Research Article

Antidiabetic and Antimicrobial Properties of Leaf and Bark Extracts of *Cerbera odollam*

Sonia Angeline Martin*, Esther Shoba, Suchithra Jayakumar, Muhammed Asharudheen

Department of Life Sciences, Kristu Jayanti College, K. Narayanapura, Kothanur, Bangalore, Karnataka, INDIA.

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ABSTRACT

Aim: The study aimed to identify the phytochemical components, antibacterial, antifungal, and antidiabetic activity in methanol and aqueous extract of *Cerbera odollam* leaves and bark using different testing techniques. **Materials and Methods:** This research work evaluated the antibacterial and anti-diabetic effects of methanol and aqueous extracts of *Cerbera odollam*, extracts were generated by drying and extracting the leaf and bark using a soxhlet apparatus. The antibacterial and antifungal activity was determined using the well diffusion method. **Results:** The antidiabetic role of the plant extracts was analyzed using alpha-amylase inhibition screening assay followed by starch iodine assay. The results were validated with UV-VIS Spectroscopy and FTIR analysis. The methanolic extract of the leaf possesses maximum antibacterial, antifungal, and antidiabetic activity. **Conclusion:** This study provides new insights and scope to analyze the methanol and aqueous bark extracts of *Cerbera odollam* for their effective antidiabetic activity.

Keywords: Antidiabetic, Antimicrobial, *Cerbera odollam*, FTIR, Phytochemicals.

Correspondence:
Dr. Sonia Angeline Martin
Department of Life Sciences, Kristu Jayanti College, K. Narayanapura, Kothanur, Bangalore-560077, Karnataka, INDIA.

Email: sonia.m@kristujayanti.com

INTRODUCTION

Cardiac glycosides such as neriifolin, cerberin, odollin, and others are found in the fruit and seed of *Cerbera odollam* (*C. odollam*), making it exceedingly toxic. It is believed to be responsible for 10% to 50% of all poisoning episodes in Kerala, India.[1] The tree has a number of therapeutic benefits. To cure itching, the oil derived from the seeds is applied to the scalp. It has purgative, emetic, irritating, and cathartic qualities and is used to treat rheumatism. Its bark contains sedative and antinociceptive effects. Previous research has reported the effects of cardiotoxicity on the central nervous system, neurological activities, cardiac stimulant activity, and cytotoxic action.[2]

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Phytochemicals are found extensively in *C. odollam* and have a variety of pharmacological activities, including anti-inflammatory, anti-cancer, antibacterial, antifungal, and free radical scavenging.[3] In recent days plant compounds are increasingly being used for therapeutic purposes and they have been used to cure and prevent a variety of ailments, as well as to enhance immunity.[4] Identifying and using phytochemicals contained in plant extracts will be tremendously important in therapeutic treatment.[5] *Cerbera odollam* has been extensively researched for its antiproliferative, anticancer, antiestrogenic, antibacterial, antinociceptive, and sedative properties *in vitro* and *in vivo*.[2,6] Though these properties were extensively studied the antidiabetic activity has not been explored much.

Diabetes manifests itself in a variety of ways, each requiring a distinct treatment strategy.[7,8] This prompted us to investigate the antidiabetic property. The goals of this study were to investigate the phytochemical elements of the extracts as well as to determine the antidiabetic properties of *Cerbera odollam* extracts of leaves and bark. We studied it by analysing the starch hydrolytic

properties of *Cerebera odallam* leaf and bark extracts in this work. The antibacterial activity was confirmed with bacteria such as *Escherichia coli, Staphylococcus aureus* and *Bacillus subtilis* species. The antifungal properties of plant extracts were determined using *Fusarium* and *Aspergillus* sp.[9]

MATERIALS AND METHODS

Collection and Preparation of Plant Extract

Leaves and bark of *C. odollam* were collected from Alleppey district (9.4126° N, 76.4100° E) of Kerala and identified by comparing with standard herbarium specimens. After collection, the leaves and bark were sundried for 10 days. A coarse powder was made by grinding the dried leaves and bark.

Aqueous Extraction

About 45.0 g of leaves and 90.0 g of bark were weighed using an electronic weighing machine and it is soaked in 500 mL aqueous solution for 1 week. After filtering using filter paper, namely Whatman no. 1, the mixture was evaporated in a water bath.

Methanol Extraction

About 45.0 g of leaves and 90.0 g of bark were weighed using an electronic weighing machine and soaked in 500 mL Methanol for 24 hr. Soxhlation was done for methanol extract. After about 10 cycles, the extract was collected. The extract was subject to rotary evaporation.[10]

Phytochemical Analysis

A preliminary qualitative phytochemical screening test was done for Alkaloids, Flavonoids, Tannins, Saponins, Phenols, Carbohydrates, Terpenoids, and Quinones by using a standard test procedure. The extracts were tested with the respective reagents at defined concentrations and amounts in order to identify specific phytochemicals using conventional technique.[11]

Antibacterial assay

Mueller Hinton Agar plates with discs containing extracts from leaves and bark were used for the antibacterial test. The acquired culture was then put on an agar plate in a sterile environment and let to stand for 10 min. Penicillin and methanol were used as controls, and test samples of 0.5 mg/mL and 1 mg/mL concentration were put onto the sterile disc. After extract diffusion, the plates were left in an incubator at 37ºC for 24 hr. The inhibitory zone was then assessed for *Escherichia coli* (GenBank: OK448495.1), *Staphylococcus aureus* (GenBank: OM779114.1), *Bacillus subtilis* (GenBank: ON243966.1).

Antifungal assay

Antifungal assay was determined by disc diffusion method on Potato Dextrose Agar plates with disc containing plant extracts. The acquired culture was then put on an agar plate in a sterile environment and let to rest for 10 min. Samples of 0.5 mg/mL and 1 mg/mL, together with controls of nystatin and methanol, were placed onto the sterile disc for testing. The agar plates were maintained for 24 hr at 37ºC, after the extract was diffused into the wells. The inhibition zone was evaluated for *Aspergillus flavus* (GenBank: ON237608.1) and *Fusarium oxysporum* (GenBank: ON237606.1) and they were tested for antifungal activity against the leaf and bark extract.[12]

Alpha amylase inhibition screening assay

This experiment included combining amylase enzyme (160 µL) with plant extract (120 µl) and incubating the mixture at 37 ºC for 45 min. The mixture was incubated, and then added to a well in a petri dish that had been pre-prepared with 3% agar (w/v) and 1.2% starch (w/v). After incubating for three days at 25ºC, the plates were saturated with an iodine solution and let to stand for 15 min. Hydrolysis zone diameter for starch was measured. As a control, enzyme was applied to a well of the plate that did not contain plant extract.[13] The following equation was used to calculate the percentage of inhibition.

$$\%\ \text{amylase inhibition} = \frac{Diameter\ of\ control - Diameter\ of\ test \times 100}{Diameter\ of\ control}$$

Starch Iodine Assay

1 mL of plant extract was mixed with 0.04 mL of amylase enzyme for 10 min at 37ºC. The combination was then incubated for an additional hour at 37ºC with the addition of 1% starch solution. The test tube was filled with 200 µL of 1% iodine. Acarbose, a well-established, very effective-amylase inhibitor was used as a reference standard. Starch was represented by a dark blue colour; its absence was represented by a yellow hue; and a brownish colour was indicative of starch that had been partially destroyed in a reaction mixture. Absorbance measurements showed a shift in hue to 565 nm.[14]

UV-VIS Spectroscopy

The extracts were filtered using the Whatman No.1 filter paper, after 10 min of centrifugation at 3000 rpm. A 1:10 sample dilution was made using the same solvent.

The extract's characteristic peaks were identified by scanning them between 200 and 800 nm on a Perkin Elmer Spectrophotometer. UV-VIS peaks were measured and recorded.[15]

Fourier Transform-Infrared Spectroscopy (FTIR)

In this method, the sample was exposed to infrared radiation, which the molecules absorbed. The sample transforms the radiation it has absorbed into rotational or vibratory energy, which is then measured by the detector. The frequency ranges are measured as wave number range from 4000-400/cm which represents the molecular fingerprint of the molecule. Each molecule has a unique fingerprint. FTIR is the analytical technique used to identify the functional group of the chemical compound present in the sample. Both qualitative and quantitative assessments are provided. It is done to support the qualitative methodologies. The absorption corresponds specifically to the bond present in the molecule. FTIR is based on the molecule that absorbed light in the infra-red region of the electromagnetic spectrum.[16]

RESULTS

Phytochemical Analysis

Various qualitative tests are used to identify the phytochemicals present in the (leaf and bark) aqueous and methanolic extract of *C. odollam*, including Mayer's tests for alkaloids, Salkowski's tests for terpenoids, foam tests for saponins, ferric chloride tests for phenols, Fehling's tests for carbohydrates, Ninhydrin tests for proteins, alkaline reagent tests for flavonoids, and tests for tannins, oils and resins, and steroids (Table 1). The aqueous and methanolic leaf and bark extracts for the qualitative test contain alkaloids, flavonoids, saponins, terpenoids, tannins, carbohydrates, phenols, glycosides, and quinones.[12]

Table 1: Phytochemical Analysis.

Phyto-constituents	Observation
Alkaloids	+
Flavonoids	+
Saponins	+
Tannins	+
Carbohydrates	+
Phenols	+
Cardiac glycosides	+
Terpenoids	+
Quinones	+

The phytochemicals present in (leaf and bark) aqueous and methanolic extract of *C. odollam* is confirmed by different qualitative tests.

Antibacterial Assay

The disc diffusion method assessed the antibacterial activity of methanolic and aqueous extracts against several bacterial strains. Bacterial strains are the organisms that cause infectious illnesses. The zone of inhibition was evaluated, and it revealed that methanolic extract of *C. odallam* leaf demonstrated antibacterial activity against *E. coli, S. aureus, and B. subtilis*, with the maximum zone of inhibition for *E. coli* being 26 mm zone diameter. Methanolic extracts of the leaf demonstrated the greatest antibacterial activity (Figure 1).

Antifungal Assay

The disc diffusion method tested the antifungal activity of methanolic and aqueous leaf and bark extracts against fungus strains. These fungal strains are commonly seen in infectious illnesses. Figure 2 reveals that methanolic extract of leaf has antifungal action against *Aspergillus flavus* and *Fusarium oxysporum* (zone of inhibition). Both leaf and bark extracts showed antifungal activity, however methanolic extracts of leaf with a zone of 22 mm diameter showed the most activity against *Aspergillus flavus*.

Figure 1: Antibacterial activity of leaf and bark extracts of *C. odallam*.
Methanolic extracts of the leaf demonstrated the greatest antibacterial activity against *B. subtilis, S. aureus,* and *E. coli.*

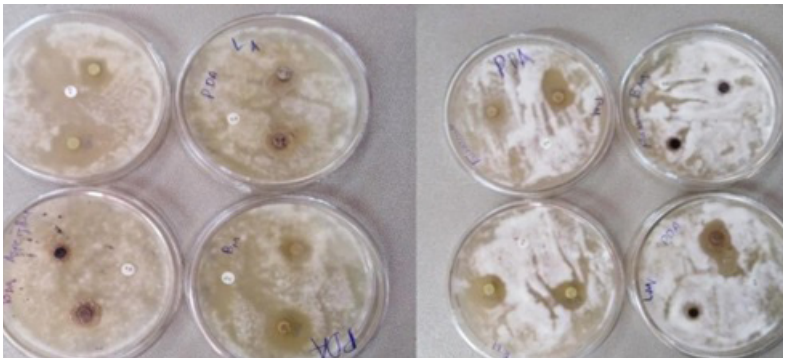

Figure 2: Antifungal activity of leaf and bark extracts of *C. odallam*.
Methanolic extract of leaf has antifungal action against *Aspergillus flavus* and *Fusarium oxysporum*.

Amylase Inhibition Screening Assay

The enzyme amylase is essential in the conversion of starch to sugar. Because of its ability to hydrolyze complex polysaccharides into oligosaccharides and disaccharides, which are then converted to monosaccharides by glycosidase and absorbed into the hepatic portal vein,[13], postprandial glucose levels rise. In this experiment, methanolic extracts of the leaf demonstrated the highest activity in inhibiting amylase enzyme, it was observed to be 50% inhibition. Methanolic extract of bark has the second highest inhibitory activity of 33.3%, while aqueous extract of leaf and bark has less inhibition of amylase enzyme showing 16.6% and 10% (Figure 3).

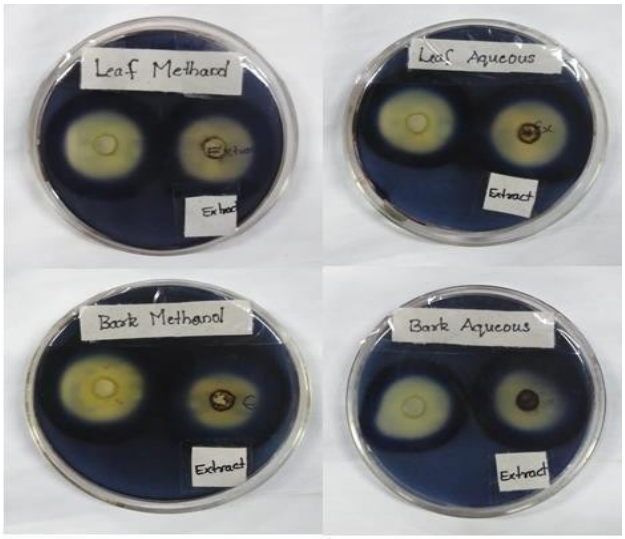

Figure 3: Inhibitory effect of leaf and bark extracts on amylase enzyme.
Methanolic extracts of the leaf demonstrated the highest activity in inhibiting amylase enzyme, it was observed to be 50% inhibition.

Starch Iodine Assay

In the present study four extracts of *C. odallam* were used. Each was tested for its ability to inhibit amylase enzyme by starch iodine method.[13] The results shows that leaf extracts show maximum inhibiting activity than bark extracts. Both leaf methanolic and aqueous extracts are showing 50.9% of inhibition of amylase enzyme at a concentration of 0.5 g/mL (Figure 4). It was also observed that the methanolic and aqueous extract of bark too showed inhibition of 45.3% at enzyme concentration of 0.5 g/mL. This helped us to understand that similar to the leaf extracts, the bark extracts also have inhibitory effects, paving way for its antidiabetic activity.

UV-vis Spectroscopy

The plant extracts were scanned in the wavelength ranging from 200-800 nm by using UV-vis spectroscopy and the characteristic peaks were detected. The various peaks represented the phytochemicals present in the extracts as shown in Figure 5a (Leaf methanolic), 5b (Bark methanolic), 5c (Leaf aqueous), 5d (Bark aqueous). This confirmed that the extracts are enriched with phytochemicals.

FTIR Analysis

FTIR analysis revealed the presence of various compounds in the leaf and bark extracts.[16] Figure 6a represents the peak assignments and peak values of the methanolic leaf extract followed by the different stretch and bending patterns corresponding to the respective compounds such as alcohols, amines, aromatic compounds, alkenes, carboxylic acids, esters etc., Figure 6b represents the peak assignments and peak values of the methanolic bark extract followed by the different stretch and bending patterns corresponding to the respective compounds such as alcohols, aliphatic primary amine, amine salt, alkenes, carbon dioxide, primary alcohol, etc., Figure 6c represents the peak assignments and peak values of the aqueous leaf extract followed by the different stretch and bending patterns corresponding to the respective compounds such as alcohols, sulfoxide, alkenes and halo compounds. Figure 6d represents the peak assignments and peak values of

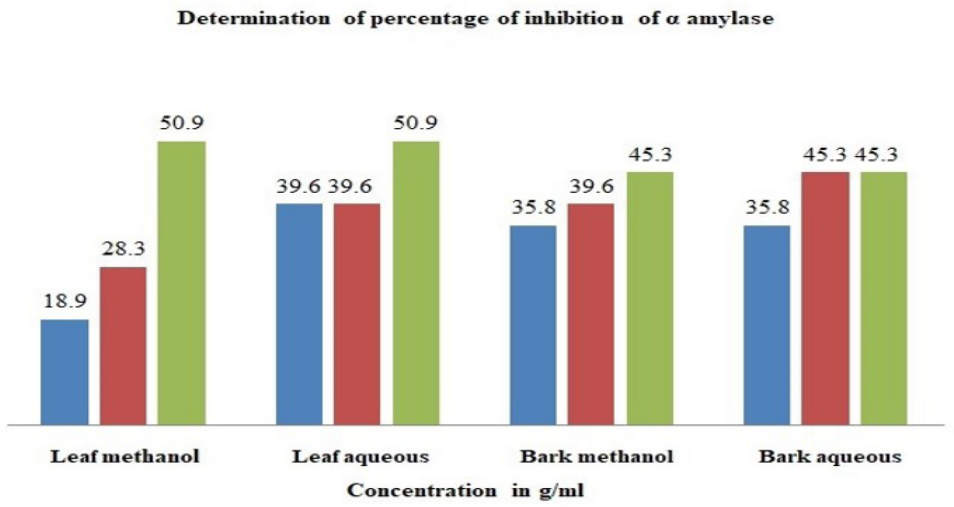

Figure 4: Percentage of inhibition of amylase by extracts of plant at different concentrations.
Both leaf methanolic and aqueous extracts are showing 50.9% of inhibition of amylase enzyme at a concentration of 0.5 g/mL.

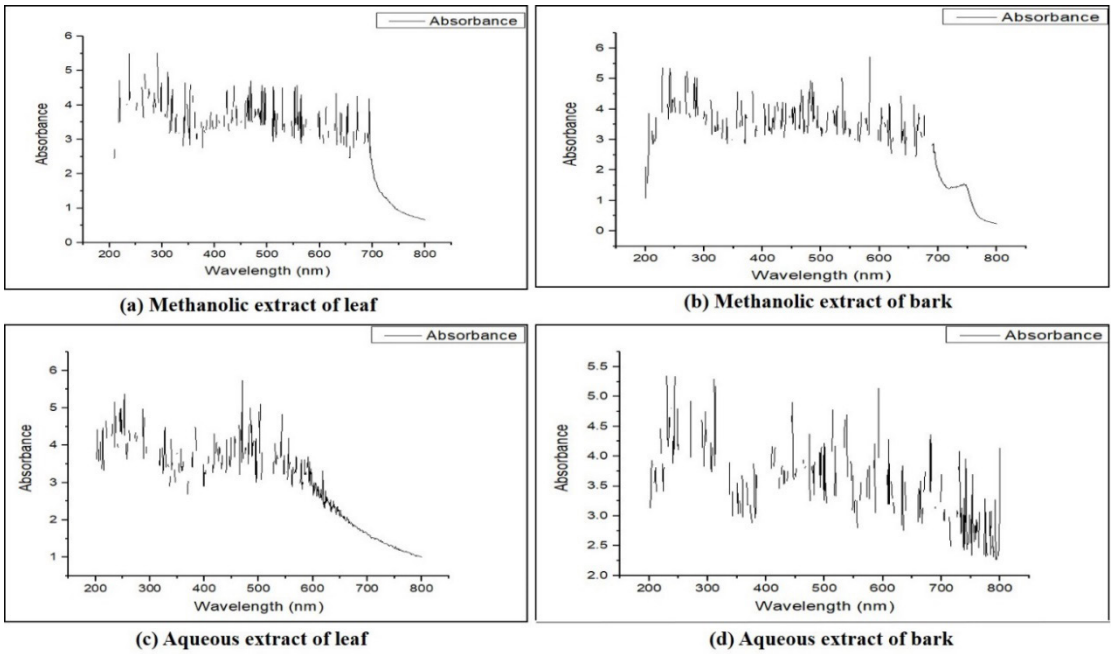

Figure 5 (a-d): UV-vis spectra of methanolic and aqueous extract of leaf and bark
UV-vis Spectroscopy of the methanolic and aqueous leaf and bark extract of *C. odallam.*

the aqueous bark extract followed by the different stretch and bending patterns corresponding to the respective compounds, such as alcohols, aromatic compounds, sulfoxide, alkenes and halo compounds. These results confirm that similar to the protective nature of leaf extracts, the bark extracts too, have the efficacy to be used as a potential therapeutic agent.

DISCUSSION

Plant extracts are gaining popularity as therapeutics because of the presence of secondary metabolites. In this study, phytochemical analysis of aqueous and methanol extracts of leaf and bark of *C. odallam* revealed the presence of alkaloids, flavonoids, tannins, carbohydrates, phenols, cardiac glycosides, terpenoids, quinones and saponins. Similar results were observed by many groups except for the presence of saponins in the present work.[12] However other species of Cerebra have reported the presence of saponins.[17-19]

Presence of tannins, alkaloid, phenol and terpenoids along with steroids have been reported to show antimicrobial properties.[9] For the antibacterial

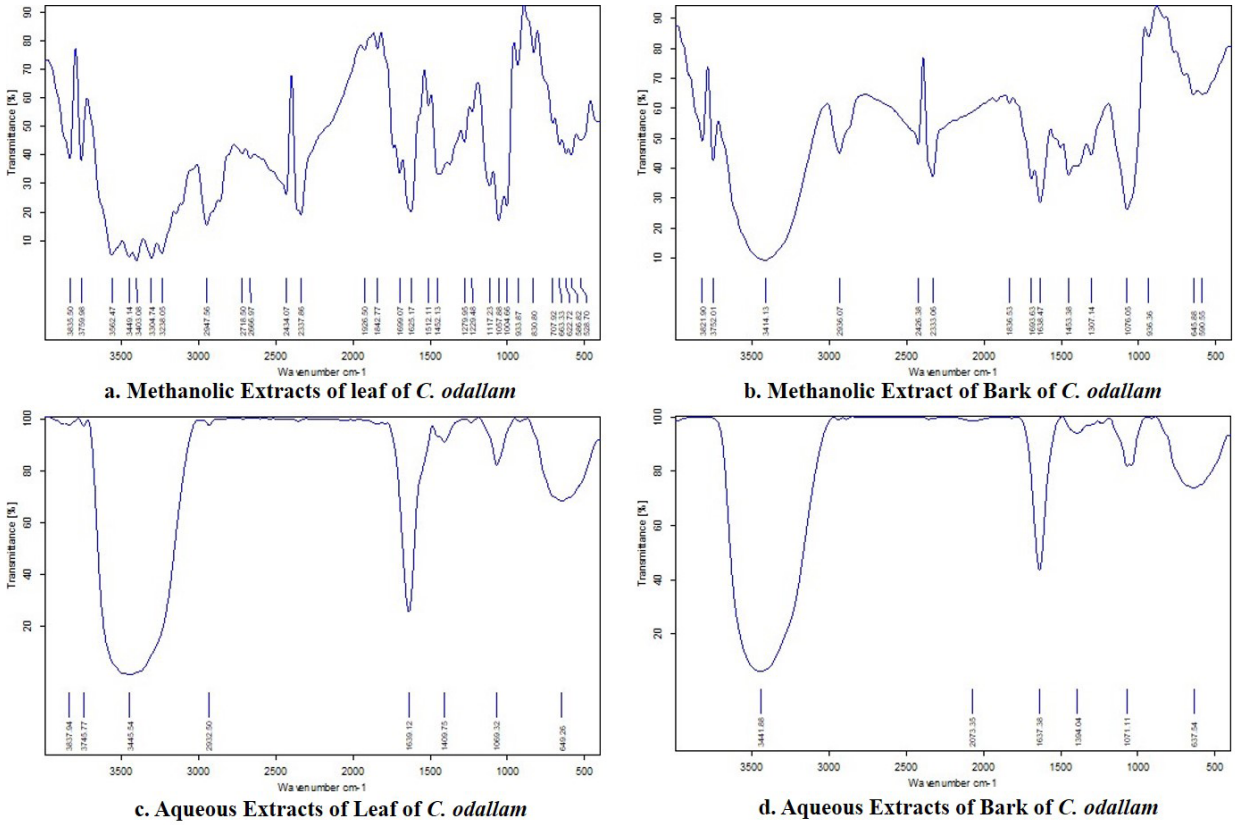

Figure 6 (a-d): FT-IR spectrum of aqueous and methanolic leaf and bark extracts of *C. odallam*.

The peak assignments and peak values of the methanolic and aqueous extracts of leaf and bark of *C. odallam* showing the different stretch and bending patterns corresponding to the respective compounds.

study reported against *E. coli, S. aureus,* and *B. subtilis*, maximum zone of inhibition was found for *E. coli* with methanol extract of the plant as compared to the aqueous extract. In comparison with the work by Sahoo and Marar (2018),[12] there was no zone of inhibition observed for the aqueous extract and for methanol leaf extract of *C. odollam* indicated the presence of low antibacterial activity which led them to mention that the plant extracts had no antibacterial property.[12] Still Bintaro (*C. odollam, C. manghas*) leaf ethyl acetate and dichloromethane extract showed antibacterial effect against gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria.[20] Butanol and hexane extract from Bintaro leaves showed strong antibacterial effect on *K. pneumonia*.[21] Thereby this work suggests that the plant leaf extract has antibacterial property.

Plants from the genus Cerbera also have potential antifungal properties.[22,23] Aqueous extract of *C. manghas* leaf has the effect of killing fungi like *Aspergillus* species and *Penicillium species*.[24] Ethanol extract of the leaves and the fruit of the plant *C. odollam* has shown to be effective against *Aspergillus niger*, *Fusarium oxysporum* and *Penicillium citrum*.[9,25] Methanol leaf extract of *C. odollam* shows the presence of antifungal effect higher than the preferred standard antifungal drug fluconazole, as the leaf extract possesses a fantastic ability to apply as an antifungal agent against fungus like *Saccharomyces cerevisiae* and *C. albicans*.[12] Antifungal action against *Aspergillus flavus* and *Fusarium oxysporum* was revealed with the methanolic leaf and bark extracts by zone of inhibition around 22 mm diameter against *Aspergillus flavus* in this work.[26]

Plant medicine is of great interest in the prevention and cure of diabetes by the inhibition of amylase.[27] Both the methanolic and aqueous extract showed amylase inhibition activity. Maximum α-amylase inhibition percentage was found to be with the methanol bark extracts and aqueous bark extract showed minimum activity. Literature reveals that the phenols, flavonoids are potential inhibitors of glucose absorption and thus, this study also implies on the same.[28] The findings of the work accentuate the necessity of conducting additional research to delve deeper into a broader spectrum of bioactivities present in *C. odallam* that have not yet been thoroughly examined. To fully explore the range of potential applications for these fascinating botanical

species, more research into their pharmacological qualities is necessary.

CONCLUSION

C. odallam is a well-known poisonous plant, but it is a species with wide range of phytochemical and pharmacological potentials. Previous researches have proved its antioxidants, anticancer, antimicrobial, insecticidal, termiticidal, and larvicidal properties. The current study emphasizes on the antidiabetic property of the methanolic extracts of leaf and bark. The results obtained from amylase inhibitory and starch iodine assays pave way in further proving the antidiabetic property of *C. odallam*. It also assures the remarkable therapeutic potential of *C. odallam*. Extended research is needed to produce a potentially effective antidiabetic medication based on plant-derived alpha-amylase inhibitors. Currently, researchers are interested in purified plant bioactive compounds. This work provides evidence that in addition to the recognized benefits of *C. odallam* leaf extracts, *C. odallam* bark extracts can also be employed for anti-diabetic activities.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

UV-vis: Ultra violet visual spectroscopy; **FTIR:** Fourier Transform Infrared Spectroscopy.

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