

A STUDY ON THE BIOEFFICACY OF *CARYOTA URENS* L.**D. Vanaja¹ and *Dr. S. Kavitha²**

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ABSTRACT

The present study is aimed to investigate the popular palm *Caryota urens* leaves for its phytochemical composition, anti-oxidant, anti-microbial, anti-inflammatory and FT-IR evaluation. Three different solvents aqueous, methanol and hexane were used in this study. The qualitative tests for phytochemicals confirmed the presence of terpenoids, alkaloids, steroids, saponins, glycosides, phytosterols, resins, phenols, flavonoids and oxalic acid. The quantitative analysis of methanolic leaf extract of *C. urens* revealed the presence of phenols (29.6 mg GAE/g), terpenoids (70mg/g) and oxalic acid (0.0612mg/g). In vitro anti-oxidant activities were evaluated by DPPH, hydrogen peroxide and reducing power methods. The antimicrobial activity of different concentrations (25, 50, 75, 100 µg/g) of methanol leaf extract

were tested against two gram positive and two gram negative bacterial strains (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*) and 4 fungal strains (*Rhizopus* sp., *Aspergillus niger*, *Penicillium* sp. and *Fusarium* sp). The larvicidal activity of methanol leaf extracts against *Aedes aegypti* was studied by direct contact method. In vitro anti-inflammatory activity of methanol leaf extract was studied by inhibition of protein denaturation method. The FTIR spectroscopic studies showed the characteristic peak values for various functional compounds in the methanol extract. The present study suggests that, *C. urens* has therapeutic potential that can be used as a resource of different bioactive compounds and antioxidants.

KEYWORDS: Phytochemicals, anti-oxidants, anti-microbials, larvicidal, anti-inflammatory and FT-IR.

INTRODUCTION

Plants have provided humans with medicines since time immemorial. The majority of world populations still rely on plant based medicine due to their easy availability, safe use and affordability. Medicinal plants, the world's oldest known health care products, play a key role in traditional medicine. These plants are not only used for primary health care, but also as pharmaceuticals. Traditional knowledge on medicinal plants is of great significance and forms the basis for the development of plant based drugs. Majority of the hints to plant based drugs come from ethnobotanical studies and traditional knowledge. The traditional system of medicine still continues to serve, in spite of the advent of modern medicine, a large portion of the population, particularly in the rural areas.^[1]

Though traditional medicine is used the world over, it is particularly relied on in developing countries.^[2] India is one such country which is endowed with a rich mega diversity of flora due to the extreme variations in geographical and climatic conditions prevalent in it. India is bestowed with a rich herbal heritage dating back to 3000 B.C. The pharmacopeias of these systems have mainly drawn upon the indigenous flora for the preparation of wide variety of herbal medicaments and about 800 plants are mentioned in the relevant systems. One such popular ornamental plant *Caryota urens* L. (Fishtail palm) used in ancient medicinal systems was chosen for the present study.

Caryota urens is a lofty handsome palm growing up to a height of 22m and a girth of 2.8 m with a smooth, cylindrical annulated trunk. The tip innate leaves are triangular in shape resembling a fish tail and the plant bears clusters of white, unisexual flower at maturity. The fruit matures to round, 1cm (0.39) drupe and is red in colour.^[3] The tender leaves are sweet and cooling and are useful in vitiated condition of pitta. The pulp of fruit is good for hyperdipsia and fatigue. A paste made from the nut is good for hemicrania. A porridge prepared from the seed flour is prescribed by local physicians to treat gastric ulcers, migraine headaches, snake-bite poisoning and rheumatic swellings. The root is used for treating tooth ailments. The bark and seed are used to treat boils. The tender flowers are used for promoting hair growth. *C. urens* flower is used to treat gastric ulcer, migraine headaches, snake bite poisoning as well as rheumatic swellings. In Ayurveda, it is recommended for seminal weakness and urinary disorders. Palm heart is used locally as flour, especially for control of diabetes and in ayurvedic medicines.^[4] The plant is less exploited medicinally and hence in the present study an attempt was made to determine the bioefficiency of its leaves.

MATERIALS AND METHODS

PLANT COLLECTION AND IDENTIFICATION

Healthy leaves of *Caryota urens* L. were collected from Ethiraj College for Women, Chennai. The plant samples were identified by Dr. D. Narasimhan, a taxonomist, Department of floristic research, Madras Christian College, Chennai.

PREPARATION OF PLANT EXTRACT

The plant samples were shade dried for 2 weeks. After shade drying, the samples were ground into a fine powder using an electrical blender. 10 g of sample was extracted with 100 ml of each solvent -methanol, aqueous and hexane separately using Soxhlet apparatus and the extract was concentrated using rotary evaporator at 50 C.^[5]

QUALITATIVE ANALYSIS OF PHYTOCHEMICALS (SECONDARY METABOLITES)

Phytochemical tests were performed to identify the phytoconstituents present in leaf extracts of different solvents prepared in Methanol, Hexane and Aqueous medium using standard procedures.^{[6][7][8]}

Estimation of total phenolic content

The total phenolic content of methanolic leaf extracts of *Caryota urens* was determined by Folin-ciocalteu reagent method.^[9]

Estimation of total terpenoids

The total terpenoids was calculated by Ferguson's method.^[10]

Estimation of oxalic acid

Oxidation of KMnO₄ by oxalic acid

Standard oxalic acid solution (1 mg/ml) was prepared with distilled water. 100 ml of 0.003 M KMnO₄ was prepared from appropriate dilution of 0.01 M KMnO₄ in distilled water and 50 ml of 2 N H₂SO₄ was prepared in distilled water. Assay mixtures contained different concentrations of oxalic acid ranging from 0.1 to 1 mg, 5 ml of 2 N H₂SO₄ and 2 ml of 0.003 M KMnO₄. This mixture was incubated for 10 min. at room temperature (27 ± 2 C). After 10 min. absorbance was recorded at 528 nm using Shimadzu, UV 1650 spectrophotometer. Reagent blank was prepared with distilled water. Absorbance for blank solution was recorded as Ab and for sample it was recorded as As. The calibration curve obtained is linear in

concentration range of 0.1 to 1 mg/ml of oxalic acid. The concentration of oxalic acid is calculated using difference in the absorbance $\Delta A = \Delta b - \Delta s$.^[11]

ANTI-OXIDANT ACTIVITY

Hydrogen peroxide scavenging activity

The hydrogen peroxide (H₂O₂) scavenging activity was determined by Ruch *et al.*, 1989 method.^[12] Ascorbic acid was used as standard. The extent of H₂O₂ scavenging activity of the leaf extract was calculated as

$$\text{H}_2\text{O}_2 \text{ scavenging effect (\%)} = \frac{A_c - A_o}{A_c} \times 100$$

Where A_c is the absorbance of control and A_o is the absorbance of leaf extract.

Reducing power assay

The reducing power assay was carried out according to the method of Oyaizu (1986).^[13] The assays were carried out in triplicates. The activity was compared with ascorbic acid standard.

DPPH ASSAY

The antioxidant activity was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH (α , α -diphenyl- β -picrylhydrazyl). The reduction of the radical is followed by a decrease in the absorbance at 517 nm. The methanol extract of *Caryota urens* leaves of varying concentration (100 – 500 μg / ml) was taken into test tubes and 2 ml of 1 mM DPPH solution was added. The tubes were kept in the dark for 30 min. Absorbance at 517 nm was measured with a UV-VIS spectrophotometer and compared to an ascorbic acid calibration curve.^[14] The percentage inhibition of the DPPH radical was calculated using the following formula:

$$\text{DPPH inhibition (\%)} = \frac{A_c - A_o}{A_c} \times 100$$

Where A_c is the absorbance of control and A_o is the absorbance of sample

ANTIMICROBIAL STUDY

Antibacterial activity

Antibacterial activities of *C. urens* extract were carried out against different bacterial pathogens such as *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The bacterial cultures were obtained from the Department of Microbiology, Ethiraj College for Women. Antibacterial activity of methanol leaf extract was carried out by Agar well diffusion method in nutrient agar medium.^[15] Streptomycin was

used as positive control and methanol was used as negative control and all the experiments were carried out in duplicates.

Antifungal Activity

Antifungal activity of methanolic leaf extract was carried out against four fungal species - *Rhizopus sp.*, *Aspergillus niger*, *Penicillium sp.* and *Fusarium sp.* The fungal cultures were obtained from the Department of Microbiology, Ethiraj College for women and maintained as pure culture in potato dextrose agar (PDA) medium.

The methanol leaf extracts were screened for antifungal activity by agar well diffusion method. Fungal cultures (48 hold) grown on PDA medium, were used for plate assays. Carbendazim was used as positive control. All experiments were repeated twice and the results were recorded after 48-78 h.

LARVICIDAL ACTIVITY

The *Aedes aegypti* mosquito larvae were obtained from the Entomology Department, Loyola College, Chennai. The insecticidal activity was determined by direct contact application.^[16]

Small sized petridishes (60 mm) were used for conducting direct contact activity. The positive and negative controls used were DMSO and methanol respectively. Various methanol concentrations of *C. urens* (100-500 µg) was used to test the larvicidal activity. 1 ml of leaf extract and 25 ml of water was added in a petriplate and in each petriplate 10 larvae were placed. The mortality rate of larvae was calculated after 24 h. Percentage mortality was calculated using the formulae.

$$\text{Percentage mortality} = \frac{\text{No. of dead larvae} \times 100}{\text{No. of larvae introduced}}$$

EVALUATION OF IN VITRO ANTI-INFLAMMATORY ACTIVITY

Inhibition of protein denaturation method

Anti-inflammatory activity of the *C. urens* extract was evaluated by protein denaturation method.^[17] Diclofenac sodium, a powerful non-steroidal anti-inflammatory drug was used as a standard. The reaction mixture consisting of 2 ml of different concentrations of *C. urens* methanol leaf extract (100-500 µg/mL) or standard diclofenac sodium (100 and 200 µg/ml) and 2.8 ml of phosphate buffered saline (pH 6.4) was mixed with 2 ml of egg albumin (from fresh hen's egg) and incubated at (27±1) C for 15 min. Denaturation was induced by keeping

the reaction mixture at 70 °C in a water bath for 10 min. After cooling, the absorbance was measured at 660 nm by using double distilled water as blank. Each experiment was done in triplicate and the average was taken. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\text{Percentage of inhibition} = \frac{A_c - A_o}{A_c} \times 100$$

Where A_c is the absorbance of control and A_o is the absorbance of leaf extract.

UV-SPECTROPHOTOMETRIC ANALYSIS

The methanol extracts were analyzed for the confirmation of secondary metabolites using UV-1650 Shimadzu spectrophotometer in the range set between 200-400 nm.

FTIR SPECTROSCOPIC ANALYSIS

Dried methanol leaf extract of *C. urens* was used for FTIR analysis. 10 mg of dried extract powder was mixed with KBr salt using a mortar and pestle. The mixture was then compressed into a thin pellet. Infrared spectra and peak values were recorded on a Shimadzu IR (Japan), between 4000 – 400 cm^{-1} .

RESULTS AND DISCUSSION

The constant rising demand of plant-based drugs is unfortunately creating heavy pressure on some selected high-value medicinal plant populations in the wild due to over-harvesting. Several of these medicinal plant species have slow growth rates, low population densities, and narrow geographic ranges and therefore they are more prone to extinction.^{[18] [19]} Furthermore, the indigenous knowledge on the use of lesser-known medicinal plants is also rapidly declining. Continuous erosion in the traditional knowledge of many valuable plants for medicine in the past and with a renewed interest, currently there is a need to review the valuable knowledge with the expectation of developing the medicinal plants sector.^[20] Much easily grown and propagated ornamental plants with potential phytochemicals provide a better solution to overcome such problems. Hence, the ornamental palm *Caryota urens*, which has been reported in traditional medicine but much exploited for its therapeutic potential was used in the present study.

PHYTOCHEMICAL ANALYSIS

The phytochemical screening of the leaf extract of *C. urens* in various solvents revealed the presence of different phytochemicals. Methanol and aqueous extracts revealed the strong

presence of alkaloids, terpenoids, steroids, glycosides, phytosterols, oxalic acids and phenols. A moderate presence of saponins, tannins, flavonoids, gum and mucilage were observed in the aqueous extracts of *C. urens*. In hexane extract, terpenoids, phytosterols, saponins, steroids were present in trace amounts (Table1).

Table 1: Phytochemical analysis of *C. urens* leaf extracts

S.NO	PHYTOCHEMICAL CONSTITUENTS	AQUEOUS	METHANOL	HEXANE
1	Alkaloids	+	—	—
2	Carbohydrates	—	—	—
3	Terpenoids	+	+	+
4	Saponins	+	+	+
5	Steroids and Triterpenoids	+	+	—
6	Anthroquinone	—	—	—
7	Glycosides	+	+	—
8	Protein and Aminoacids	—	—	—
9	Phlobatannin	—	—	—
10	Cardiac glycosides	+	+	—
11	Gums and Mucilage	+	—	—
12	Fixed oils and Fats	—	—	—
13	Phytosterol	+	+	+
14	Anthocyanin and Betacyanin	—	—	—
15	Resin	+	+	—
16	Fatty acids	—	—	—
17	Coumarin	—	—	—
18	Phenols	+	+	—
19	Flavonoids	+	—	—
20	Tannins	+	+	—
21	Oxalic acid	+	+	+
22	Inorganic acids	—	—	—

Key: (+) indicates presence; (-) indicates absence.

QUANTITATIVE ANALYSIS OF PHYTOCHEMICALS

Estimation of phenol, terpenoids and oxalic acid content

The total phenolic content of methanolic leaf extract was expressed in terms of gallic acid equivalents (standard curve equation $y = 0.001 + 0.014x$, $R^2 = 0.993$). The dry weight of terpenoid present in leaf extracts was represented in mg/g. The amount of oxalic acid in leaves of *C. urens* per 100 g was estimated using the regression equation $\Delta A = 0.966C - 0.027$, where C is the concentration of oxalic acid in mg/ml and ΔA is $A_b - A_s$ (Table 2).

Table 2 Estimation of phenol, terpenoid and oxalic acid

<i>Caryota</i> leaf extract	TPC mg GAE/g	Terpenoid mg/g	Oxalic acid mg/100g
	29.6	70	6.12

Phenolic compounds have redox properties, which allow them to act as antioxidants, as their free radical scavenging ability is facilitated by their hydroxyl groups.^[21] Terpenes play an important role as signal and growth regulators of plants. Since, terpenoids promote glutathione s-transferase and cell apoptosis, they have been used for their anti-cancer properties.^[22] Traditionally, plant-based terpenoids have been used by humans in the food, pharmaceutical and chemical industries and more recently in the development of biofuel.^[23] Oxalic acid is a primary chelator of calcium. Oxalic acid has been detected in various organisms, plants and fungi. It has long been investigated from a medicinal view point. Besides this, it has been receiving much attention for its various ecological qualities such as bioremediation of a wide variety of organic pollutants.^[24] The present study indicated that methanolic leaf extracts of *Caryota urens* contains such potential metabolites in considerable quantities.

ANTIOXIDANT ACTIVITY

The role of free radicals and tissue damage in diseases such as atherosclerosis, heart failure, neuro degenerative disorders, aging, cancer, diabetes mellitus, hypertension and several other diseases, is gaining a lot of recognition.^[25] In recent years, considerable attention has been directed towards the identification of plants with antioxidant ability that may be used for human consumption. Therefore, much research has been focused on the use of antioxidants with particular emphasis on naturally derived antioxidants, which may inhibit reactive oxygen species production and may display protective effects.^[26]

In the present study, the methanolic leaf extract of *C. urens* was investigated for its antioxidant activity at different concentration using H₂O₂, reducing power and DPPH methods.

Hydrogen scavenging activity

H₂O₂ has strong oxidizing properties. The percentage of inhibition of *C. urens* leaf extract at various concentrations (100-500µg) was evaluated and compared with ascorbic acid using Ms Excel 2007. The maximum percentage of inhibition observed in *C. urens* is 68 (Fig.1).

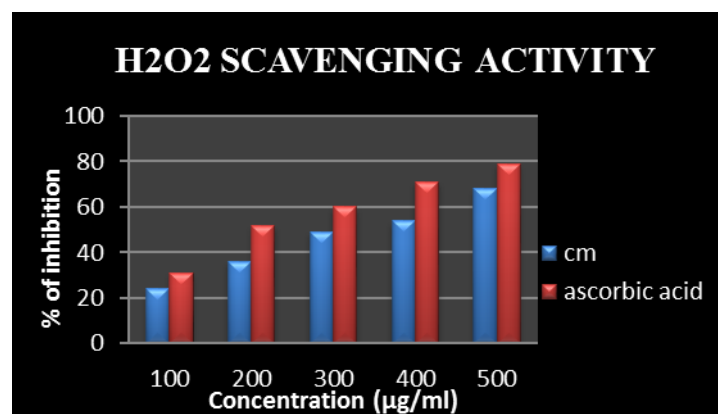


Fig.1 H₂O₂ scavenging activity showing % of inhibition of ascorbic acid and *C. urens* at various concentrations

Hydrogen peroxide occurs naturally at low concentration levels in the air, water, human body, plants and microorganisms. H₂O₂ is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals (OH) that can initiate lipid peroxidation and cause DNA damage. The assay revealed that the methanolic fraction of *C. urens* leaf (LC₅₀ value of 276.45 µg/ml) efficiently scavenged H₂O₂ and this may be due to the presence of phenols and terpenoids.

Reducing power assay

The reducing power activity of methanolic extract of *C. urens* was evaluated and compared with ascorbic acid. The maximum absorbance observed in *C. urens* is 0.146 at 500 µg/ml concentration.

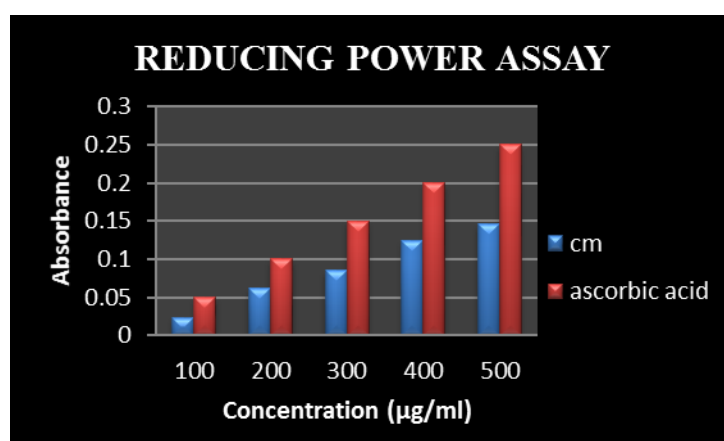


Fig.2 Reducing power assay of *C. urens* and ascorbic acid

In reducing power assay, the presence of the reductants in the solution causes the reduction of the Fe³⁺/ferricyanide complex to the ferrous form. The reducing properties have been shown

to exert antioxidant action by donating a hydrogen atom to break the free radical chain. Increasing absorbance indicates an increase in reducing ability.^[27] The reducing property of methanolic extract and ascorbic acid were found to be nearly equivalent with increasing concentration.

DPPH ASSAY

In the DPPH assay, the antioxidants were able to reduce the stable radical DPPH to the yellow colored 1, 1-diphenyl-1, 2-picryl hydrazine. The graph of *C. urens* and ascorbic acid showing % of inhibition of DPPH is represented in fig.3.

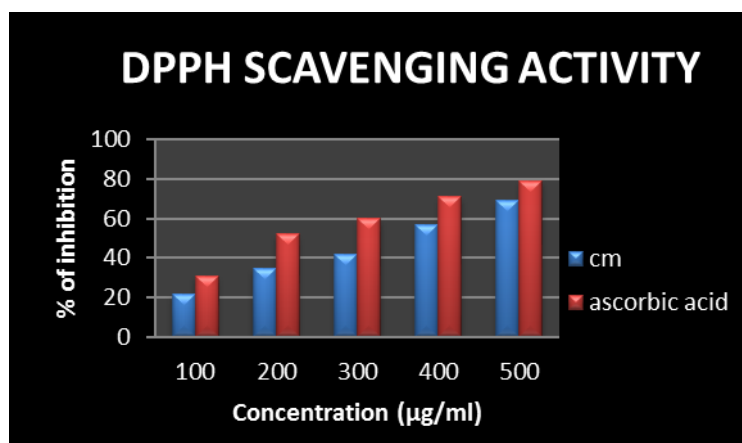


Fig.3 DPPH assay of *C. urens* and ascorbic acid

The electron donation ability of natural products can be measured by 2,2 – diphenyl 1-picrylhydrazyl radical (DPPH) purple – colored solution bleaching. The method is based on scavenging of DPPH through the addition of a radical species or antioxidant that decolourized the DPPH solution. The degree of colour change is proportional to the concentration and potency of antioxidants. Methanolic leaf extract of *C. urens* showed considerable inhibition percentage with a LC₅₀ value of 266.6 µg/ml. Results of this study suggest that the plant extract contain phytochemical constituents that are capable of donating hydrogen to a free radical that may cause potential damage.

ANTIMICROBIAL ACTIVITY

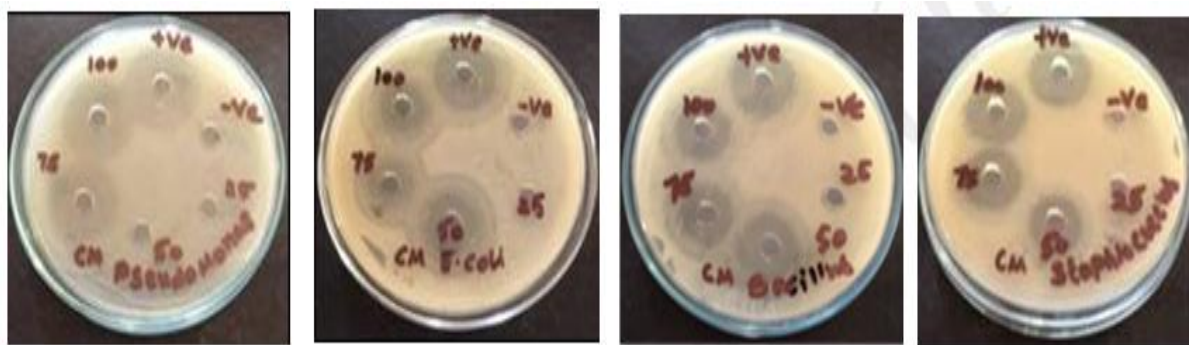
Antibacterial activity

The antibacterial activity of the methanol extracts of *C. urens* was studied in different concentrations (25, 50, 75 and 100 µg/ml) against four pathogenic bacterial strains viz. *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas* sp. (Table 3 and fig. 4).

Table 3. Antibacterial activity of methanol leaf extracts of *C. urens*

S.no	Bacterial strains	Diameter of inhibition zone (mm)				
		Positive control	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml
1.	<i>E.coli</i>	24	5	13	14	16
2.	<i>S.aureus</i>	20	6	15	18	22
3.	<i>Pseudomonas</i> sp.	28	-	9	16	21
4.	<i>B.subtilis</i>	22	6	14	16	17

Positive control: Streptomycin; Negative control: methanol

**Fig. 4 Antibacterial activity of *Caryota urens* methanol leaf extracts against *Pseudomonas* sp., *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus***

In *C. urens* methanol extract maximum growth suppression was observed against *Staphylococcus aureus* (22 mm) at 100 µg/ml concentration among the four bacterial organisms. The presence of phytochemicals like terpenoids, steroids, phenolics and oxalic acid was responsible for the antibacterial activity.^{[28] [29]} Moreover, it is also reported that such compounds in lower quantities might be involved in some type of synergism with the active compound which might be the reason behind the high activity of antimicrobial and antioxidant in the leaf extracts.^[5]

Antifungal activity

The antifungal activity of methanol leaf extracts of *C. urens* showed the effective inhibition against *Fusarium* sp. (19 mm) and poor inhibition against *Penicillium* sp.(15 mm). The results are represented in Table 4 and fig. 5.

Table 4. Antifungal activity of methanol leaf extracts of *C. urens*

S.no	Fungal strains	Diameter of inhibition zone (mm)				
		Positive control	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml
1.	<i>Rhizopus</i> sp.	21	15	17	22	24

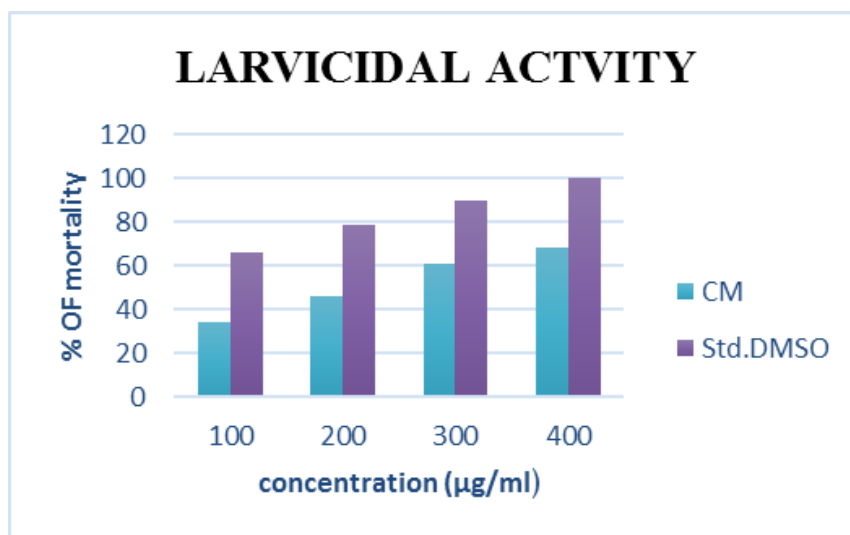


Fig.6 Larvicidal activity of *C. urens* and DMSO

The high larvicidal activity of methanol leaf extracts of *Caryota* leaves is supported by the presence of phytochemicals such as steroids, saponins and triterpenoids which are known to have insecticidal and pesticidal properties.

IN VITRO ANTI-INFLAMMATORY ACTIVITY

Inhibition of protein denaturation

In the present investigation, the in vitro anti-inflammatory effect of leaf extract was evaluated against denaturation of egg albumin. Diclofenac sodium was used as the reference drug. The maximum percentage of inhibition observed in *C. urens* was 68 % at 500 µg/ml. The results are shown in Fig.7.

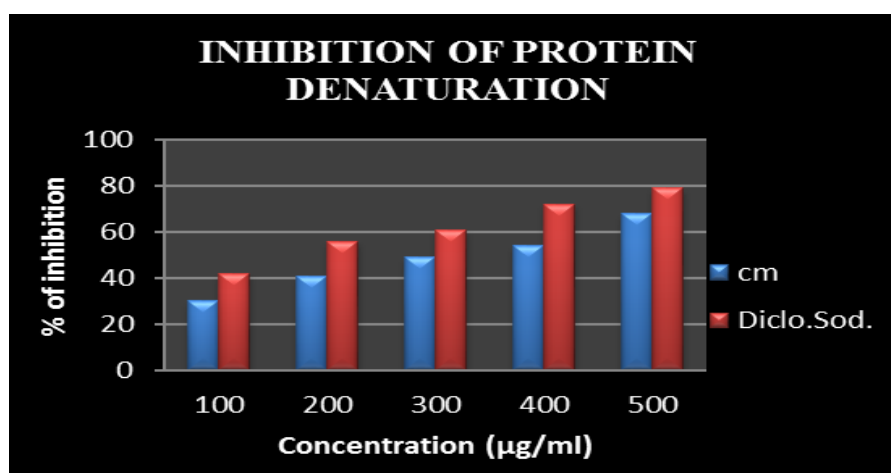


Fig.7: In-vitro anti-inflammatory activity of *C. urens* and diclofenac sodium

Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound. Most biological proteins lose their biological function when denatured. Denaturation of protein is a well-documented cause of inflammation. The ability of methanol leaf extracts of *C. urens* to inhibit protein denaturation was studied. The standard anti-inflammatory drug diclofenac sodium showed maximum inhibition of 82.78% at the concentration of 400 µg/ml. The maximum percentage of inhibition 62.6% was observed in the extract at 400 µg/ml. The methanolic leaf extracts of *Caryota* showed significant results due to its antioxidant property.^[17] It has been reported that the presence of phenols, terpenoids and saponins in plants are related to anti-inflammatory activity.^[32]

THIN LAYER CHROMATOGRAPHY

The presence of phenols and oxalic acid in *C. urens* leaf extracts were confirmed by TLC technique. The R_f values 0.36 and 0.46 confirms the presence of phenol and oxalic acid in *C. urens* leaf extracts.

UV-VIS SPECTRAL ANALYSIS

The secondary metabolites present in methanol leaf extracts of *Caryota* were confirmed using UV-Vis spectroscopy. The UV-Vis spectrum showed the peaks at 209.8 nm, 270.2 nm, 329.4 nm and 341.4 nm with the absorption of 1.729, 0.532, 0.539 and 0.527 respectively. The spectrum of the extract is shown in Fig.8. The UV spectrum of *C. urens* confirmed the presence of oxalic acid, phenols, flavonoids and terpenoids with the peak values 209.8, 270.2, 329.4 and 341.4 nm respectively.

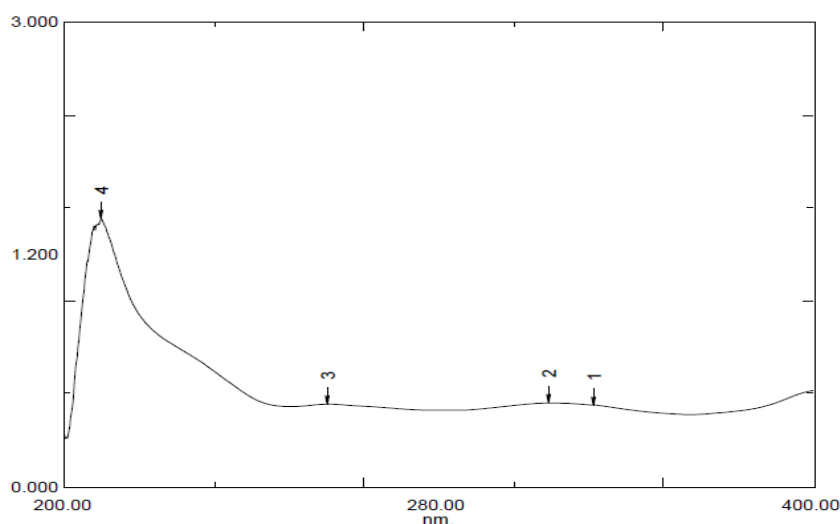


Fig.8 UV spectrum of *Caryota urens* methanol leaf extract

FTIR SPECTRAL ANALYSIS

The FTIR spectrum of *Caryota* leaf extracts confirmed the presence of alkyl halides, aromatics, amines, alkanes, aldehydes, carboxylic acids and phenol. The data on the peak values and functional groups are represented in Table 5 and spectrum is given in Fig.9.

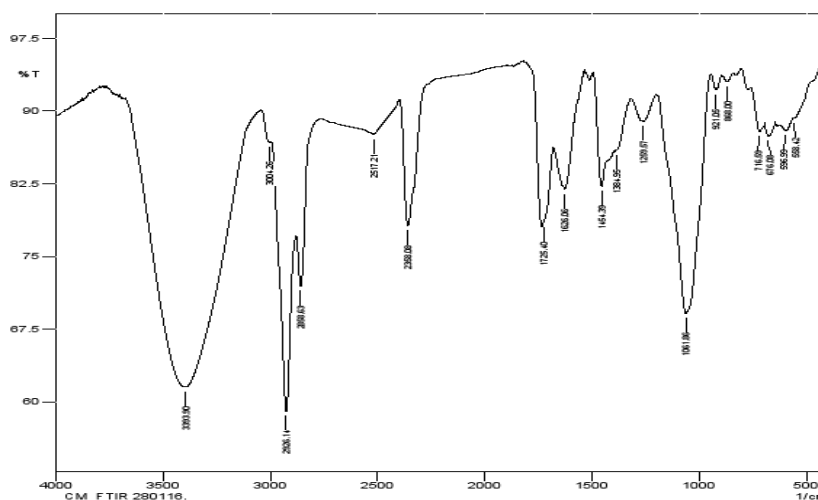


Fig .9: FTIR spectrum of *C. urens* leaf extract

Table 5: FTIR spectral peak values and functional groups of *C. urens* methanolic leaf extract

S.No	Peak Value	Stretch	Functional Groups
1	558.42, 596.99, 676. 08	C-Br	Alkyl halides
2	716.59, 868	C-H	Aromatics and amines
3	1259.47,1384.95, 2858.63, 2926.14	C-H	Alkanes
4	1061.86, 1259.57	C-O	Esters and ethers
5	1725.4	C=O	Aldehydes and Carboxylic acids
6	921. 05, 2858.	O-H	Carboxylic acids
7	3393.9	O-H	Phenol

The presence of various functional groups may be attributed to the existence of variety of potential phytochemicals. The multiple functional groups reflect either the complex structure or it indicates the nature of sample as mixture.^[33]

CONCLUSION

The present study suggests that methanolic leaf extracts of *Caryota urens* L. reveals the presence of potential phytochemical constituents. The palm exhibited multidimensional characters as it possessed antioxidants, antimicrobials, anti-inflammatory and larvicidal activities. Hence, *C. urens* leaves can be used as a rich and cheapest source of antioxidants,

anti-inflammatory and antimicrobial agents. Also, it can serve as a potential larvicidal agent against the dengue vector *A. aegypti*, which can be promoted in the dengue vector control program. However, the bioactive compounds can be further analyzed for their therapeutic properties.

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