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Investigation of phytochemicals, antibacterial and antioxidant assays of *Cryptocarya amygdalina* Nees leaf extract

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Abstract

Cryptocarya amygdalina Nees, a medicinal tree of the Lauraceae family, is widely distributed across Peninsular Myanmar, Southern China, Bhutan, Thailand, Nepal, and several Indian states including Odisha, West Bengal, Arunachal Pradesh, Assam, Meghalaya, Sikkim, and Tripura. This plant is reported to possess several pharmacological properties like anti-inflammatory, cholinesterase inhibitory, anti-fungal, anti-oxidant, anti-plasmodial, anti-tuberculosis, insecticidal, and anti-cytotoxic. Hence, this study is undertaken to determine the phytochemical composition present in leaf extracts using various solvents (aqueous, methanol, acetone, chloroform, and n-hexane) and to evaluate the antibacterial activity and antioxidant properties of the methanolic leaf extract. Preliminary phytochemical analysis revealed the presence of several groups of bioactive compounds, such as alkaloids, saponins, phytosterols, phenolic compounds, glycosides, tannins, flavonoids, coumarins, steroids, quinones and terpenoids. The aqueous leaf extract showed the highest diversity of compounds. Antibacterial activity of the methanolic leaf extract was tested against four human pathogenic bacteria such as *Vibrio cholerae* and *Salmonella typhi* (Gram-negative), and *Streptococcus mutans* and *Streptococcus pyogenes* (Gram-positive). The methanolic leaf extracts demonstrated significant antibacterial activity compared to the standard antibiotic ciprofloxacin. Additionally, the methanolic leaf extract exhibited antioxidant potential in DPPH radical scavenging assays with an IC_{50} value of 64.97±0.37 µg/ml. These findings highlight the therapeutic potential of *C. amygdalina* leaves as a source of natural antibacterial and antioxidant agents, providing a foundation for further drug discovery and pharmacological exploration.

Keywords: *Cryptocarya amygdalina,* Lauraceae, phytochemicals, DPPH, antibacterial activity

Introduction

Plants have been considered as a reservoir of a wide range of bioactive phytochemicals that can be used as herbal medicines to cure a diverse range of human diseases. As per a report by the WHO (World Health Organization), around 80% of the world population is currently using plant-based therapies to treat primary health issues. All these are results of ethno-pharmacological advancements. Additionally, plant-based medicine has become an alternative due to its effectiveness, safety, and cost-effectiveness (Mwangi *et al,* 2024; Semenescu *et al,* 2024) [9, 16]. In addition to Primary metabolites, Plants also contain secondary metabolites such as flavonoids, alkaloids, phenolic compounds, steroids, glycosides and tannins. The presence of these compounds is a key factor in the therapeutic properties of plants (Diab *et al,* 2021) [4]. In the current world, the spread of infectious diseases and the development of drug resistance as well as the nature of their agent, give all possible reasons to explore and develop new antimicrobial agents. Plant derived compounds have the potential to minimize the damage caused by the generation of unbalanced free radicals (Mahmood *et al,* 2019) [7].

Cryptocarya is a genus comprising around 300 evergreen plant species belonging to the family Lauraceae, widely distributed across tropical and subtropical regions. This genus is well-known for its rich diversity of secondary metabolites, including alkaloids, α-pyrones, and flavonoids. In recent years, the phytochemical composition of *Cryptocarya* has gained significant attention due to its wide range of pharmacological activities, such as cytotoxic, anti-inflammatory, antimalarial, and anti-Trypanosoma effects (Manh and Son *et al,* 2023) [8]. These phytochemical compounds are found to be safer for many diseases. Around 177 new bioactive compounds with their chemical structures have been reported from the genus. These compounds are believed to be safer to treat many diseases caused by cellular mutation. (Sargazifar *et al,* 2025) [15].

Cryptocarya amygdalina Nees is an endangered medium-sized tree species of the Lauraceae family, distributed across Southern China, Bhutan, Myanmar, Thailand, Peninsular Malaysia, Indonesia, India and Bangladesh. In India, this species is found in Odisha, the Andaman Islands, and the northern regions. It is an ethnomedicinal plant having therapeutic potential, including spasmolytic, antioxidant, anticancer, and anti-inflammatory properties (Dey *et al,* 2020; Ray *et al,* 2021a) [3, 12]. The leaf essential oil of *C. amygdalina* is rich in different sesquiterpenes with β-caryophyllene as the major component (Ray *et al,* 2021b) [13].

This study aimed to examine the preliminary phytochemicals of *Cryptocarya amygdalina* by different solvent extracts and the methanolic extract was taken into consideration for both antimicrobial and antioxidant properties analysis.

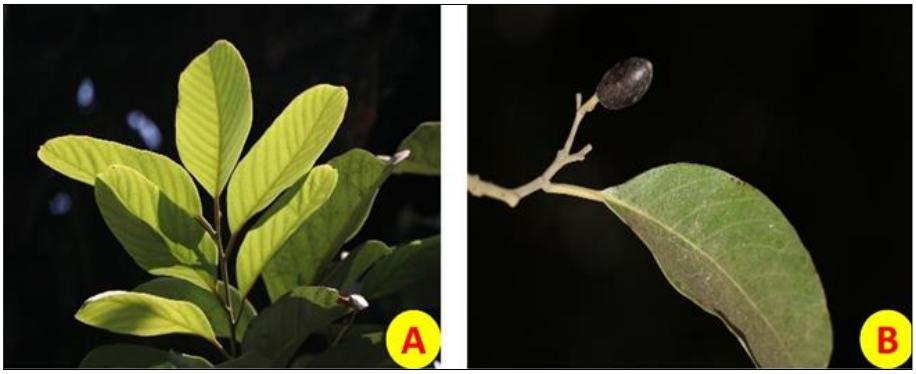

Fig 1: *Cryptocarya amygdalina* A - Leaf; B - Fruit

Materials and Methods

Preparation of Plant Extracts for Phytochemical Analysis

Healthy leaves of *Cryptocarya amygdalina* were collected, washed thoroughly under running tap water and shade-dried at room temperature. After complete drying, the leaves were ground thoroughly. Then, 5 g of leaf powder was added to 100 ml of different solvents including aqueous, acetone, methanol, chloroform, and n-hexane in separate conical flasks and kept for 24 hours at room temperature. Then the plant extracts were filtered using Whatman No.1 filter paper. The extracts were used for further screening.

The preliminary phytochemical analysis of *C. amygdalina* leaf extracts was done as per the documented methodologies (Yadav and Agarwala, 2011; Shaikh and Patil, 2020) [18, 20].

Preparation of Methanolic Extract for Antimicrobial Assay

The methanolic leaf extract was prepared by taking 10g of leaf powder in 200ml of methanol in a Soxhlet apparatus; then the obtained extract was lyophilized to obtain the dried form. Different concentrations (10, 20, 40 and 80 mg/ml) of the methanolic extract were made by dissolving the leaf extract in DMSO (Dimethyl sulfoxide).

For antibacterial activity analysis, the agar well diffusion method was used. Bacterial strains were spread evenly over the agar plate and wells were made in the agar plates with a diameter of 6 mm each. Into each well 60µl of the samples was pipetted and 60µl of DMSO was pipetted into a well in the centre as a negative control. Ciprofloxacin was taken as a positive control. All plates were incubated at 37°C for a period of 16 hours. Zones of inhibition were measured against both gram-negative and gram-positive bacteria, such as *Vibrio cholera* and *Salmonella typhi*, *Streptococcus pyogenes* and *Streptococcus mutans*.

Antioxidant Assay

For the antioxidant assay of the methanolic leaf extract, the DPPH scavenging method was used**.** Different concentrations of the methanolic leaf extract were taken; the volumes were made up to 1ml. The solution was mixed with 1ml of methanolic DPPH solution. Absorbance was taken at 517nm after 30 min of incubation in the dark at room temperature. The Ascorbic Acid was taken as a standard. The percentage of DPPH Inhibition was calculated by using the following formula:

% DPPH inhibition = {(Blank Absorbance – Sample Absorbance)/Blank Absorbance} × 100

IC_{50} values (Half maximum inhibitory concentration values) of the methanolic leaf extract and also the standard ascorbic acid was calculated by using a line of regression (Rahman *et al,* 2023) [11].

Results and Discussion

Preliminary phytochemical screening of *Cryptocarya amygdalina* leaves revealed the presence of several classes of secondary metabolites, including alkaloids, saponins, phytosterols, phenolic compounds, glycosides, tannins, flavonoids, coumarins, steroids, quinones and terpenoids by treating with different solvents like aqueous, acetone, methanol, chloroform, and n-Hexane. The aqueous leaf extract showed the presence of the highest number of compounds such as saponins, glycosides, flavonoids, coumarins, steroids and terpenoids (Table 1).

According to previous studies, the ethanolic stem extract of *C. chingii* contains compounds such as cryptogiones, which are flavonoids with having anti-inflammatory properties (Feng *et al,* 2012) [5]. The roots and aerial parts of *C. alba* and the bark of *C. nigra, C. chinensis* contain several types of alkaloids (Nasrullah *et al,* 2013; Castro-Saavedra *et al,* 2016; Manh and Son *et al,* 2023) [1, 8, 10]. Seedlings of *C. alba* were found to have phenolic compounds in response to allelochemical stress (Rodríguez-Cerda *et al,* 2023) [14]. Sesquiterpenes are found in the leaf essential oil of *C. amygdalina* which are a class of terpenes (Ray *et al,* 2021b) [13]. Many compounds belonging to coumarins are found in the leaves of *C. obovata* and the bark of *C. amygdalina* and *C. bracteolate. C. chinensis* leaves were found to have α-Tocopheryl quinone (Manh and Son *et al,* 2023) [8].

Table 1: Preliminary phytochemical analysis of leaf extracts of *C. amygdalina*

Phytochemicals	Solvents				
	Aqueous	Acetone	Methanol	Chloroform	n-Hexane
Alkaloids	-	-	-	-	+
Saponins	+	-	+	-	-
Phytosterols	-	-	-	-	+
Phenolic compounds	-	+	+	-	-
Glycosides	+	+	-	-	-

Tannins	-	+	+	-	-
Flavonoids	+	-	+	-	-
Coumarins	+	-	-	-	+
Steroids	+	+	-	-	-
Quinones	-	-	+	+	+
Terpenoids	+	+	-	-	-

The antibacterial evaluation of methanolic leaf extracts of *Cryptocarya amygdalina* demonstrated notable inhibitory effects against selected pathogenic bacteria. The methanolic leaf extract of *C. amygdalina* showed inhibitory activity against all four tested bacterial strains (*Vibrio cholera, Salmonella typhi, Streptococcus pyogenes* and *Streptococcus mutans*) in a concentration-dependent manner (Table 2; Figure 2). *V. cholerae* exhibited the highest zone of inhibition (17 mm), followed by *S. pyogenes* (16 mm), *S. mutans* (12 mm) and *S. typhi* (12 mm) at a concentration of 80mg/ml. At 10 mg/ml, the methanolic extract showed minimal or no zone of inhibition (ZOI ≤ 7 mm).

The leaf oil of *C. impressa* and *C. infectoria* has demonstrated antimicrobial activity against *Salmonella enterica* (Manh and Son, 2023) [8]. Phytoconstituents such as flavonoids, alkaloids, coumarins, terpenoids and pyrones present in the genus *Cryptocarya* have demonstrated antimicrobial activities (Sargazifar *et al,* 2025) [15]. Das *et al*. (2025) [2] have suggested that an inhibition zone below 7 mm indicates minimal or no significant antibacterial activity of a sample.

Table 2: Antibacterial Activities of *C. amygdalina* Methanolic Leaf Extract

Bacterial strains	**Zone of inhibition (mm) in different concentrations of the methanolic leaf extract of *C. amygdalina***			
	10 mg/ml	**20 mg/ml**	**40 mg/ml**	**80 mg/ml**
Vibrio cholera	11	13	13	17
Salmonella typhi	6	10	11	12
Streptococcus pyogenes	11	14	14	16
Streptococcus mutans	10	11	11	12

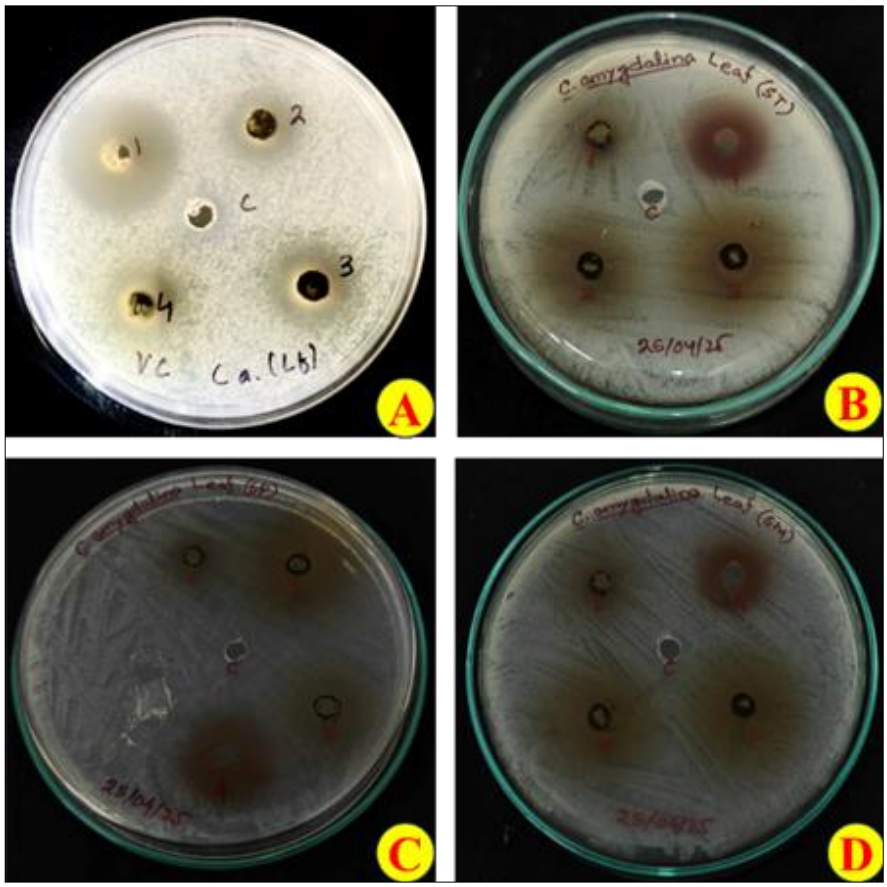

Fig 2: Antibacterial activity of *C. amygdalina* methanolic leaf extracts against *V. cholerae* (A); *S. typhi* (B); *S. pyogenes* (C) and *S. mutans* (D)

The antioxidant potential of *C. amygdalina* methanolic leaf extract was evaluated using the DPPH radical scavenging assay at different concentrations (5, 10, 20, 40 and 80 µg/ml) (Figure 3). The extract showed a concentration-dependent increase in DPPH inhibition. Ascorbic acid was used as the standard, exhibiting consistently higher inhibition values across all tested concentrations. The half maximum inhibitory concentration values of the standard ascorbic acid and the methanolic leaf extracts are found to be 6.17±0.20 µg/ml and 64.97±0.37 µg/ml, respectively.

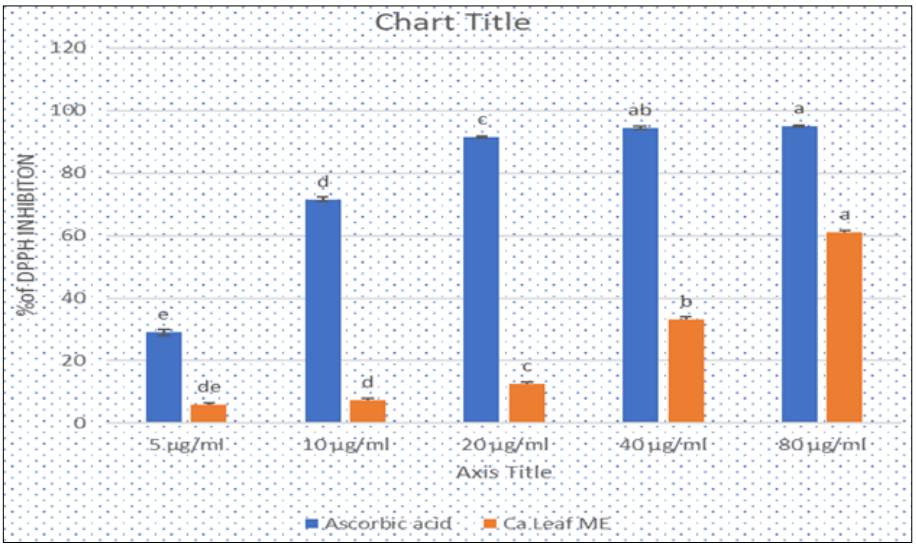

Fig 3: Percentage of DPPH inhibition of methanolic extract, compared with ascorbic acid. The data are represented as mean value ± SE from three independent replications. Mean values shown by different lowercase letters are significantly different at $p < 0.05$ (DMRT)

Table 3: The DPPH free radical scavenging activity by *C. amygdalina* methanolic leaf extract

Standard/Extract	DPPH IC_{50} (µg/ml)
Ascorbic acid	6.17±0.20
Methanol leaf extract	64.97±0.37

In a previous study, it was found that the bark essential oil of *C. amygdalina* exhibited better reducing power abilities as compared to the standard ascorbic acid against DPPH free radicals (Ray *et al,* 2021a) [12]. Antioxidant properties were also seen in the leaves of *C. latifolia* (Hamza *et al,* 2016) [6], bark of *C. nigra* (Nasrullah *et al,* 2013) [10], *C. alba* essential oil and fruits (Simirgiotis *et al,* 2013; Touma *et al,* 2020) [17, 19]. *C. amygdalina* leaf essential oil possesses strong antioxidant activity with an IC_{50} value of 6.97±0.24 µg/ml. The lower IC_{50} value indicates higher antioxidant activity (Ray *et al,* 2021b) [13].

Conclusion

The present study demonstrated that *Cryptocarya amygdalina* leaves are a rich source of bioactive secondary metabolites, including alkaloids, saponins, phytosterols, phenolic compounds, glycosides, tannins, flavonoids, coumarins, steroids, quinones and terpenoids. The phytochemical diversity observed across different solvent extracts highlights the solvent-dependent efficiency of metabolite extraction, with aqueous, methanolic, and acetone extracts showing the highest variability. The methanolic leaf extract exhibited significant antibacterial activity against all tested pathogens, with *Vibrio cholerae* and *Streptococcus pyogenes* showing the highest susceptibility, while *Salmonella typhi* was least affected. In addition, the methanolic leaf extract exhibited antioxidant activity against DPPH free radicals with an IC_{50} value of 64.97±0.37 µg/ml.

Collectively, these findings confirm that *C. amygdalina* possesses considerable antimicrobial and antioxidant potential, attributable to its rich phytochemical profile. The results provide scientific support for its traditional medicinal uses and suggest that *C. amygdalina* leaves may serve as a promising source of natural therapeutic agents. Further studies could be done by focusing on compound isolation, structural characterization, and *in vivo* evaluations.

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