presence of terpenoids, steroids and alkaloids. GC-MS analysis of the alcoholic extract showed presence of 20 compounds with Silane, dimethoxydimethyl, Borazine, phosphine oxide, n-Hexadecanoic acid, 9-Octadeccenoic acid are in high concentration. The extract was then tested for its Aphrodisiac activity using experimental model viz, Sexual behavior on prolonged immobilization- induced stress in rats. The acute toxicity study of alcoholic extract of *Trianthema decandra* was carried out for determination of LD_{50} up to the dose level of 2000mg/kg, extract was administered orally to rats as per the OECD guidelines no.425. The different dose were selected $1/20^{th}$, $1/10^{th}$, $1/5^{th}$ of the lethal dose were taken as effective dose for the study. In prolonged immobilization-induced stress model, parameters like Mount latency, number of mounts, Thrusting were recorded simultaneously by two investigators with light provided by a 40-watt red lamp. Alcoholic extract of *Trianthema decandra* showed Aphrodisiac activity in a dose dependant manner. The medium and high dose treated groups have shown a significant increase in the number of mounts, thrusting and decrease in the latency. The results suggest the Aphrodisiac activity of aerial parts of *Trianthema decandra* and may be attributed to elevation of Testosterone, Adrenergic, Cholinergic & Dopamine levels elevation.

KEYWORDS: GC- MS, Aphrodisiac, *Trianthema decandta*, Tentex fort.

INTRODUCTION

The sexual dysfunction in male i.e. Erectile dysfunction, is either functional or organic and commonly referred as impotency. It may result from psychological, neurologic, hormonal, arterial or cavernosal impairment or from a combination of these factors.[1] There is an increase in incidence of erectile dysfunction as a result of old age, increase in prevalence of degenerative diseases and stress associated life styles.[2] It is estimated that about 20 to 30 million men are affected by this condition and the prevalence increases with age.[3] Both medical and surgical treatment modalities are available for treating this condition and the agents which cause the arousal of sexual desire are known as aphrodisiacs. However despite the availability of a number of effective conventional aphrodisiac agents the plant derived herbal aphrodisiacs continue to be a popular alternative to allopathy to improve sex life.[4] The plant *Trianthema decandra* commonly known as Gadabandi in Hindi and Punarnavi in Sanskrit belonging to the family Aizoaceae. This plant is globally distributed tropical and sub tropical regions. In India it grows in dry-soil lands. It has been known since ancient times for curative properties and has been utilized for treatment of various ailments such as burns and wounds. The leaf extract has been used in the treatment of chronic pain of osteoarthritic patients. The juice of the leaves dropped in to nostrils relives one-sided headache. The roots are aperients and said to be useful in hepatitis, asthma and suppression of the menses. This plant is mainly used as antioxidant, analgesic, antimicrobial and anti-inflammatory.[5] In the present study, we have evaluated the effect of alcoholic extract of *Trianthema decandra* on the sexual behavior and general short term toxicity studies in rats.

MATERIALS AND METHODS

Plant material

The fresh aerial parts of *Trianthema decandra* were collected and authenticated by Dr. K.Madhava Shatty, Assitant Professor, Dept. of Botany, S.V. University, Tirupathi, A.P. The plant herbarium was prepared (PRIP-01/13) and deposited in the Dept. of Pharmacognosy, Pulla Reddy Institute of Pharmacy for further reference.

Preparation of Extract

The whole plant was dried at room temperature under shade and coarse powdered. The powered material were subjected to hot extraction process using soxhlet apparatus by successive solvent extraction method based on the increasing order of solvent polarity and

solvent was removed by Buchi 461 Rotary vacuum evaporator. The alcoholic extract was then used for GC-MS and Aphrodisiac activity studies.

Preliminary phytochemical screening[6,7]

The crude extract obtained from the pilot scale were subjected to preliminary phytochemical screening to characterize the different constituents present in them.

GC-MS analysis of Alcoholic extract of aerial parts of Trianthema decandra

The GC-MS analysis of alcoholic extract of *Trianthema decandra* was carried out in a GC-MS-QP2010. Mark: SHIMADZU gas chromatograph fitted with ZB-624 30 m X 1.4mm ID 0.25µm film thickness or equivalent column. Carrier gas was helium with a flow rate of 2.5 ml/min; column temperature initially was at 120^0C for 2 min. then rose to 250^0C at the rate of 10^0C per minute maintained at 250^0C for 20min; injector temperature 220^0C, detector temperature 260^0C, volume injected was 1µl with liquid injector alcoholic extract; The Mass spectra operating parameters were, ionization potential, 70eV; ion source temperature; 250^0C, solvent delay 3.0 min, program run time:31 min and scan range 30-350 amu, EV voltage 3000 volts. Finally structural fragments were identified on the basis of retention time and mass spectral data by computer matching with commercial library software Wiley-229.

Selection and housing of animals

Healthy albino rats (150-200g) of either sex were selected for acute oral toxicity studies and for investigation of Aphrodisiac property of the alcoholic extract. Animals were housed individually under standard environmental condition, fed with standard diet and water *ad libitum*, and were acclimatized for 7 days before the experiment.

Determination of Acute Toxicity[8,9]

Acute toxicity study of alcoholic extract of *Trianthema decandra* Linn was carried out for determination of LD_{50} up to the dose level of 2000mg/kg, extract was administered orally to rats as per the OECD guidelines no.425. The LD_{50} of the test extract was calculated using aot 425 software provided by environmental protection agency, USA. From the LD_{50} dose 1/20, 1/10 & $1/5^{th}$ doses were selected and considered as low, medium and high dose respectively for the study. During the first four hours after the drug administration, the animals were observed for gross behavioral changes if any for 7 days. Parameters such as hyperactivity, grooming, convulsions, hypothermia, sedation and mortality were observed.

APHRODISIAC ACTIVITY STUDIES

Sexual behavior on prolonged immobilization- induced stress in rats.

A total of thirty Prepubertal (40 days of age) male albino rats were housed for 7 days under standard husbandry condition like room temperature 26 ± 2^0c, relative humidity 45-55% and light/ dark cycle of 12 hours. All the animals were fed with synthetic standard diet (amrut laboratories pranava agro industries ltd. sangli) and water was supplied ad libitum under strict hygienic conditions[10,11] after obtaining permission from Institutional Animal Ethical Committee (IAEC) of Smt. Sarojini Ramulamma college of Pharmacy, Mahabubnagar, (Telangana State), animal studies were performed as per rules and regulations in accordance to guidelines of CPCSEA with registration number 51/01/C/CPCSEA.

Rats were randomly divided into five groups of six animals each. Rats were trained with sexually active were selected for main experiment. Rates in group I was administered Gum acacia 10ml/kg, p.o., and served as control, while group II, III and IV received 100mg/kg, 200mg/kg and 400mg/kg of alcoholic extract of *Trianthema decandra* dissolved in Gum acacia and group V received Tentex forte 1000mg/kg as a standard drug. Different groups of animals were treated as mentioned above in the morning. Stress immobilization was attained by wrapping the animals in wire mesh for 3 hrs a day during the light period, starting at 8:00 a.m., for 15 days. Control animals were left undisturbed in their cages. The males were placed in the observation 2 hrs after the beginning of the dark phase and 10 min before the females for adaptation. The latency, number of mounts and thrusting were recorded at the same time by two investigators with light provided by a 40-watt red lamp. In the mount behavior the male places his forepaws on the female without pelvic movements, while in the thrusting behavior he executes repeated deep pelvic thrusts.[12,13]

Statistical Analysis[14]

Data was shown as the mean ± SEM and analysed by one way ANNOVA followed by students T-test. The level of significance for all experiments was P<0.05*, 0.01** and 0.001**.

RESULTS AND DISCUSSION

The preliminary phytochemical investigations showed the presence of terpenoids, steroids, flavonoids, alkaloids, saponins and carbohydrates in the alcoholic extract of *Trianthema decandra*. GC-MS analysis of the extract showed presence of fragments like silane, sym-tetramenthyldimethoxydisiloxane, Omega-Dimethoxytetramethyl cyclopentasiloxane,

haetpamethyl-phenyl-cyclotetrasiloxane, borazine,2,4,6-triphenyl-1,3,5-tripropyl, phosphine oxide, bis-pentamethylphenyl, n-Hexadecanoic acid, 9-Octadecenoic acid, 9, 12-Octadecadienoic acid. A complete analysis report is given in Table 1.

Table 1. GC-MS analysis of Alcoholic extract of *Trianthema decandra.*

S.No.	Retention	Name	% Area
1	1.169	Ethyl alcohol tecsol jaysol alcohol algrain	1.55
2	1.1248	2-Propanol Isopropyl alcohol propan-2ol, Propol Lutosol Alcojel Avantin Imsol	3.35
3	1.647	Silane, dimethoxydimethyl, Dimethoxydimethylsilane	4.55
4	1.841	Silane, dimethoxydimethyl, Dimethyldimethoxysilane	3.09
5	6.443	Sym-Tetramethyldimethoxysisiloxane, Disiloxane, 1,3-dimethoxy-1,1,3,3-tetramethyl, alpha, Omega-Dimethoxytetramethyl	1.83
6	9.575	Cyclopentasiloxane, decamethyl	0.56
7	9.809	Cyclopentasiloxane, decamethyl	1.93
8	21.698	Hepamethyl-phenyl-cyclotetrasiloxane	0.45
9	21.867	Borazine,2,4,6-triphenyl-1,3,5-tripropyl	0.55
10	21.992	Borazine,2,4,6-triphenyl-1,3,5-tripropyl	1.66
11	22.183	Phosphine oxide, bis(pentamethylphenyl)	1.56
12	22.308	Borazine,2,4,6-triphenyl-1,3,5-tripropyl	1.21
13	22.447	Borazine,2,4,6-triphenyl-1,3,5-tripropyl	5.96
14	23.558	n-Hexadecanoic acid	13.06
15	23.725	n-Hexadecanoic acid	1.20
16	23.847	Nonamethyl, Phenyl-Cyclopentasiloxane	2.89
17	24.623	9-Octadecenoic acid, Methyl ester, methyl oleate, Methyl cis-9-ocadecenoate, Oleic acid methyl ester, Oleic acid	0.83
18	24.675	9-Octadecenoic acid, Methyl ester, Oleic acid, methyl ester, Emery oleic acid ester, Methyl cis-9-octadecenoate.	0.93
19	25.477	9-Octadecenoic acid, trans-delta- Octadecenoic acid, trans-delta-9- Octadecenoic acid, trans-Octadec-9-enoic acid	52.40
20	26.110	9,12- Octadecadienoic acid, cis-9-cis-12-Octadecadienoic acid, cis-cis-Linoleic acid	0.45

Pretreatment with alcoholic extract of *Trianthema decandra* and Tentex Fort were able to significantly decrease intromission mounting latencies, when compared to control group. It also significantly increases the number of mounts and thrusting in comparison with control and standard drug but Alcoholic extract of *Trianthema decandra* with 100mg/kg did not show a significant effect on mounting latencies, number of mounts and thrusting in comparison with control and standard drug. Shown in Table no.2,3,4 and graphically depicted in Fig no. 1,2, 3.

Table 2: Effect of AETD Mounting latency in stress induced altered sexual behavior in Rats.

S.NO	GROUP	H	B	T	HB	BT	HT	MEAN ± SEM
1	CONTROL	905	1050	968	958	854	820	925.83 ± 34.169
2	STANDARD	71	60	48	64	59	53	59.167** ± 3.301
3	AETD(100 mg/kg)	987	952	958	958	980	960	965.83^{NS} ± 5.764
4	AETD (200mg/kg)	87	60	75	90	67	63	204.50** ± 20.013
5	AETD (400mg/kg)	70	55	45	70	60	50	73.667** ± 5.136

Table 3: Effect of AETD on Number of Mounts in stress induced altered sexual behavior in Rats.

S.NO	GROUP	H	B	T	HB	BT	HT	MEAN ± SEM
1	CONTROL	11	12	9	10	9	8	9.833 ± 0.6009
2	STANDARD	35	39	28	25	38	34	33.167 ** ± 2.272
3	AETD (100mg/kg)	20	19	21	28	25	19	14.167^{NS} ± 1.302
4	AETD (200mg/kg)	32	34	29	33	30	31	22.000** ± 1.506
5	AETD (400mg/kg)	40	45	32	30	42	40	31.500** ± 0.7638

Table 4: Effect of AETD on Thrusting in stress induced altered sexual behavior in rats.

S.NO	GROUP	H	B	T	HB	BT	HT	MEAN ± SEM
1	CONTROL	8	7	7	6	7	5	6.667 ± 0.4216
2	STANDARD	33	38	24	26	35	32	31.333** ± 2.186
3	AETD(100mg/kg)	18	17	20	24	24	17	11.167^{NS} ± 0.6009
4	AETD (200mg/kg)	30	32	24	32	29	27	20.000** ± 1.342
5	AETD (400mg/kg)	38	32	30	25	40	38	29.000** ± 1.265

Table 5: Effect of AETD on Mounting latency, Number of Mounts and Thrusting in Stress induced altered sexual behavior in Rats.

S.no	Group	Dose mg/kg	Mounting latency mean ± sem	Number of mounts mean± sem	Thrusting mean ± sem
1	Control	10 ML	925.83 ± 34.169	9.833 ± 0.6009	6.667 ± 0.4216
2	Standard tentex forte	100	59.167** ± 3.301	33.167 ** ± 2.272	31.333**± 2.186
3	Low dose aetd	100	965.83^{NS} ± 5.764	14.167^{NS} ±1.302	11.167^{NS} ±0.6009
4	Medium dose aetd	200	204.50** ±20.013	22.000** ±1.506	20.000** ± 1.342
5	High dose aetd	400	73.667** ± 5.136	31.500** ±0.7638	29.000** ± 1.265
f one way anova df					704.35 35

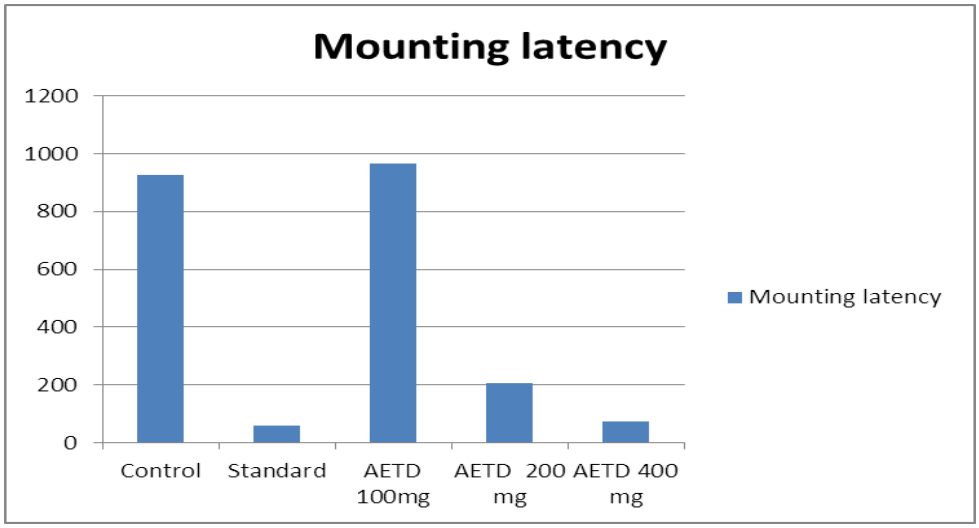

Fig. No.: 1. Effect of *Trianthema decandra* linn on Mounting latency in Rats.

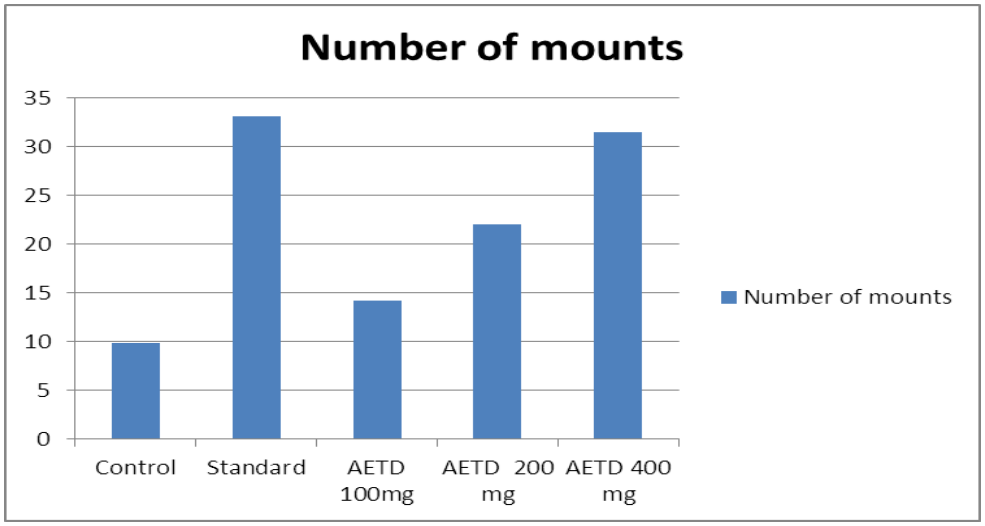

Fig. No.: 2. Effect of *Trianthema decandra* linn on Number of Mounts in Rats.

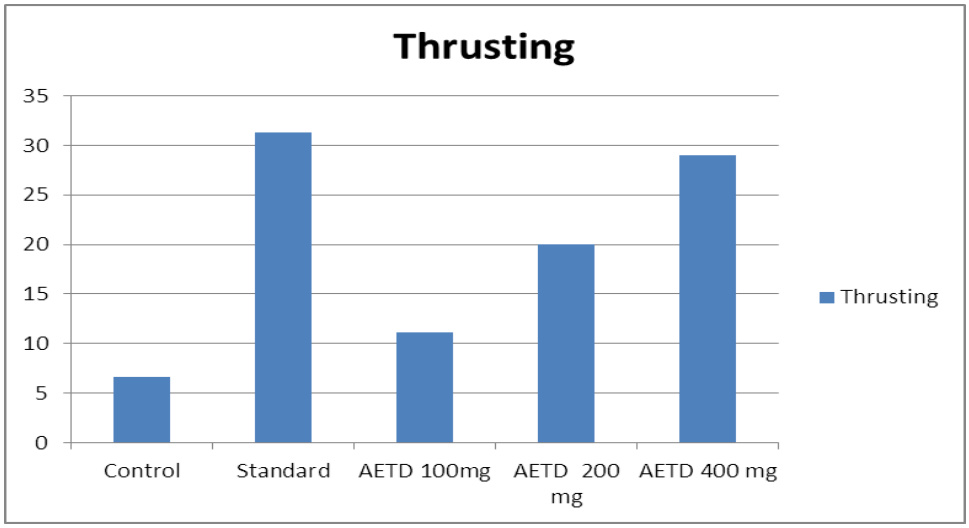

Fig. No.: 3. Effect of *Trianthema decandra* linn on Thrusting in Rats.

Erectile dysfunction is more prevalent in males than in females and thus, it is conventional to focus more on male sexual difficulties. Male sexual behavior is regulated by a range of redundant mechanism involving several neuropeptides (oxytocin and galanin, with inhibitory activity) and neurotransmitters (mainly dopamine, serotonin, noradrenaline and norepinephrine). The stimulatory effect of oxytocin on male sexual behavior is proportionately greater in sexually sluggish than in sexually potent animals. Low non-stereotypy-inducing doses of direct or indirect dopaminiergic drugs improve the copulatory performance of sluggish/impotent males, while a further improvement of the sexual behavior of vigorous copulators is not always clearly apparent. Finally, facilitation of central noradrenergic transmission, either by blockade of alpha 2-adenoreceptors or by stimulation of beta 2-adrenorecpetors, while having either no effect or a worsening effect in sexually potent animals, improves copulatory behavior in sexually sluggish animals.

Sexual behavior in prolonged immobilization induced stress model, the animals were cohabitated with one oestrous female for 30 min after 15 days of immobilization stress. Standard Tentex forte and Alcoholic extract of *Trianthema decandra* (100, 200 & 400 mg/kg) treated groups have shown a significant increase in number of mounts and thrusting and decrease in mounting latency as these were considered as a positive signs for aphrodisiac activity.

ACKNOWLEDGMENT

The authors are thankful to the Principal and Chairman GNITC, School of Pharmacy, Hyderabad, for providing necessary facilities to carry out the research work.

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