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Phytochemical Investigation and Anxiolytic Evalution of *Carissa Carandas*

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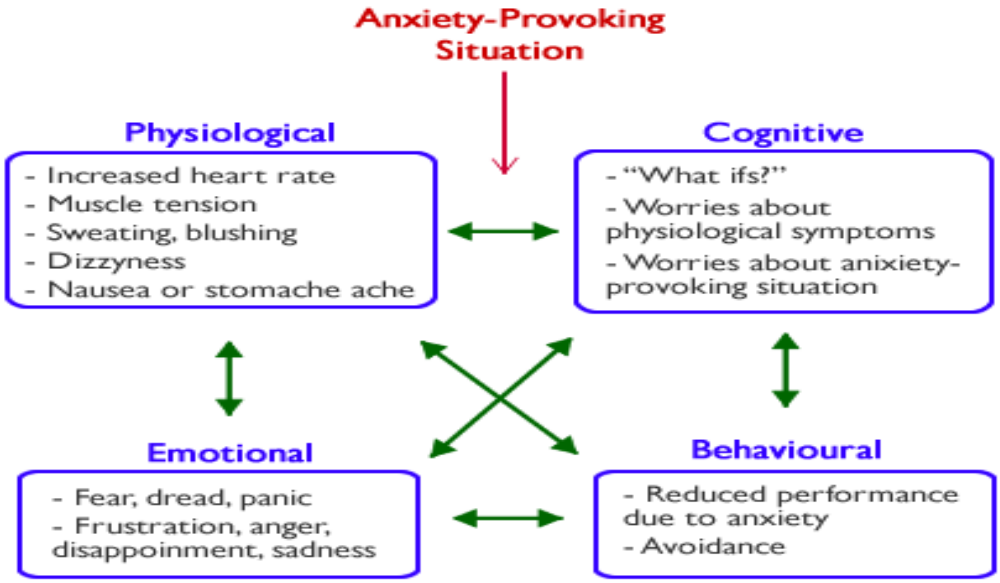
Abstract:
About 13-14 % of world's population has mental health disorders.2 Mental stress leads to various psychiatric disorders like depression, cognitive dysfunction and anxiety.It was found that prevalence rate of anxiety disorder in India is variable.The primary use of sedative–hypnotic and anxiolytic drugs is to encourage calmness (anxiolytics or sedatives) or to produce sleep (sedative–hypnotics). All people are subjected to states of emotional tension and uneasiness. Screening of seed extract of *Carissa carandas* was carried for anxiolytic property. The extracts screened for anxiolytic activity were found to be potent agent against anxiety by increasing the behavioural activity.

Keywords: Anti-anxiety, Carissa carandas, Anxiolytics, Sedatives, Hypnotics



Introduction:

In general, anxiety is protective, but excessive anxiety can prove disabling and could manifest in anxiety disorders. Although effective treatments for anxiety disorders are available, a vast majority of anxiety patients are unresponsive to classical anti-anxiety medications and also having side effects that limit the-

ir adherence to prescribed regimens.

2. Review of Literature

Anupama *et al* (2020). investigated eleven compounds, which were screened by gas chromatography/mass spectroscopy (GC-MS) analysis, namely dichloroacetic acid-2-ethylhexyl ester; 1-pentatriacontanol; myo-inositol-4-c-methyl; heptacosanoic acid; 1-methoxy-25-methylheptacosan-1-ol-methyl ester; methyl-13-octadecenoate; Z, Z-6,28-heptatriactontadien-2-one; 12-oleanen-3-yl acetate, (3-alpha); 2R-acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexanol; β-amyrin,2,4,4-trimethyl-3-hydroxymethyl-5a-(3-methyl-but-2-enyl)-cyclohexene; & 2R-acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexanol, from the methanol extract of dried fruits of *C. carandas*. They possessed significant anti-inflammatory effect using carrageenan-induced hind paw oedema model in rats.

Parvin MN. *et al*., (2018) investigated methanol leaf extract of crude drug for antinociceptive, anthelmintic and cytotoxic activities. Antinociceptive effect of C. carandas was determined using acetic acid-induced writhing assay in Swiss albino mice, whereas in case of anthelmintic activity, the fresh juice of plant leaves was evaluated by recording the time duration of paralysis and the death of Pheretima posthuma, earthworms. The cytotoxic activity of methanol leaf extract was analysed using brine shrimp lethality bioassay. Results revealed the potency of the plant that it possesses significant antinociceptive and anthelmintic activity. Methanol leaf extract showed marked cytotoxic property as compared with vincristine sulfate used as standard drug.

3. Research Envisaged

Traditional medicines based mostly on medicinal plants have been used for the treatment of various diseases by mankind for centuries. Plants are also well known to be the rich sources of biologically active compounds. Therefore, one approach that has been used for the discovery of antimicrobial agents from natural sources is based on the evaluation of traditional plant extracts. Also however, the genus *Carissa carandas* L. still remains totally unexplored.

It is hypothesised that *Carissa carandas* reduces the oxidative and mental stress in patients of anxiety disorder.

Favourable outcome of this study will generate evidence for incorporation of *Carissa carandas* along with antianxiety drug to reduce stress in patients of anxiety disorder.

4. Aim and Objectives

The aim of the present study is to evaluate the phytochemical constituents, antianxiety activity of fruits and seeds of *Carissa carandas* L.

- To evaluate the bioactive compounds from fruit and seeds of *Carissa carandas* L.
- To analyse and identify of different components.
- To evaluate the antianxiety activity.

5. Plant Profile

- *Carissa carandas* is an evergreen thorny shrub belongs to Apocynaceae family, which is commonly known as karonda.

Class : Dicotyledonae
Sub class : Gamopetalae
Series : Bicarpellatae
Order : Gentianales
Family : Apocyanaceae
Genus : Carissa
Species : carandas

- It has small berry-shaped fruits, used as additive in many pickles or as a spice in northern India. It is drought resistant plant that can be grown in a wide range of different types of soils. Approximately more than 25 species of genus Carissa are known, out of which five species are native to India.
- It has been found that the fruit is the richest source of iron, vitamin C and pectin. Even it is used as an ingredient in most of the edible preparations such as jam, jelly, squash and syrup. The demand of the plant has been increased tremendously in the market as it contains antiscorbutic property and found very beneficiary effect in the treatment of anaemia. It has been utilised in many ayurvedic preparations. Root extract is used in chest pain.

Fig:- Fruits of Carissa carandas

Properties and use:

The plant Carissa spinarum used locally in Indian and Chinese system for various painful inflammatory and arthritic conditions was assessed for its anti- arthritic effect. Family- Apocynaceae is a throny, evergreen shrub, widely and rocky soils of India, The root of this plant has long been prescribed in the indigenous system of medicine as purgative, for the management of rheumatism, ambush worm infested wound of animals and in snake bite.

Materials and methods

6.1. Chemicals

DPPH (1,1–diphenyl–1,2–picrylhydrazyl), TPTZ (2,4,6,-tripyridy-striazine), potassium ferricyanide, trichloroacetic acid (TCA), FeCl3, sodium nitroprusside, sulphanilamide, N 1 napthylethylenediaminedihydrochloride, ascorbic acid, NBT (nitro blue tetrazolium), reduced NADH (nicotinamide adenine dinucleotide), PMS (phenazinemetho sulphate), ascorbic acid/standard Vitamin C and quercetin were obtained from the central store of DIPS.

6.2. Plant material

Carissa carandas (Fruits) were purchased from local Market and were authenticated by Botanist.

6.3 Preparation of plant extract

250.0 g of *Carissa carandas* (Fruits), were used for extraction. The samples were soaked overnight in 70% ethyl alcohol and filtered using Whatman No.1 paper. This process was repeated twice by adding fresh solvent each time. The pooled extract was subjected to flash evaporation followed by lyophilization. The lyophilized samples were used for all the *in vitro* anti-oxidant and *in vivo* assays.

6.4 Animal experiment

Animal studies were conducted according to the institute animal ethical committee regulations approved by the committee for the purpose of the control and supervision of experiments on animals. Male mice weighing 25-30 g were selected and were obtained from Animal House facility of Daksh Institute of Pharmaceutical Science Chhatarpur MP, India, housed in an acryl fiber cage in a temperature controlled room temperature (25 ± 2º C) and was maintained in 12 h light/ dark cycle with free access to diet and drinking water.

6.5 Experimental design

The extracts of *Carissa carandas* were separately suspended in a vehicle comprising 1% w/w Tween 20 in distilled water. Various doses viz., 100, 200 or 400 mg/kg body weight of 70% ethanol extract of plants were prepared by suspending the dried extracts in vehicle.

Six mice were taken in each group. The doses of extracts were so adjusted as to administer 0.25 ml of the suspension of extracts. Diazepam 1 mg/kg suspended in the vehicle was used as standard anxiolytic. The suspending vehicle (0.25 ml) was used as control. The administration of all extracts and standard were done one hour before the test commencement through intraperitonially for 7 days (i.p).

6.5.1.1 Behavioural analysis

Elevated plus maze, Marble burying test, and Hyponeophagia test were carried out for *Carissa carandas* extracts. The procedure for behavioural analysis was followed.

6.6 Preliminary Phytochemical analysis of *Carissa carandas*

6.7 Qualitative phytochemical analysis of *Carissa carandas*

6.7. a. Saponins (frothing test): To 0.5ml filtrate add 5ml distilled water; frothing persistenceindicated presence of saponins.

6.7. b. Tannins: 2 ml of extract was added to few drops of 1% lead acetate. A yellowishprecipitate indicated the presence of tannins.

6.7. c. Amino acids: 200 µl of extract a few drops of Ninhydrin reagent was added. Purple colour appearance indicates the presence of amino acids.

6.7. d. Alkaloids: 2ml of filtrate+1%HCl+Dragendroff reagent, orange precipitate indicate the presence of alkaloids.

6.7. e. Steroids:- liebermann-burchard reaction: 2ml filtrate +2ml acetic anhydride + concentrated sulphuric acid; green colour indicates the presence of steroids.

6.7. f. Terpenoids:- 4ml of filtrate +concentrated sulphuric acid 3ml was added to form a layer; reddish brown colour at ion interface indicates the presence of terpenoids.

6.7. g. Total phenol content

Total phenol content was determined by the method adapted from Singleton & Rossi, (1965) with some modifications using the Folin-Ciocalteu reagent. The reaction mixture consisted 5ml of diluted sample to which 3 ml of distilled water and 0.5ml Folin –Ciocalteu reagent was added.

After 3minutes, 2ml of 20% Na_2CO_3 solution was added and the tubes were placed in boiling water bath for one min, cooled and the absorbance was measured at 760 nm. Standard graph was drawn by using different concentrations of gallic acid.

6.7. h. Total flavonoid content

The flavonoid content was determined as described by Zou, et al., (2004). 0.5mlof sample was mixed with 0.5 ml of 2% $AlCl_3$ and incubated for 10 mins. The absorbance was measured at 415 nm. The measurement was compared to a standard graph of quercetin.

Shinoda's test:- The test solution of the extracts was dissolved in 95% ethanol. To this, a small piece of magnesium foil metal was added; this was followed by 3-5 drops of the concentrated HCl. The intense cherry red color indicated the presence of flavonoids.

Zinc-HCl reduction test : Add a pinch of zinc dust and few drops of concentrated HCl to 1 mL of an alcoholic solution of crude extract using a test tube. The appearance of magenta colour indicates the presence of flavonoids

Shibita's reaction test): One gram (1 g) of the water extract was dissolved in methanol (50%, 1-2 mL) by heating, then metal magnesium and 5-6 drops of concentrated hydrochloric acid were added. The solution when red is indicative of flavonols and orange for flavones.

6.8. Biochemical studies

6.8.1. *In vitro* antioxidant activity

6.8.1. a. Reducing power (Fe^{3+} - Fe^{2+} transformation ability)

The reducing power of a compound serves as significant indicator of potential anti-oxidant activity as described by Oyaizu, (1986). Increased absorbance of the reaction mixture indicates increased reducing power. Various conc. of the extracts in 1ml of water were mixed with phosphate buffer (2.5 ml, 0.2 M; pH 6.6) and 1% potassium ferricyanide (2.5 ml). The mixture was incubated at 50ºC for 20 min.

Aliquots of trichloroacetic acid (2.5 ml, 10%) were added to the mixture, which was then centrifuged at 3000×g for 10 min. supernatant obtained (2.5 ml) was mixed with distilled water (2.5 ml) and freshly prepared $FeCl_3$ solution (0.5ml, 0.1%). The absorbance was measured at 700nm.

6.8.1. b. Super oxide anion scavenging activity

Superoxide anion scavenging activity of a compound serves as significant indicator of potential anti-oxidant activity described by Shivkumar et al., (2006). 1ml of NBT solution (144µM in 100mM phosphate buffer , pH 7.4), 1ml of reduced NADH (677µM in 100mM phosphate buffer, pH 7.4) and 0.5 ml of sample extract was mixed and the reaction was started by adding 100µl of PMS solution (60µM PMS in100mM phosphate buffer, pH 7.4) . The reaction mixture was incubated at 25°C for 5 min, and the absorbance was measured at 560 nm.

6.8.1. c. 1,1–diphenyl–1,2–picrylhydrazyl (DPPH) radical scavenging activity

The assay is based on the measurement of the scavenging ability of antioxidant towards the stable radical DPPH as described by Chen et al., (2007). When DPPH radical reacts with a suitable reagent, the electrons become paired off and the solution loses colour stoichiometrically depending on number of electrons taken up.

A volume of 2ml of sample was added to 2ml of phosphate buffer (0.02M, pH 6) and 2ml of 0.2mM DPPH in 95% ethanol. The mixture was shaken and left for 30 min at room temperature and the absorbance was measured at 517nm.

6.9. Screening of plant extracts for anti-anxiety property.

6.9. a. Elevated plus-maze test

The test procedure and scoring methodology for the elevated plus-maze test have been described by Kulkarni (2002). In brief, the apparatus composed of two open (30 × 5 × 0.25 cm) and two enclosed (30 × 5 × 15 cm) arms that radiated from a central platform (5 × 5 cm) to form a plus sign. A slightly raised edge on the open arms (0.25 cm) provided an additional grip for the animals.

Figure:- Plus Maze Apparatus

The maze floor and the closed arms were covered with black adhesive tape. The plusmaze was elevated to a height of 40 cm above floor level by a single central support.

The mice were administered with drugs or vehicle and sixty minutes later, the trial was started by placing the animal on the central platform of the maze facing an open arm. The number of entries into, and the time spent, in each of the two types of arm, were counted during a 5 min test period. The open-arm entries and open-arm time were used as indices of anxiety.

6.9. b. Marble Burying Test

A modified procedure based on Yamada et al. (2002) was employed. Mice were placed individually in plastic cages with the designated bedding material for 30 min (habituation period) and then placed into waiting cages. Twelve glass marbles were then evenly spaced 3 cm apart on a 4-cm layer of bedding material in the habituation cages. Mice were then reintroduced into the same cage in which they had been habituated. After 30 min, the marble burying period was terminated by removing the mice, and the number of marbles that were more than two-thirds covered with bedding material was counted.

Fig:- Marble Burrrying Test

6.9. c. Hyponeophagia test

A modified procedure based on Deacon, (2011) was employed to carry out hyponeophagia test. The open field apparatus was used for this experiment. The animals were fasted on the previous night. Novel food like sweet corn was used to induce hyponeophagia. The entire room, except the open field was kept dark during the experiment. One hour after the treatment of vehicle/standard/extract each animal was placed at one corner of the apparatus. The latency to eat and the behavioural aspects were measured in the next 5min.

Fig:- Hyponeophagia test

Results and Discussion

7.1 Preliminary Phytochemical Analysis

The approximate composition of *Carissa carandas* is given in (Table 7.1). It has good amount of vitamin C, which contribute to the antioxidant property.

Proximate composition / 100gm	
Moisture (g)	93.5
Ash (g)	1.2
Fat (g)	0.3
Dietary Fibre (g)	0.8
Protein (g) (Nx6.25)	1.8
Carbohydrate (g)	2.4
Ascorbic acid (mg)	3.5

Table 7.1. The approximate composition of *Carissa carandas*

7.2 Qualitative phytochemical analysis of *Carissa carandas*

The *Carissa carandas* was found to contain glycosides, tannins, flavonoids, polyphenols, proteins, and sterols through preliminary photochemical screening (Table 7.2).

The results obtained in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and these plants are proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.

Assays	**Ethanol extract**
Tannins	+
Amino acids	++
Polyphenols	+++

Flavonoids	+++
Saponins	++
Terpenoids	+
Alkaloids	-
Steroids	+

Table 7.2:- Qualitative phytochemical analysis of *Carissa carandas*

7.2. a. Flavonoids and Total polyphenols

Flavonoids are a class of secondary plant phenolics with powerful antioxidant properties. Therefore it is valuable to determine the total flavonoids content of the extracts in the study. Several studies have shown that many flavonoids contribute significantly to the total anti-oxidant activity and anxiolytic activity of plants. There is abundant evidence that flavonoids are effective in blocking oxidant induced neuronal injury.

Se. No.	Extract	Test	Result
1.	Hydro-alcoholic Extract (*Carissa carandas*)	**Shinoda's test:-**	++
		Zinc-HCl reduction test	++
		Shibita's reaction test):	++

Table 7.3: Flavonoidal analysis

7.3. Biochemical studies

7.3.1. *In vitro* antioxidant activity

7.3.1. a. Reducing power (Fe^{3+} - Fe^{2+} transformation ability)

This method is based on the principle of increase in the absorbance of the reaction mixture. The increase in the absorbance indicates increase in the anti-oxidant activity and in turn, the increase in absorbance of the reaction mixture indicates the reducing power of the samples. In the reducing power assay the presence of anti-oxidants in the samples would result in the reduction of Fe III to Fe II by donating an electron. Amount of Fe II complex can be then be monitored by measuring the formation of Perl's Prussain blue at 700nm. Increasing absorbance at 700nm indicates an increase in reductive ability.

Figure 7.1 shows dose dependent response curves for the reducing powers of the extracts. The reducing powers of all the extracts also increased with the increase in their concentrations. *Carissa carandas* extracts had shown good reducing power. Our data on the reducing power of the tested extracts suggest that it is likely to contribute significantly towards the observed anti-oxidant effect. The reducing power of bioactive compounds has been reported to be associated with their anti-oxidant activity.

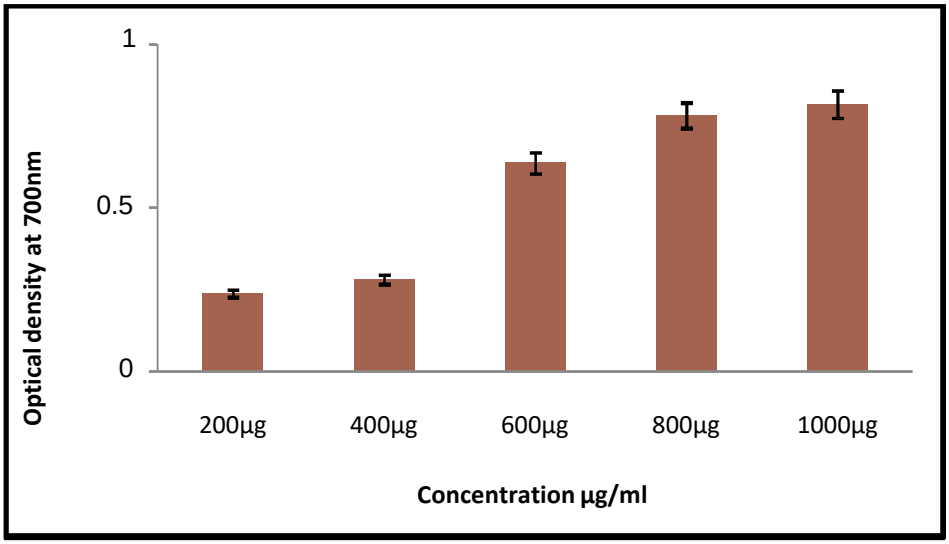


Figure 7.1, Reducing power activity of *Carissa carandas*

7.3. b. Super oxide anion scavenging activity

Superoxide anion radicals ($O_2^{\cdot -}$) are generated by one electron transfer to molecular oxygen. This process can generate other more harmful ROS, such as hydrogen peroxide (H_2O_2), hydroxyl radicals (OH•), hypochlorous acid (HOCl) and singlet oxygen ($^1O_2$). Superoxide anion is also harmful reactive oxygen species as it damages cellular components in biological systems. The ability of the *Carissa carandas* and the reference compound ascorbic acid to quench superoxide radicals from reaction mixture is reflected in the decrease of absorbance at 560nm. The IC_{50} value of standard ascorbic acid was 17.4 µg/ml. The IC_{50} value of *Carissa carandas* given in Table 7.5). It can be put forward that *Carissa carandas* is a more potent scavenger of superoxide radical.

7.3. c. DPPH radical scavenging activity

The stable DPPH radical model is a widely-used, relatively quick method for the evaluation of free radical scavenging activity. DPPH radicals react with suitable reducing agent as a result of which electron become paired off forming the corresponding hydrazine. The effect of anti-oxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability. DPPH· is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Because of its odd electron, DPPH· gives a strong absorption maximum at 517nm by visible spectroscopy. As the odd electron of the radical becomes paired off in the presence of a hydrogen donor, that is, a free radical scavenging anti-oxidant, the absorption strength is decreased and the resulting decolourization is stoichiometric with respect to the number of electrons captured.

7.4. Antianxiety Testing

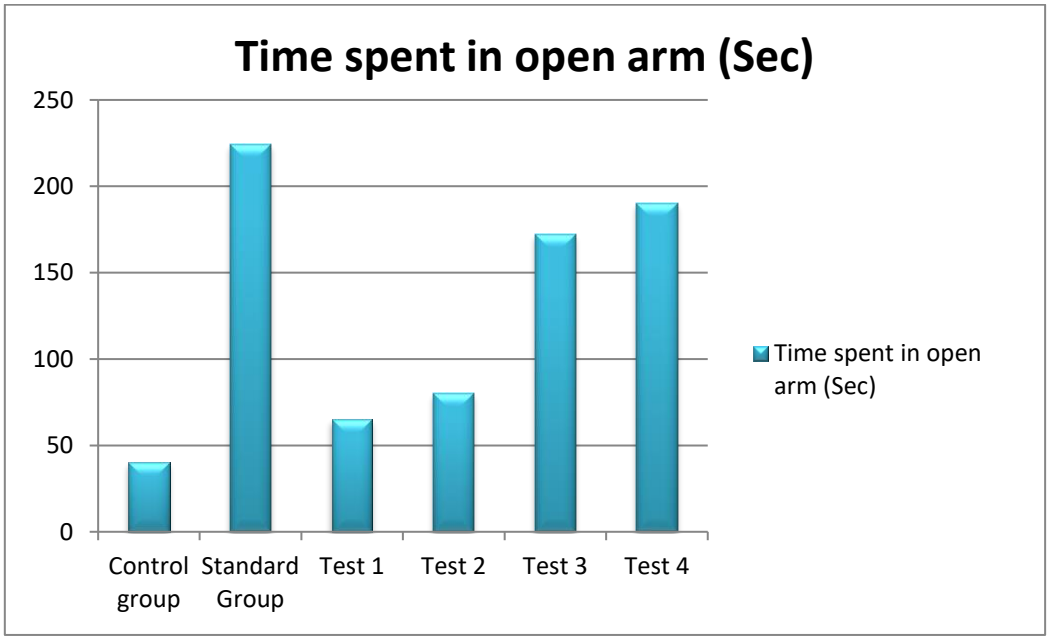
7.4. a. Elevated Plus Maze Test

EPM is considered one of the most widely validated tests for assaying new benzodiazepine-like anxiolytic agents. It is well known that the anxiolytic agents increase the motor activity which is measured by time spent by the animal in the open arms. Animals treated with diazepam showed a significant increase in the time spent in the open arms and decreased time spent in closed arms, as well as an increase in the number of entries in the open arms.

The values of the group treated with *Carissa carandas* at 400mg/kg body weight were higher than that of the treatment with lower doses. However, the standard drug diazepam showed higher locomotor activity/ time mobile comparable to that of 400mg/kg body weight in open field test (Figure 13).

Table:- 7.5 Hydro-alcohol extract of *Carissa carandas* showed increase in time spent in open arm compared to untreated group. Though the lower doses did not affect the time spent in open arms, the 200 and 400mg/kg body weight treated group showed significant increase which is comparable to diazepam drug adminisistered group of mice (Figure 7.2).

Se. No.	Group	Drug	Time spent in Open Arm (Sec)	Time spent in closed Arm (Sec)
1	Control group	Normal saline	40 ± 06	260 ± 06
2	Standard Group	Diazepam (1mg/kg)	224 ± 22	76 ± 22
3	Test 1	100 mg/kg (Extract)	65 ± 17	235 ± 17
4	Test 2	200 mg/kg (Extract)	80 ± 21	220 ± 21
5	Test 3	400 mg/kg (Extract)	172 ± 14	128 ± 14
6	Test 4	600 mg/kg (Extract)	190 ± 26	110± 16



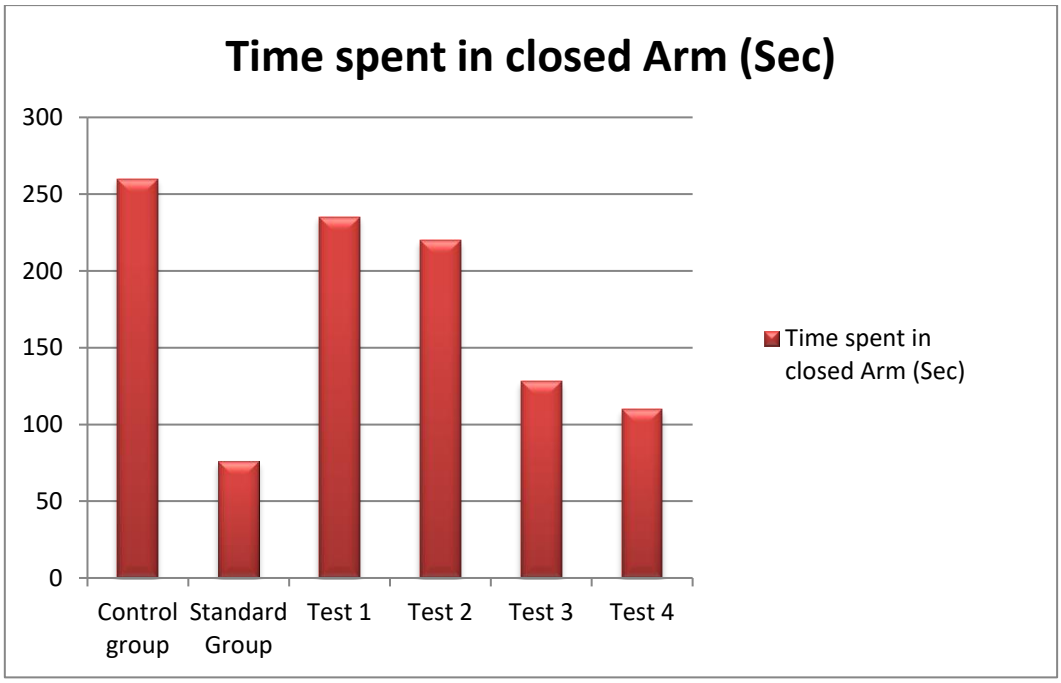


Figure:- 7.2. Effect of hydro-alcohol *Carissa carandas* extract on the time spent in the open arms of the elevated plus- maze during a 5 min test in mice. Data are presented as mean values (±SD.) from group of six mice.*p < 0.05 compared with vehicle-treated control.

Table 7.6. Effect of hydro-alcohol extract of *Carissa carandas* on marble burying test and Light & Dark Test.

Group	Marble Burying	Light dark box Test	
		Time spent in light	Time spent in Dark
Control group	4.50±0.9	53.68±9.6	126.45±10**
Standard Group	1.55±0.9*	192.97±09.7**	42.67±11.8
Test 1	3.25±1.2	99.80±10.0*	111.45±10**
Test 2	2.50±0.5*	110.88±12.9**	99.82±15*
Test 3	2.25±0.9*	172.88±12.9**	60.72±13*
Test 4	1.75±0.9*	87.32±10.3	56.25±9.7

Data are presented as mean values ±SD, n=6, * p<0.05 and **p<0.01 compared with control.

Behavioral Studies:

Table:-7.7. Effect of hydro-alcohol extract of *Carissa carandas* on the elevated plus maze test for15 and 30 days treatment. The plant extracts, diazepam or control, were injected 60 min prior to test. Data are presented as mean values (±SD.) from group of six mice.

Groups	15 days			30 days		
	Time in open arm (s/5min)	Time mobilein open arm (s/5min)	No of entries to open arm	Time in open arm (s/5min)	Time mobile in open arm (s/5min)	No of entries toopen arm
Control	53.00±11.2	33.12±10.9	15.75±5.3	66.95±10.1	35.00±15.08	16.00± 6.4
100mg/kg	87.72±12.2	49.15±10.7	22.25±5.4	86.90±10.8	47.05±13.06	27.75± 4.1

bwt. (CC)						
200mg/kg bwt.(CC)	113.32±13.3*	76.20±12.7*	28.25±3.3*	114.57±11.9*	78.40±12.1*	31.50± 4.3
400mg/kg bwt. (CC)	143.32±11.4**	82.27±12.8**	33.75±4.4**	155.27±13.9**	102.32± 6.6**	40.50± 7.1**
1mg/kg bwt.(D)	192.90±12.2**	116.25±13.04**	37.25±7.6**	180.72±14.6**	117.75±14.0**	46.00±11.5**

*p < 0.05 and **p < 0.001indicates significant difference from control. CC represent *Carissa carandas* and D represents diazepam.

Table:-7.8 Effect of hydro-alcohol extract of *Carissa carandas* on the marble burying test for 15 and 30 days treatment. The plant extracts, diazepam or control, were injected 60 min prior to test. Data are presented as mean values (±SD.) from group of six mice.

Groups	**15 days treatment**	**30 days treatment**
	No of marbles buried	No of marbles buried
Control	4.25±0.9	5.00±0.8
100mg/kg bwt. (CC)	3.50±1.2	3.25±1.7
200mg/kg bwt. (CC)	2.75±0.5*	2.50±1.2*
400mg/kg bwt. (CC)	2.25±0.9**	1.75±0.9**
1mg/kg bwt. (D)	1.75±0.9**	1.25±0.5**

*p < 0.05 and **p < 0.001indicates significant difference from control. CC represent *Carissa carandas* and D represents diazepam.

Table:-7.9. Effect of hydro-alcohol extract of *Carissa carandas* on the Hyponeophagia (sweet corn) for 15 and 30 days treatment. The plant extracts, diazepam or control, were injected 60 min prior to test. Data are presented as mean values (±SD.) from group of six mice.

Groups	**15 days treatment**			**30 days treatment**		
	No of entries to food zone	Latency for1 entry to food Zone (s/5min)	Time in food zone (s/5min)	No of entries to food zone	Latency for 1 entry to food zone (s/5min)	Time in food zone (s/5min)
Control	1.25±0.5	112.67±7.7	8.63± 3.4	2.00±0.8	143.35±10.3	8.63± 3.4
100mg/kg bwt. (CC)	3.00±0.8	65.80±11.7*	12.40± 5.1	3.50±1.3	92.10±12.9	12.40± 5.1
200mg/kg bwt. (CC)	8.00±4.1*	58.72±12.2*	29.18±11.5*	8.75±3.3*	59.12±10.2**	29.18±11.5*
400mg/kg bwt. (CC)	10.00±3.7*	24.72±5.2**	33.40± 6.2**	11.75±3.8*	26.20± 5.9**	31.98± 6.6**
1mg/kg bwt.(D)	10.25±1.3**	12.82±6.5**	44.13± 7.4**	13.00±4.1**	21.75± 7.7**	45.38± 7.1**

*p < 0.05 and **p < 0.001 indicates significant difference from control. CC represent *Carissa carandas* and D represents diazepam.

8. Summary & Conclusion

Screening of seed extract of *Carissa carandas* was carried for anxiolytic property. The extracts screened for anxiolytic activity were found to be potent agent against anxiety by increasing the behavioural activity.

Carissa carandas extract was found to improve exploratory behaviour and biochemical changes that occur due to anxiolytic condition. *Carissa carandas* was fed to animals for two different treatment duration (15 and 30 days) and subjected for a variety of behaviour parameters like elevated plus maze, open field test, marble burying test, rat exposure test, hyponeophagia. The plant extract treated group found to improve exploratory behaviour, lacomotory activity in mice.

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