Original Article

To Determine The Anti-Inflammatory And Anti-Pyretic Activity On The Stem Of Carissa Carandas Linn

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Abstract

Plants hold an extraordinary put in the world since they are the establishment forever. In all food frameworks, they are the essential makers. Plants and spices have been used as a crucial part of medication since long before recorded history. Inflammation is a pathologic condition that includes a wide range of diseases such as rheumatic and immune-mediated conditions, diabetes, cardiovascular accident, and etcetera. Anti-inflammatory herbs have proven beneficial by combating inflammatory responses that lead to severe abnormality in body systems. Inflammation though a protective response to infection or injury and may result in pathological outcome when aggravated or of severe degree thus needs an early intervention for proper resolution. Fever is a complex physiologic response triggered by infectious or aseptic stimuli. Elevations in body temperature occur when concentrations of prostaglandin E (2) (PGE (2) increase within certain areas of the brain. Carissa carandas L. (Apocynaceae) is a medicinal plant and is widely distributed in tropical and subtropical regions of India. Meanwhile it is an evergreen shrub that constitutes a continuous source of leaves and stem throughout the year. The present investigation attempted to find out the anti-inflammatory and anti-pyretic potentials of the methanol extract of C. carandas L. stem. The extract was evaluated for phytochemical screening, which indicated the presence of steroids, glycosides, flavonoids, tannins, terpenoids and carbohydrates.

Keyword: Carissa carandas L, Anti-inflammatory, Anti- pyretic, Herbs,

INTRODUCTION

Herbal medicines have long been used for preventing or treating diseases including inflammatory diseases and indeed a priceless source of valuable chemical compounds that developed into indispensable drugs in medical practice. Today, there are plenty of chemical drugs in the shelves of the pharmacy. This may take those modern chemical drugs for granted. However, herbal medicine has been with us from prehistoric days as a rich source of medicinal compounds. Herbal medicine is a valuable constituent of traditional medicine and modern medicine, as will be probably the same in the future. Considering that inflammation is one of the essential factors in the development of many human diseases, there is an urgent need to expand our scientific understanding for beneficial effects of herbal medicines on inflammatory diseases.[1]

Inflammation is part of the complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants,[2] and is a protective response involving immune cells, blood vessels, and molecular mediators. The function of inflammation is to eliminate the initial cause of cell injury, clear out necrotic cells and tissues damaged from the original insult and the inflammatory process, and initiate tissue repair.[3]

Inflammation is a protective strategy evolved in higher organisms in response to detrimental insults such as microbial infection, tissue injury and other noxious conditions. It is an essential immune response by the host that enables the removal of harmful stimuli as well as the healing of damaged tissue. Acute inflammation has therefore been considered as apart of innate immunity, the first line of host defese against foreign invaders and danger molecules. Mankind has known the classical symptoms of inflammation for hundreds of years, which include redness, pain, swelling and heat.[4]

Fever, also referred to as pyrexia, is defined as having a temperature above the normal range due to an increase in the body's temperature set point. [5-8] There is not a single agreed-upon upper limit for normal temperature with sources using values between 37.2 and 38.3 °C (99.0 and 100.9 °F) in humans.[9,10] The increase in set point triggers increased muscle contractions and causes a feeling of cold or chills. This results in greater heat production and efforts to conserve heat. When the set point temperature returns to normal, a person feels hot, becomes flushed, and may begin to sweat.[11] Treatment to reduce fever is generally not required.[12] Treatment of associated pain and inflammation, however, may be useful and help a person rest.[13] Medications such as ibuprofen or paracetamol (acetaminophen) may help with this as well as lower temperature.[14] Children younger than three months require medical attention, as might people with

serious medical problems such as a compromised immune system or people with other symptoms. Hyperthermia requires treatment.[15]

MATERIAL & METHOD

Pharmacology of plant

Anti-inflammatory activity

Carrageenan-induced hind paw oedema in rats was used to test the anti-inflammatory effects of methanol extract of dried fruits. Methanolic fruit extract demonstrated considerable suppression of paw volume (76.12 percent) in a dose-dependent pattern at a dosage of 400 mg/kg. GC-MS analysis was used to filter out around eleven bioactive components in a methanol extract of C. carandas dried fruits.[16]

Antiviral activity

At 6 g/ml, the ethanolic extract of C. carandas fruits has antiviral action against the poliovirus HIV-1, the Sindbis virus at 3 g/ml, and the herpes simplex virus at 12 g/ml [17].

Herbs are increasingly being used as a supplemental treatment, and they are gaining in popularity and demand across the world. The current literature review on C. carandas includes all relevant information that will aid in subsequent study investigation. Furthermore, future study should concentrate on the unique cellular and molecular targets of distinct plant elements that exhibit specific therapeutic effects. C. carandas' clinical potency and toxicological investigations need to be investigated and reported. Herbal crude medications must be well standardised, as they are frequently used in problematic investigations. As a result, secondary metabolites included in the extract must be thoroughly identified and described using contemporary methods. Furthermore, rigorous validation of Carissa carandas extract is essential in order to investigate the complex relationships between herb–drug and food–drug interactions. However, more advanced experimental research, such as DNA microarrays and other techniques, would be included, which might aid in the discovery of new possible natural-source medications.[18]

Collection And Authenication of Plant Material

The whole plant was collected from rural region of Allahabad. It was authenticated by a botanist. After authentication, stem was properly washed and cleaned with water and dehydrated beneath shadow at room environment. After completely drying, the material are subjected to evaluation using different parameters.

Then the stem was taken for the size reduction using cutter mill in order to get coarser to a fine powder. After size reduction, the powder passed through 40# sieve to get uniformly sized powder. The final powdered material are kept in air tight wide mouth bottle for future use. The powdered material are subjected to various standardization parameters as per pharmacopoeias / literatures.

Ethanolic Extraction of stem of Carissa Carandas by using Soxhlet Apparatus

were carefully separated from the plant, cleaned, shade dried, mechanically grinded and coarsely powdered. About 1000 gm of airdried leaf powder was extracted with 90% ethanol in a Soxhlet extractor for 36 hours. It was concentrated to dryness under reduced pressure and controlled temperature (40-50ºC) using rotary evaporator. The extracted material was weighed and percentage yield was calculated.

$$\textbf{\% Extraction} = \frac{m1 - m2}{m1} \textbf{ X 100}$$

The collected leaf extract was stored in a desiccator.

Where:
M1 = mass in grams of thimble and the sample before extraction
M2 = mass in grams of thimble and the sample after extraction

PHYTOCHEMICAL PARAMETERS

Determination of Loss on Drying

Accurately weighed 5 - 6 g of powder separately and placed in a tar redvanishing dish and afterward dehydrated for 4 h. at 110 ºC. The sample was cooled. The dehydrating and weighing was proceeded at one-hour interim until steady weight was arrived at. The calculation of loss on drying was based on the moisture content present in the sample and the formula to calculate are:

$$\text{Loss on Drying} = \frac{weight\ of\ powder\ after\ drying\ in\ g}{Initial\ weight\ of\ the\ powder\ in\ g} \textbf{ x 100}$$

ASH VALUES

Total ash

Accurately take 2 gram of the pulverized air dried powder and set in an at one time lighted crucible (typically platinum crucible or silica) and spread in an even layer. The crucible is then burnt by progressively growing temperature upto 600 ºC awaiting white, showing deficiency of carbon. Material was cool in a desiccator and weigh up. Ash which are carbon free is not acquired by along these lines, the cauldron was cooled and deposit was dampened with 2 ml water or soaked result of ammonium nitrate. It was dried up on water bath and again blazed to relentless mass. The surplus permitted to calm in desiccator for 35 min, and it was reweighed on the double. The total ash value was measured in fraction with respect to the dehydrated material. The formula are:[19]

$$\text{Total ash value} = \frac{weight\ of\ empty\ crucible}{weight\ of\ drug\ taken} \text{ x } 100$$

Where, Z = Weight of crucible + ash (after complete incineration)

Acid insoluble ash

Twenty-five ml HCL was mix in container containing the total ash and closed with glass plate. The crucible was immersed mildly for5-7min. on the water bath. After 5-7 min, the glass was splashed with warm water(5ml)and liquid poured to the pot. The unsolvable substance was collected on an ash-less mesh. The insoluble matter then washed with warm water till the remainder was found to be neutral. After washing unsolvable stuff, shifted to the new crucible from the filter paper. Crucible containing material was with ered upon hot plate, burnt to persistent weight. The excess was permitted for 30 min to calm in a desiccator and weigh up instantaneously. Ash (acid insoluble) was calculated in term of percentage with respect to the dehydrated plant material. [20,21]

Water soluble ash

25 ml water (purified) was poured in the crucible (silica)holding total ash. Simmered for 5 min. The unsolvable substance was transferred in sintered glass crucible and washed with hot water. After washing, the unsolvable stuff was moved to the unique new crucible and burnt for 15 minutes at 450°C. The excess was allowed to cool for 30 - 40min. in desiccator, furthermore it was weighed at once. The weight of the deposit was deducted from the weight of aggregate ash. Water dissolvable ash remains was ascertained in term of rate with reference to dehydrated plant material.[22]

Alcohol extractive value

Around 5.0 gram of coarsely pulverized airdried solid was weighed. Transferred it in conical flask with stopper. Macerated the powder for 6 h with 100 ml methanol with infrequent shaking. After it was permitted to remain for 18h. After 18 h. it was filtered speedily and care was taken not to miss any amount of solvent. After this, the 25 ml of remainder was transferred to a flat bottomed tarred dish and vaporized to dryness. The extract was dehydrated for 6 h at 105 °C, chilled in desiccators for 30 min and balanced immediately. The rate of the liquor dissolvable extractive regarding the airdried powdered medication material was ascertained.[23]

Water extractive value

About 5.0 gram of the drug was mixed with chloroform (100 ml) and allowed to macerate for 24 h in a closed flask with occasional shaking for initial 6 h and then permitted it to stand another 18 h. The solution sieved quickly, 25 ml of remainder was vaporized to dehydration in tareplane bottomed bowl, and dry up at 105 °C, weigh up. The fraction of the water extractive with respect to dehydrated powdered drug material was calculated.[24]

FORMULATION OF HERBAL SUSPENSION

Table 1 Formulation Table

S. No	Material	Formulation-1 (F-1)
1.	**Stem extract of *Carissa carandas***	**55%**
6.	Methyl cellulose (suspending agent)	**25%**
7.	Purified water q. s.	**25%**

EVALUATION OF HERBAL SUSPENSION

Viscosity

At room temperature, the example's consistency was evaluated utilizing a Brookfield viscometer at 50 cycles each moment with spindle number 3.

pH

Utilizing a pH meter, the pH of the still up in the air.

Flow rate

Utilizing the condition, the clear consistency of a suspension test going through a pipette was determined.
Flow rate = Volume of pipette (ml)/Flow time

PHARMACOLOGICAL SCREENING

Albino wistar rats of weighing in the middle of 150– 250 g were used in this investigate. They were engaged for assessing acute toxicity study, anti-pyretic activity study and anti-inflammatory activity study. Animals were given marketable laboratory animal feed stuff and water in ample quantity. All the experimental animals were maintained at normal environmental condition of work space. They were housed at 25 ºC and 12 h day / night cycle in a group of six rats in hygienic cages. The beds of the cages was changed every day.

Acute toxicity study

The acute toxicity studies were conducted as per the guidelines 425 of Organization for Economic Cooperation and Development for testing of chemicals for acute oral toxicity. Albino wistar rats treated with different doses (150, 250 mg/kg) while the control group received saline (10 ml/kg). All the groups were observed up to 6 h for any gross effect and then mortality rate was observed after 24 h of treatment.

Antipyretic activity

The antipyretic activity of **Stem extract of Carissa carandas** and its active fraction was evaluated using albino wistar rats (180–220 g) as previously explained method. The selected animals were healthy and normal body temperature of each rat was checked by using digital thermometer. Pyrexia was induced in all rats by injection of 20% aqueous suspension of brewer's yeast (Saccharomyces cerevisiae) 10 ml/kg. Subcutaneous (SC). All animal groups (6/group) were fasted with access to only water, after injection of yeast for 24 h. After that, the rectal temperature of each rat was recorded and pyrexia was confirmed by increase in temperature more than 1°C, while animals showing less than 1°C rise in temperature were excluded from the experiment. The group I received saline (10 ml/kg), group II received paracetamol (100 mg/kg) as a standard drug, while group III-IV received 150 and 250 mg/kg, doses (through feeding tubes) of Stem extract of Carissa carandas. The rectal temperature of the groups was recorded.

Anti-inflammatory activity

Carrageenan induced paw edema

The anti-inflammatory activity was performed on rats (180-220 g). The normal paw volumes of all the rats were measured ab initio and animals were divided into different groups, each consisting of six rats. The group I was treated with normal saline (10 ml/kg), Group II with the standard drugs i.e. diclofenac sodium, (10 mg/kg) respectively while rest of the groups were treated with Stem extract of Carissa carandas (150 and 250 mg/kg). After thirty minutes of the above intra-peritoneal and oral administration, carrageenan (1%, 0.1 ml) was given subcutaneously into the sub plantar tissue of the right hind paw of each rat. The paw volume was quantified using digital plethysmometer before and at 1st, 2nd, 3rd and 4th h after carrageenan administration. The edema volume of paw and percent inhibition of edema were calculated using the following formulae:

EV=PVA−PVI

EV = edema volume,

PVI = Paw volume before carrageenan administration (i.e. initial paw volume) \

PVA = Paw volume after carrageenan administration.

Percent inhibition = [(EVc−EVt) EVc] ×100

EVc = Edema volume of control animals, EVt = Edema volume of test drug animals.

RESULTS

Extractive Values

For ethanol solutions, the extractive values of the plant were assessed.

Table 2 Extractive Values of the plant extract

S.NO	Name of The Plant	Yield % w/w
1	Stem of carissa carandas	13.62

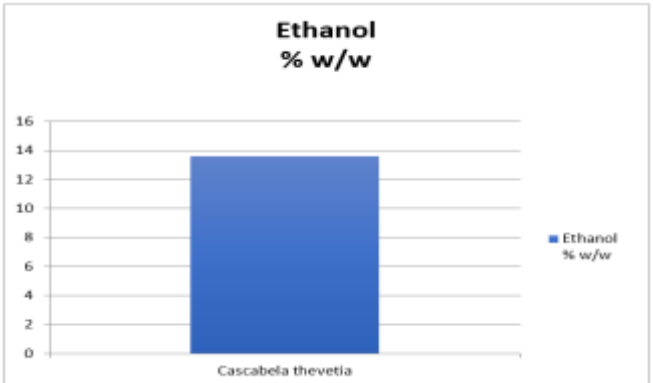

Figure 1 Graph of Extractive Values of the plant extract

PHYTOCHEMICAL PARAMETERS

Ash Values:

Water soluble ash, acid insoluble ash and total ash value of carissa carandas was discovered to be 4.7 %.

Extractive Values:

Extractive value (water and ethanol soluble) of carissa carandas were discovered to be 13.62 %.

Loss on Drying:

The loss on drying of carissa carandas was discovered to be 5.54 % w/w. All the compiled results are shown in table below.

All the compiled results are shown in table below:

Table 3 Loss on Drying And Foreign Organic Matter

Crude drugs	Loss on drying (% w/w) *	Foreign matter (% w/w) *
carissa carandas	5.54	1.28

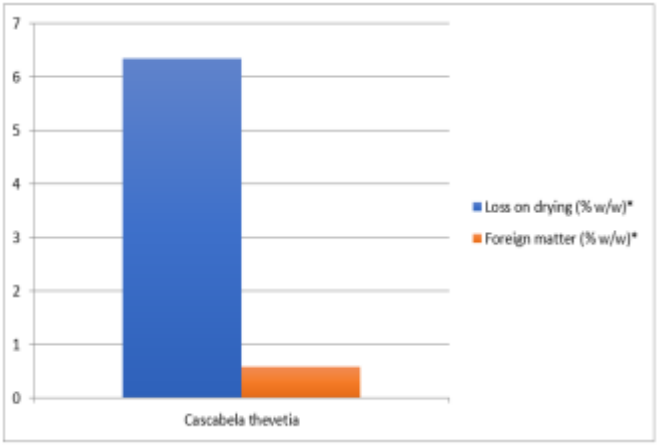

Figure 2 Graph of Loss on Drying And Foreign Organic Matter

Table 4 Total Ash, Acid Insoluble Ash And Water Soluble Ash Values

Crude drugs	Total ash value* % w/w	Water soluble ash* % w/w	Acid insoluble ash value* % w/w
carissa carandas	4.7	11.32	5.25

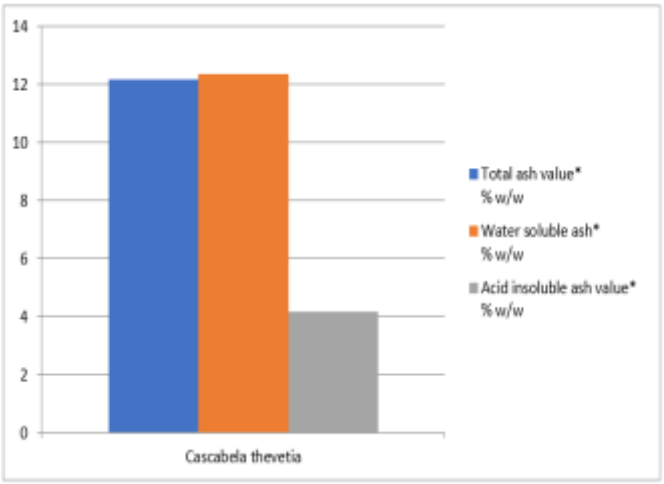

Figure 3 Graph of Total Ash, Acid Insoluble Ash And Water Soluble Ash Values

EVALUATION OF SUSPENSION

Table 5 Evaluation of suspension

S.no	Property	F1
1.	pH	4.8
2.	Viscosity	70 cP
3.	Flow rate	6 ml/5 sec

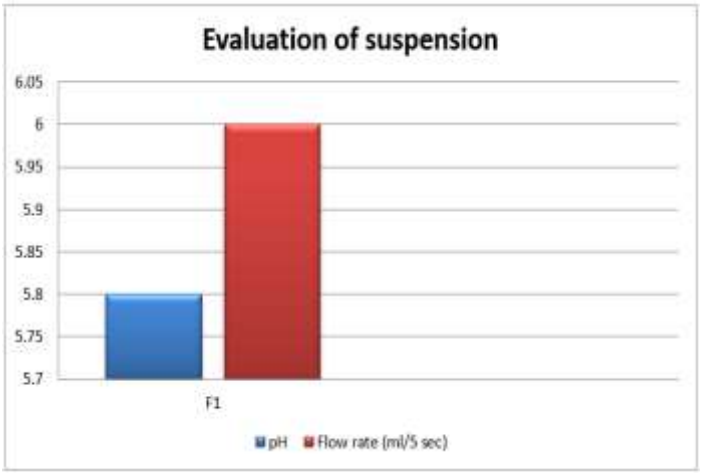

Figure 4 Graph of Evaluation of suspension

PHARMACOLOGICAL SCREENING

Screening for Anti-Pyretic Activity:

Elevation in temperature might be due to disease, contamination or one of the spin-off of tissue harm, aggravation, join dismissal and/ or an alternate ailment state. Antipyretics are executors, which diminishes the hoisted body temperature. Management of body temperature needs a delicate harmony between generation of hotness and its misfortune. The hypothalamus directs the set time when body temperature is kept up at normal condition. Hunt for safe home grown cures with strong antipyretic movement got force as of late as the accessible antipyretic, such as nimesulide, paracetamol, and headache medicine and so forth have a harmful impact to the different organ of the body. Expanded body temperature and torment are known as the principle side effects of body against a provocative incitement. In this way, it is for the most part vital to have pain relieving and antipyretic action. Yeast incited fever is known pathogenic pyrexia. Its etiology incorporates generation of prostaglandins, which fixed the thermoregulatory center at a lesser temperature.

Table 6 The results of anti-pyretic activity are tabulated in table below

S.no	Treatment Group	0 hours	8 hours	16 hours	24 hours
1.	GROUP-1 10 mg/kg				
2.	GROUP-2 150 mg/kg				
3.	GROUP-3 150 mg/kg				
4.	Group -4 150 mg/kg				

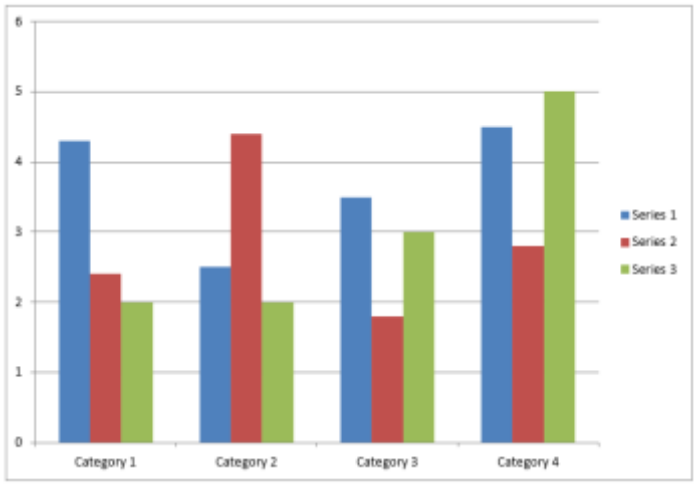

Figure 5 Graph of anti-pyretic activity

Anti-inflammatory activity

The anti-inflammatory activity at test doses (150 and 250mg/kg) of Stem extract of Carissa carandas is presented in Table 5 with the average volume of the paw edema. The percent protection of inflammation is presented. The injection of the carrageenan in paw created an inflammatory edema which increased gradually. The Stem extract of Carissa carandas at the dose of 300mg/kg exhibited an anti-inflammatory activity that became significant ($P< 0.01$) 2 h after the injection of carrageenan and was maintained all along the experiment with a maximum effect of 60.880%. The extract 150 and 250mg/kg) induced significant ($P< 0.01$) anti-inflammatory effect and the anti-inflammatory effect of diclofenac sodium (10mg/kg) was greater than that of the extract as presented in Figure below

Table 7 Anti-inflammatory activity

S.no	Treatment Group	No. of writhing (10min	No. of writhing (10min
1.	GROUP-1 10 mg/kg	64	
2.	GROUP-2 150 mg/kg	38	
3.	GROUP-3 150 mg/kg	28	
4.	Group -4 150 mg/kg	25	

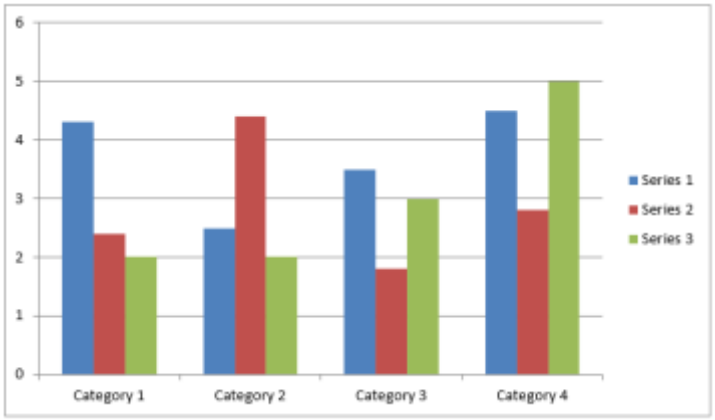

Figure 6 Graph of Anti-inflammatory activity

CONCLUSION

Results of the present study showed that the Stem extract of Carissa carandas has marked antipyretic, and anti-inflammatory effects with a reasonable safety profilet. There are several mediators for pyrexia and the inhibition of these mediators are responsible for the antipyretic effect. The administration of Stem extract of Carissa carandas significantly attenuated rectal temperature induced. The various morphological characters like colour, odour, taste, size, shape, etc. has been studied for all three plant materials i.e stem of carissa carandas. The taste of stem of carissa carandas are sour in taste. The extractive values of the plant were assessed. The yield was found to be 13.62 % w/w. The loss on drying of carissa carandas was discovered to be 5.54 % w/w. Plant material concentrates have shown that saponins, tannins, glycosides, and sugars are available. The exract pH was found to be 4.8 and flow rate 7.5 ml/5 sec The anti-inflammatory activity at test doses (150 and 250mg/kg).

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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