Evaluation of antidepressant activity of Carissa carandas leaves extract.

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1. INTRODUCTION

Depression is a common psychiatric illness and is expected to become the second leading cause of global disease burden after heart disease by 2020[1]. The monoaminergic hypothesis of depression does not fully explain its progression, causes, or treatment. A widely accepted hypothesis suggests that oxidative stress plays a role in the development of depression[2]. According to WHO estimates, 121 million people worldwide suffer from clinical depression[3], which often begins in early adulthood and is associated with a decrease in monoamine neurotransmitters[4]. Medicinal plants may offer effective alternatives for treating depression, with fewer side effects compared to synthetic medicines. Traditional medicine has significantly contributed to the development of modern medicine, and extensive research is now being conducted on various plant species and their active therapeutic compounds. The plant kingdom holds great potential as a source of new compounds with notable therapeutic benefits. The key advantages of herbal medicine include its effectiveness, minimal side effects, and low cost[5].

Carissa congesta Wight (syn. *C. congesta Auct*. formerly widely shown as *C.congesta L*.) belong to Apocynaceae. It is called kerenda in Malaya, karaunda in India; Bengal currant or Christs thorn in South India; namdaeng in Thailand; caramba, caranda, caraunda and perunkila in the Philippines . This species may be a rank-growing, straggly, woody, ascent ligneous plant sometime growing to ten or fifteenft. (3-5 m) high, sometimes ascending to the tops of tall trees; and rich in white, gummy latex. The branches, numerous and spreading, forming dense masses, are set with sharp thorns, simple or forked, up to 2 in (5 cm) long, in pairs in the axils of the leaves. The leaves are evergreen, opposite, oval or elliptic, 1 to 3 in (2.5- 7.5 cm) long; dark-green, leathery, glossy on the upper surface, lighter green and dull on the underside. The scented flowers are hollow with five hairy lobes that are twisted to the left within the bud rather than to the right as in alternative species. They are white, often tinged with pink, and borne in terminal clusters of 2 to 12. The fruit, in clusters of 3 to 10, is oblong, broad-ovoid or round, 1/2 to 1 in (1.25-2.5 cm) long; has fairly thin but tough, purplish-red skin turning dark-purple or nearly black when ripe; smooth, glossy; enclosing very acid to fairly sweet, often bitter, juicy, red or pink, juicy pulp, exuding flecks of latex. There may be 2 to 8 small, flat, brown seeds.

In India, leaf juice is applied to bee or wasp stings. In the Philippines, a decoction of young leaves is used to treat stomach cramps and diabetes. Root decoctions are used for intestinal parasitism,

while leaf infusions are used to treat heavy menstrual bleeding (menorrhagia). Crude extracts from the leaves and roots have shown anticancer properties. Roots are also used to treat dysentery. The bitter and astringent leaves are used as an emetic, while the roots are used as purgatives, hemostatics, blood purifiers, vermifuges, and remedies for toothache in Madagascar.

The juice of ***Carissa carandas*** leaves is used in Mauritius to treat upset stomach and indigestion. In the West Indies and South Africa, the plant is used to manage diabetes. Flower extracts are used in Cuba and Jamaica as an eye wash for infants. In the Bahamas, a flower decoction is used to treat tuberculosis, asthma, and flatulence.

In Africa, the leaves of ***Carissa carandas*** are used to treat menorrhagia and rheumatism. Traditionally, this plant has been used to relieve muscle pain and depression of the central nervous system. The plant has various pharmacological activities, including anticancer, anti-diabetic, antioxidant, antimicrobial, antidiarrheal, wound healing, and antiulcer effects. Alkaloids isolated from the plant have been found to possess hypotensive, sedative, tranquilizing, and anticancer properties

The current study was conducted to validate the traditional claims and folk uses of ***Carissa carandas*** leaves.

2. MATERIAL AND METHODS

2.1 Plant material:

The leaves of *Carissa carandas* was collected from the Sangamner, Maharashtra, India. The leaves were identified by Dr. P.G. Diwakar, Joint Director, Botanical Survey of India; Pune with a voucher specimen (MTS01) has been kept in herbarium botanical survey of India, Pune.

2.2 Preparation of extracts:

The leaves of *Carissa carandas* were collected, air-dried in the shade, and then coarsely powdered using a mechanical grinder. A total of 500 grams of the powdered material was evenly packed into a Soxhlet apparatus. It was then successively extracted using solvents of increasing polarity, starting with petroleum ether, followed by ethyl acetate, chloroform, ethanol, and finally water.

2.3 Preliminary phytochemical evaluation:

The preliminary phytochemical screening of the petroleum ether, ethyl acetate, and ethanolic extracts of *Carissa carandas* leaves was conducted to qualitatively identify the types of phytoconstituents present.

2.4 Selection of Animals:

Healthy albino mice of either sex, weighing 25-30g, were used for the study. The animals were housed individually under standard conditions of temperature (25±1ºC), a 12-hour light/dark cycle, and were fed a standard pellet diet with water available ad libitum. Approval was obtained from the Institutional Animal Ethics Committee. The approved protocol number for the experiment, according to CPCSEA guidelines, is MES/COP/IAEC/13/2015-16.

2.5 Acute toxicity studies:
Swiss albino mice (weighing 25-30g) of both sexes were used for acute oral toxicity studies. Mortality and behavioral changes in the animals were monitored for 48 hours, following the OECD guidelines.

2.6 Drugs and Solvents:

Solvents such as petroleum ether, chloroform, and ethanol were obtained from Merck. Diazepam and chlorpromazine were used as standards to study depressant activity.

2.7 Pharmacological Screening:

Locomotor activity:

Locomotor activity was measured using an actophotometer. Male Swiss albino mice, weighing between 20-25g, were divided into eleven groups, with six animals in each group. Each animal was placed individually in the actophotometer for 10 minutes, and the baseline activity was recorded. Extracts were administered orally 60 minutes before the test, while chlorpromazine (3 mg/kg) was given as the standard treatment 30 minutes before the test via the intraperitoneal route. The control group received distilled water 60 minutes before the test. After treatment, the animals were placed back in the actophotometer, and changes in locomotor activity were recorded.

2.8 Statistical analysis:
All the data were given as means ± S.E.M. Data were analysed by one way ANOVA. Whenever ANOVA was significant, further comparisons between vehicle and drug treatment groups were performed using Dunnette's multiple comparison test. The level of statistical significance adopted was $P < 0.05$.

Table 1: Preliminary phytochemical evaluation of *Carissa carandas* leaf extract

Sr. No.	Phytochemical Constituents	Pet. ether extract	Chloroform extract	Ethyl acetate extract	Ethanol extract	Aqueous Extract
1	Steroids	+	-	-	+	+
2	Saponins	+	_	+	+	+
3	Tannins	+	+	+	+	+
4	Alkoloids	+	+	+	+	+
5	Carbohydrates	-	-	-	+	-
6	Proteins	-	-	-	-	-
7	Amino acids	-	-	-	-	-
8	Flavonoids	-	+	+	+	+
9	Diterpenes	-	-	+	+	-

10	Phenols	-	+	+	+	+

The positive (+) sign indicates Presence and negative (-) indicates absence of group of phytochemicals.

3. RESULT AND DISCUSSION:

3.1 Preliminary phytochemical evaluation:

Chemical tests were carried out according to the literature mentioned in method. Group of chemicals were identified in chemical test as shown in table 1. Preliminary phytochemical findings of *Carissa carandas* leaves showed presence of alkaloids, terpenoids, flavonoids and phenolics.

3.2 Acute toxicity study (LD50) of *Carissa carandas* leaves extracts:

Oral administration of *Carissa carandas* G. Don leaves extracts at doses upto 2000 mg/kg produced no signs of toxicity. No mortality was observed up to 14 days. Thus the median lethal dose (LD50) of the extracts was then greater than 2000 mg/kg body weight as shown in table 2.

Table 2: Acute toxicity study of *Carissa carandas* leaves extracts

Groups	**Dose**	**D/T**	**Symptoms**	**Mortality**
Control	D/W 10 ml/kg p.o	None	None	None
Extracted	2000 ml/kg p.o	None	None	None

3.3 Effect of *Carissa carandas* leaf extracts on locomotor activity:

The petroleum ether, ethyl acetate and ethanol extract of *Carissa carandas* leaves at a dose of 200 and 400 mg/kg shows decrease in locomotor activity score (*P<0.05). The petroleum ether extract at a dose of 400 mg/kg shows maximum reduction in locomotor activity as compared with control group as shown in table 3.

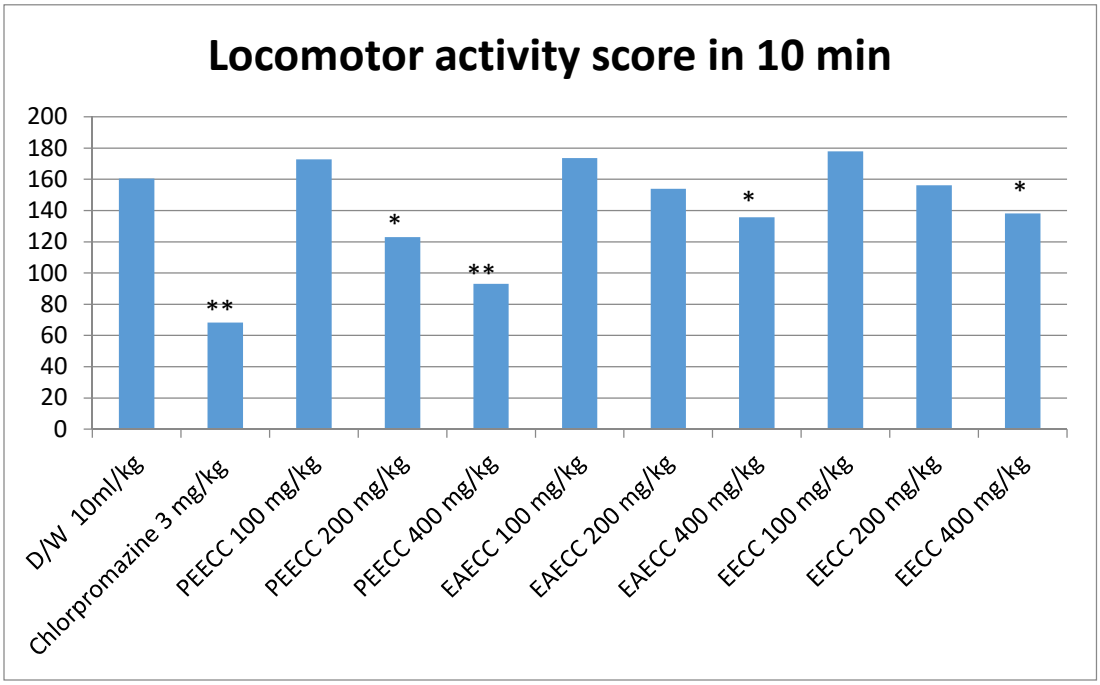
The chlorpromazine used as standard shows significant (**P<0.01) reduction in locomotor activity. Mostly the CNS depressant drugs decrease the locomotion by increasing the activity of inhibitory neurotransmitter GABA in the brain. In Previous studies it has been stated that the various phytochemicals in plants such as alkaloids, flavonoids, saponins may produces the CNS depressant action by enhancing GABA transmission in the brain[19].

Table 3: Effect of *Carissa carandas* leaf extract on locomotor activity

Sr. No.	Groups	Treatment	Locomotor activity scores in 10 min
1	Control (D/W)	10ml/kg, p.o.	160.50±2.14
2	Standard (Chlorpromazine)	3 mg/kg i.p.	68.33±1.52**

3	PEECC	100 mg/kg, p.o.	172.83±3.84
4	PEECC	200 mg/kg, p.o.	123.00±2.76*
5	PEECC	400 mg/kg, p.o.	93.00±3.43**
6	EAECC	100 mg/kg, p.o.	173.50±3.24
7	EAECC	200 mg/kg, p.o.	154.00±3.75
8	EAECC	400 mg/kg, p.o.	135.63±3.00*
9	EECC	100 mg/kg, p.o.	178.00±1.57
10	EECC	200 mg/kg, p.o.	156.10±2.82
11	EECC	400 mg/kg, p.o.	138.17±2.98*

* P< 0.05, **P<0.01 Values are Mean± SEM , n=6,when compared with control by using one way ANOVA followed by Dunnette's multiple comparison tests where, PEECC- Petroleum Ether Extract of *Carissa congesta* leaves, EAECC-Ethyl Acetate Extract of *Carissa congesta* leaves, EECC- Ethanol Extract of *Carissa congesta* leaves.



The petroleum ether extract of *Carissa carandas* leaves may facilitate the action of GABA and shows the decrease in locomotor activity. The mixture of petroleum ether extract of *Carissa carandas* shows significant reduction in locomotor activity may be due to presence of various phytochemicals such as flavonoids, steroids etc.

4. CONCLUSION:

These experimental results have established a pharmacological evidence for the folklore claim about the usefulness of *Carissa carandas* leaves extract. Further the study of isolation of active principles responsible for such activity is essential.

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