

Research Article

Phytochemical composition and biological activities of *Crotalaria hebecarpa* (DC.) Rudd.

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Abstract

In this study, *Crotalaria hebecarpa* (Fabaceae), an ethnomedicinal plant commonly used to treat skin infections, fever and digestive disorders by the ethnic communities was selected to study the phytochemical composition and biological activities. Ethanolic-whole plant extract was used to study the preliminary phytochemical compounds followed by GC-MS analysis. Preliminary phytochemical analysis revealed the presence of alkaloids and phenols while the GC-MS analysis revealed the presence of 17 different phytochemicals, among which dodecanoic acid, trifluoro acetic acid and cyclopentene were significant compounds with various biological activities. The IC₅₀ values obtained for *C. hebecarpa* extract for H₂O₂, DPPH and ABTS antioxidant activities were 788.24 ppm, 825.05 ppm and 1087.68 ppm, respectively. The IC₅₀ inhibition concentration of *C. hebecarpa* extract was still lower, 335.4 ppm for alpha-amylase activity and 541.54 ppm for alpha-glucosidase activity. Furthermore, the mosquito larvicidal and pupicidal efficacy of *C. hebecarpa* extract comprehend the effectiveness of the plant in vector control. Overall, the study implies that the ethnomedicinal plant could be useful for biomedical applications with further scientific investigations.

Keywords: Antidiabetic activity, Antioxidants, Ethnomedicinal plants, Mosquito larvicidal activity, Phytochemicals, Plant extract

Introduction

The benefits of herbal medicine for chronic illnesses are that it offers safe, effective, and well-tolerated treatments (Kaur *et al.*, 2020). Herbal medicine should be used wisely. There is no risk associated with the use of the most commonly used herbs. There are, however, some plants that can cause side effects, and herbal remedies should be treated as medicines (Ganesan, 2015). A well-trained practitioner should be consulted before taking or using certain plants. The health benefits of plants include increasing stamina, supporting the nervous system, providing vitamins, and maintaining good health (Moise *et al.*, 2024). Traditional healers employ a variety of methods for treating ailments, including hot and cold infusions, powders that are applied to incisions, poultices, lotions, ointments, vapour baths, emetics, and enemas (Sivasankari *et al.*, 2013). Healthcare has always been shaped heavily by indigenous medical systems. The knowledge base of indigenous peoples now depends on using household medicinal plants (Alum, 2024).

Indigenous knowledge regarding the curative properties of household medicinal plants in the surveyed area plays a vital function in the primary healthcare systems (Mahwasane *et al.*, 2013). Besides the fact that home remedies are easily accessible, they are also easy to prepare and in addition to that, they can be used at home. Further, in addition to the ethnic knowledge from the family and spiritual health care practice, it is also related to indigenous knowledge, traditional health care practices, and household medicinally important plants (Pandey *et al.*, 2013; Batool *et al.*, 2023).

The Indian healthcare system relies heavily on medicinal plants. The World Health Organization (WHO) estimates that 80% of rural populations in developing countries use medicinal plants as their primary healthcare resources (Yogeshwari & Kumadha, 2017). Forest habitats support 90% of the country's medicinal plants. Medicinal plants are only found in and around freshwater bodies and in open grasslands and agricultural pastures, which constitute only 10% of the landscape (Sundararajan & Vasudevan, 2018).

Many centuries of research have been devoted to accumulating knowledge about medicinal plants, mainly based on Ayurveda, Unani, and Siddha systems of medicine (Muthu *et al.*, 2006). The ethnic community of the study area relies on indigenous knowledge about herbal remedies to prevent and treat a variety of maladies in humans and livestock (Jayakumar *et al.*, 2018; Mekonnen *et al.*, 2022). Global dissemination of indigenous medicinal plants knowledge could be achieved through clinical studies that prove the validity of the recorded treatments (Davis & Choisy, 2024). Phytochemical analysis, various biological and pharmacological studies on medicinal plants and their derivatives are comprehensive ways to find out the chemical composition, antioxidant activities, antidiabetic efficiency, toxicological studies such as mosquito larvicidal and pupicidal efficacy are some of the examples reported (Hasan *et al.*, 2024; Wadaan *et al.*, 2024; Gokul *et al.*, 2025).

The ethnobotanical exploration conducted during 2022-2025 revealed that there were more than 100 plants and their parts have been used for the ailments of several

illnesses in Salem district, Tamil Nadu, India. Based on the ethnobotanical explorations and literature survey conducted on the ethnobotanicals and their applications in various biomedical and pharmacological applications revealed that there exists a lacuna in the phytochemicals and pharmacological activities of *Crotalaria* species of the family Fabaceae. According to the findings of ethnomedicinal survey, *Crotalaria hebecarpa* has been utilized for treating fever, skin infections and digestive disorders. Recent literature undoubtedly reported that the chemical composition and pharmacological properties of *C. hebecarpa* is least studied. Therefore, in the present study, the phytochemical composition of *C. hebecarpa* extract and its biological activities such as antioxidant potential (DPPH, H₂O₂ and ABTS), antidiabetic activity (alpha-amylase and alpha-glucosidase) along with mosquito larvicidal and pupicidal activities (*Aedes aegypti* and *Culex quinquefasciatus*) were investigated using standard methods to comprehend the plant's utility in traditional medicine.

Material and Methods

The ethnomedicinal plant, *Crotalaria hebecarpa* (DC.) Rudd. (Fabaceae) (Figure 1) was collected from foothills of Kanjamalai, Salem district, Tamil Nadu, India (11°37' 13.47" N and 78°2' 8.755" E) and identified with the help of local floras and confirmed with World Flora Online (WFO) and Plants of the World Online (POWO). The whole plant was collected, air dried for a week and coarsely powdered. The plant powder was subjected to ethanolic extraction using Soxhlet apparatus. The extract of *C. hebecarpa* was subjected to preliminary phytochemical analyses, alkaloids, flavonoids, carbohydrates, proteins, phenols, phytosterols and saponins using Harborne's methods (Harborne, 1998).

The extract of *C. hebecarpa* was subjected to GC-MS analysis and a Clarus 680 GC system was employed, which featured a fused silica capillary column with Elite-5MS stationary phase (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250 µm df). Constituent separation was performed by utilizing Helium as the carrier gas, maintaining a steady flow of 1 mL/min with the injection port at a temperature of 260 °C. One µL of the extract of each plant species was added to the instrument. GC-MS NIST (2008) database was used to compare the mass spectra of the separated compounds with those of known substances.



Figure 1: a) Habit with flowers and b) a fruiting twig of *Crotalaria hebecarpa* (DC.) Rudd

In vitro antioxidant activity of *C. hebecarpa* extract

The antioxidant potential of ethanolic extracts of *C. hebecarpa* was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method. This assay is based on the decrease in absorbance at 517 nm, which occurs when DPPH radicals are neutralized by antioxidants in the sample (Brand-Williams *et al.*, 1995). The ability of the ethanolic extract of *C. hebecarpa* to scavenge hydrogen peroxide (H₂O₂) was assessed following the protocol outlined by Keshari *et al.* (2016). The final absorbance was then measured at 230 nm using a UV-Vis spectrophotometer. ABTS Radical Scavenging Assay: The ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] assay was performed according to the standard method (Re *et al.*, 1999), with slight modifications. The antioxidant activity was expressed as the percentage inhibition. Various extract concentrations (62.5, 125, 250, 500 and 1000 ppm) were tested. All measurements were performed in triplicate for each sample and concentration.

In vitro antidiabetic activity of *C. hebecarpa* extract

Alpha-amylase and alpha-glucosidase inhibition assays were carried out with the plant extract. In this method, a 0.2% alpha-amylase solution was pre-incubated with 1.5 mL of either the extract sample or a standard inhibitor in 0.2 M phosphate buffer (pH 6.9) at 25 °C for 10 mins. This step facilitated interaction between the enzyme and the test material. The reaction mixtures were read for absorbance at 540 nm (Min *et al.*, 2023). For alpha-glucosidase (maltase) inhibition assay following a standardized protocol (Dahlqvist, 1964). The antidiabetic activity was expressed as the percentage inhibition of enzyme activities and the IC₅₀ value was also calculated. Acarbose was used as control. Various extract concentrations (62.5, 125, 250, 500 and 1000 ppm) of *C. hebecarpa* were tested. All measurements were performed in triplicate for each sample and concentration.

Mosquito larvicidal and pupicidal efficacy of *C. hebecarpa* extract

Fourth instar larvae and pupae of *Aedes aegypti*, and *Culex quinquefasciatus* were collected and carefully transferred to plastic trays containing dechlorinated water and maintained under controlled laboratory conditions at room temperature (25±2 °C) and fed with a mixture of dog biscuit and yeast powder in a 3:1 ratio. The larvicidal and pupicidal potential of the extract was evaluated using a concentration-response bioassay following the World Health Organization protocol (Sharma *et al.*, 1998; WHO, 2005), with minor modifications. For the assay, 20 healthy fourth instar larvae of each mosquito species were placed in a 250 mL, containing 200 mL of double-distilled water. Various extract concentrations (62.5, 125, 250, 500 and 1000 ppm) of *C. hebecarpa* were tested. All measurements were performed in triplicate for each sample and concentration. A control group, containing only double-distilled water without extract was maintained simultaneously to account for natural larval and pupae mortality.

The treated larvae and pupae were observed for mortality at time intervals of 12 h and 24 h. Various extract concentrations (62.5, 125, 250, 500 and 1000 ppm) of *C. hebecarpa* were tested. All measurements were performed in triplicate for each sample and concentration. Larval mortality data were corrected for any mortality in the control group using Abbott's formula (Abbott, 1925). Lethal concentration values (LC₅₀ and LC₉₀) were determined at 12 h and 24 h using Probit analysis (Finney, 1971), performed with SPSS software.

Results and discussion

Preliminary phytochemical and GC-MS analysis of *C. hebecarpa* extract

The preliminary phytochemical analysis revealed that the whole plant extract of *C. hebecarpa* showed positive for alkaloids, proteins, carbohydrates and phenols, and negative for flavonoids, phytosterols and saponins. The GC-MS analysis of the ethanolic extract of whole plant showed 17 different compounds with the molecular weight ranging from 71-282 (Figure 2 & Table 1). The phytochemicals include: Propanenitrile, 2-hydroxy; 1h-imidazole, 4,5-dihydro-2-methyl; hex-4-enylamine; [2-(n,n-dimethyl)]-1,2-propanediamine; 2-norbornanone; 2-cyclopentyl ethanol; 1,3-bis(hydroxymethyl)urea; bicyclo[4.1.0]heptan-2-one, 1-methyl; 4-morpholineaceto-

nitrile; cyclopentene, 3-pentylchl; 4-penten-1-ol, 3-methyl-, acetate; 1-undecyne; 2-propenoic acid, cyclohexyl ester; cis-3-hexenylpyruvate; trifluoroacetic acid, cyclohexyl ester; carbonochloridic acid, 1,6-hexanediy ester; dodecanoic acid, 5-hexen-1-yl ester; Among the phytochemicals, dodecanoic acid, trifluoro acetic acid and cyclopentene are significant compounds with various biological activities. While examining the GC-MS chromatogram, more than 20 phytocompounds have been reported for *Crotalaria medicaginea* extract (Kusar *et al.*, 2024). The study also revealed the antioxidant and anti-inflammatory activity which could have been attributed to the dichloromethane, acetone and methanol fractions.

Antioxidant activities of *C. hebecarpa* extract

In the present study, the antioxidant activities, DPPH, H₂O₂ and ABTS were studied employing rutin as a routine standard and the results are shown in Figure 3. The ethanolic extract of *C. hebecarpa* showed 788.24 ppm of IC₅₀ value as the lowest percentage inhibition in H₂O₂ scavenging activity followed by DPPH (825.05 ppm) and ABTS (1087.68 ppm). Ethanolic extract of *C. hebecarpa* whole plant extract showed 21.70, 32.03, 38.23, 43.40 and 52.70% of inhibition with DPPH activity in 62.5, 125, 250, 500 and 1000 ppm concentration, respectively. Similarly, H₂O₂ and ABTS antioxidant studies also exhibited concentration oriented effect. Several studies concerned with antioxidant activities

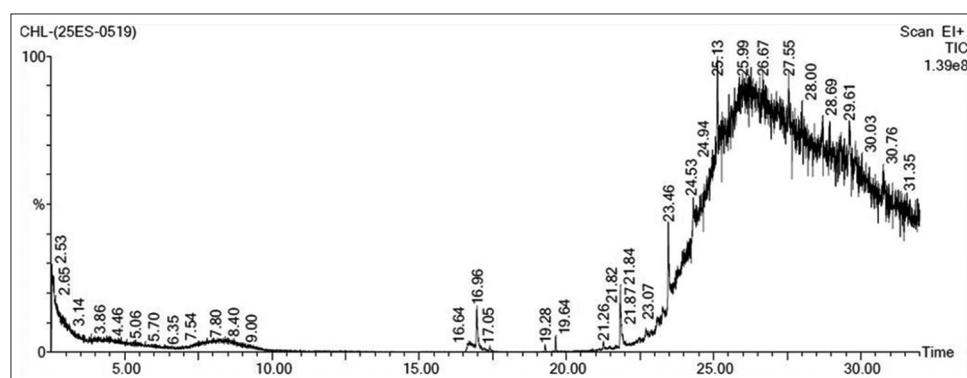


Figure 2: GC-MS chromatogram of ethanolic whole plant extract of *C. hebecarpa*

Table 1: Chemical compounds identified in the ethanolic whole plant extract of *C. hebecarpa* using GC-MS analysis

Compound name	Mol. wt.	Formula	CAS no.
Propanenitrile, 2-hydroxy	71	C ₃ H ₅ ON	78-97-7
1h-imidazole, 4,5-dihydro-2-methyl	84	C ₄ H ₈ N ₂	534-26-9
Hex-4-enylamine	99	C ₆ H ₁₃ N	55108-01-5
[2-(n, n-dimethyl)]-1,2-propanediamine	102	C ₅ H ₁₄ N ₂	900133-38-7
2-norbornanone	110	C ₇ H ₁₀ O	497-38-1
2-cyclopentylethanol	114	C ₇ H ₁₄ O	766-00-7
1,3-bis (hydroxymethyl) urea	120	C ₃ H ₈ O ₃ N ₂	140-95-4
Bicyclo[4.1.0]heptan-2-one, 1-methyl	124	C ₈ H ₁₂ O	14845-40-0
4-morpholineacetonitrile	126	C ₆ H ₁₀ ON ₂	5807-02-3
Cyclopentene, 3-pentylchl	138	C ₁₀ H ₁₈	37689-14-8
4-penten-1-ol, 3-methyl-, acetate	142	C ₈ H ₁₄ O ₂	71487-16-6
1-undecyne	152	C ₁₁ H ₂₀	2243-98-3
2-propenoic acid, cyclohexyl ester	154	C ₉ H ₁₄ O ₂	3066-71-5
Cis-3-hexenylpyruvate	170	C ₉ H ₁₄ O ₃	68133-76-6
Trifluoroacetic acid, cyclohexyl ester	196	C ₈ H ₁₁ O ₃ F ₃	1549-45-7
Carbonochloridic acid, 1,6-hexanediy ester	242	C ₈ H ₁₂ O ₄ Cl ₂	2916-20-3
Dodecanoic acid, 5-hexen-1-yl ester	282	C ₁₈ H ₃₄ O ₂	900160-10-7

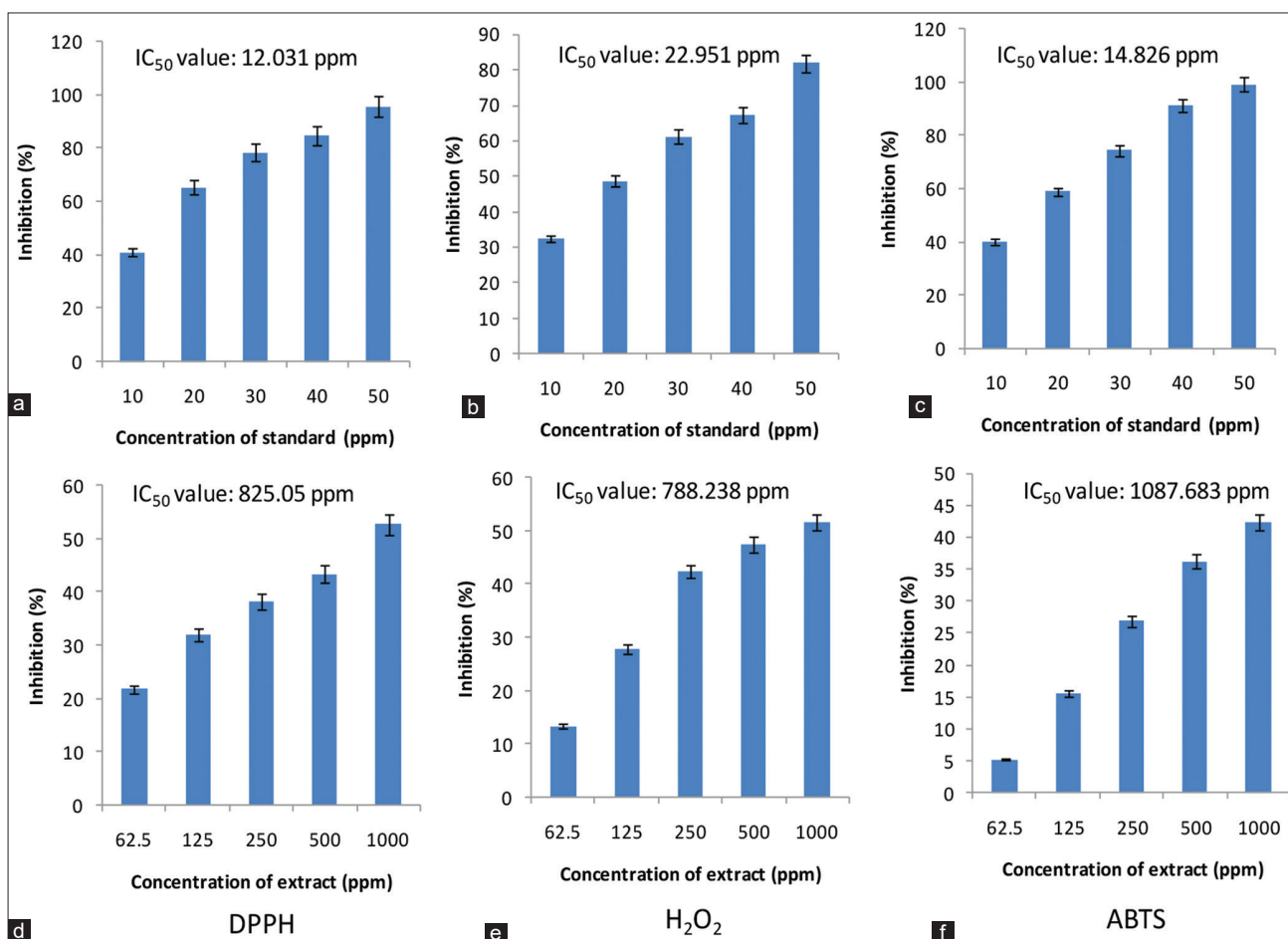


Figure 3: (a-f) Antioxidant activity of *C. hebecarpa* whole plant ethanolic extract. Standard drug - Rutin. Values are mean±standard deviation

of ethnomedicinal plants revealed wide range of IC₅₀ values ranging from <50 ppm to >1000 ppm. In the present study the IC₅₀ values of DPPH, H₂O₂ and ABTS ranged between 788.24 ppm and 1087.68 ppm. Even much higher IC₅₀ values have been reported in ginger and garlic varieties (Akullo *et al.*, 2023). This shows that the present results on the antioxidant activities of *C. hebecarpa* extract is considerable and further studies may highlight the plant's indigenous utility.

Antidiabetic activity of *C. hebecarpa* extract

Alpha-amylase and alpha glucosidase are the two important activities to study the antidiabetic activities of drugs, extracts and medicinal plants (Febriyanti *et al.*, 2025). In this study, ethanolic extract of *C. hebecarpa* was used to study alpha-amylase and alpha glucosidase inhibition as a measure of antidiabetic activity. Compared with the antioxidant activities of *C. hebecarpa* extract, antidiabetic activity was considerable. The alpha amylase inhibition by *C. hebecarpa* ranged from 21.38-88.71% in the extract concentration range of 62.5 ppm to 1000 ppm which was concentration dependent; while the alpha-glucosidase inhibition range was found between 10.62% and 73.45% with an IC₅₀ value of 541.44 ppm (Figure 4). By inhibiting the alpha-glucosidase and alpha-amylase enzymes, ethnomedicinal plants can help manage postprandial hyperglycemia (Febriyanti *et al.*, 2025). In the present study, both the inhibition assays crossed 50% inhibition limit. Earlier studies reported that *Swietenia*

mahagoni (IC₅₀=214 ppm) and *Momordica charantia* (274 ppm) reached 50% alpha-amylase inhibitory activity while *Physalis angulata* showed an IC₅₀ value of 438 µg/mL for alpha-glucosidase activity (Febriyanti *et al.*, 2025). This falls in line with the present study findings, signifying the efficiency of *C. hebecarpa* and its traditional use.

Mosquito larvicidal and pupicidal activity of *C. hebecarpa* extract

The fourth instar larvae and early pupa stage of the mosquitoes, *A. aegypti* and *C. quinquefasciatus* were tested with different concentrations (T1=0 ppm; T2=62.5 ppm; T3=125 ppm; T4=250 ppm; T5=500 ppm and T6=1000 ppm) of *C. hebecarpa* for 12 h and 24 h toxicity studies. The bioassay studies revealed concentration dependent and duration of exposure based effects on the tested larvae and pupae (Figure 5 & Table 2). A maximum of 90% and 100% mortality of fourth instar larvae of *A. aegypti* was observed in 1000 ppm concentration of the *C. hebecarpa* extract at 12 h and 24 h exposure, respectively; while the pupal mortality was lesser than that the larvae (70% in 12 h; 96.6% in 24 h). However, similar and closer mortality range was recorded for both the larvae and pupae of *C. quinquefasciatus* exposed *C. hebecarpa* extract for 12 h and 24 h exposure. The LC₅₀ concentration of 145.12 ppm was recorded against iv instar larvae of *A. aegypti* at 24 h exposure with a highly significant Chi-square value (0.01% level) followed by the LC₅₀ concentration of 198.15 ppm against *C. quinquefasciatus*

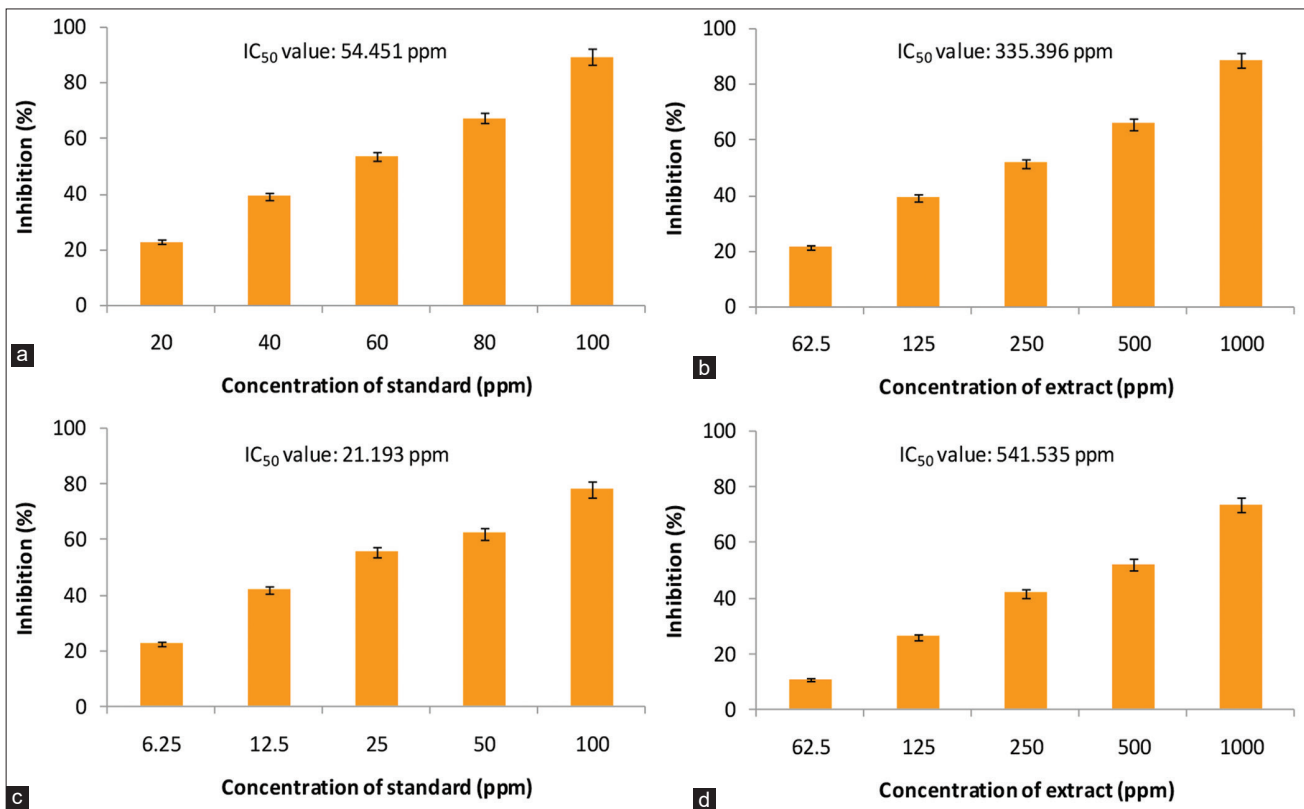


Figure 4: (a-d) Antidiabetic activity of *C. hebecarpa* whole plant ethanolic extract. Standard drug used - acarbose. Values are mean±standard deviation

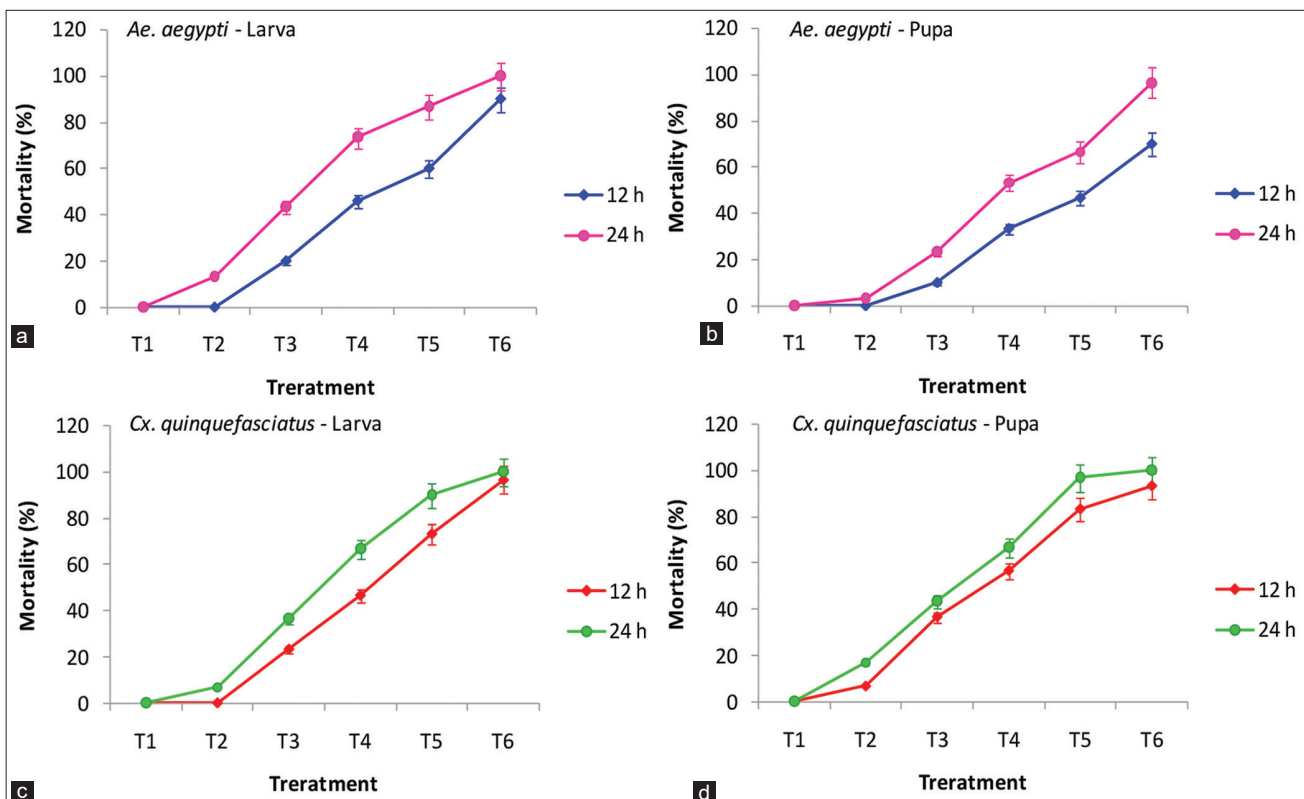


Figure 5: (a-d) Mosquito larvicidal and pupicidal activities of *C. hebecarpa* whole plant ethanolic extract. Values are mean±standard deviation. Treatments: T1=0 ppm; T2=62.5 ppm; T3=125 ppm; T4=250 ppm; T5=500 ppm; T6=1000 ppm

(24 h). With respect to the larvae of both the mosquito species exposed, 24 h exposure had higher effect (Table 2). The lowest and significant IC₅₀ value of 131.68 ppm was observed against the pupae of *C. quinquefasciatus* upon 24 h exposure.

A variety of phytochemicals (secondary metabolites) found in ethnomedical plants possess significant larvicidal and pupicidal effects against mosquitoes. Compounds such as alkaloids, terpenoids, essential oils, and phenolic acids

Table 2: Mosquito (*A. aegypti* and *C. quinquefasciatus*) larvicidal and pupicidal activities of whole plant extract of *C. hebecarpa*. χ^2 - Chi-square value. Significance levels: 0.001, 0.01 and 0.05

Exposure	LC ₅₀ (ppm)	Fiducial limit (LC ₅₀)		LC ₉₀ (ppm)	Fiducial limit (LC ₉₀)		χ^2 -value	Significance
		Lower	Upper		Lower	Upper		
<i>Aedes aegypti</i>								
IV instar larvae								
12 h	376.45	365.00	398.04	993.18	876.51	1010.23	52.03	0.001
24 h	145.12	130.96	166.70	545.83	556.04	631.35	91.65	0.001
Pupae								
12 h	564.55	530.00	612.18	1135.82	1124.73	1189.12	58.34	0.001
24 h	241.78	235.96	270.86	814.90	778.07	891.04	31.12	0.01
<i>Culex quinquefasciatus</i>								
IV instar larvae								
12 h	268.18	245.63	280.16	952.76	944.20	969.32	44.06	0.001
24 h	198.15	185.08	223.34	494.33	481.68	515.89	22.71	0.05
Pupae								
12 h	176.01	168.49	192.77	923.34	904.60	943.33	63.50	0.01
24 h	131.68	117.01	146.59	423.06	415.13	447.20	49.16	0.001

act as natural toxins, killing or disrupting mosquito larvae and pupae (Rawani *et al.*, 2014). The alkaloids and phenols present in *C. hebecarpa* and the phytochemicals might have been attributed to the toxic effect of the extract against the larvae and pupae of *A. aegypti* and *C. quinquefasciatus*. The LC₅₀ values of 158.93 ppm, 169.18 ppm and 203.48 ppm respectively for the methanol, chloroform and n-hexane extracts of *Peltophorum pterocarpum* leaves extract against *C. quinquefasciatus* larvae while it was 204.041 ppm, 239.50 ppm and 284.04 ppm against the pupae (Yagoo *et al.*, 2023). The findings of Yagoo *et al.* (2023) falls in line with the present findings. However, much higher LC₅₀ values of 76840-94200 ppm were obtained for the solvent extracts of *Catharanthus roseus* against *C. quinquefasciatus* (Subarani *et al.*, 2013) where these LC₅₀ values were several folds higher than that of the present study. The findings of the current study with reference to mosquito larvicidal and pupicidal activities of *C. hebecarpa* ethanolic extract imply that the *C. hebecarpa* possesses substantial efficacy against the vector mosquitoes, *A. aegypti* and *C. quinquefasciatus*.

Conclusions

The study of phytochemical constituents and the studied biological properties of ethanolic whole plant extract of *C. hebecarpa* signify the potential of the ethnomedicinal plant traditionally used by ethnic communities in Salem district of south India. The DPPH, H₂O₂ and ABTS antioxidant activities of *C. hebecarpa* extract revealed an IC₅₀ inhibition range of 788.24 ppm to 1087.68 ppm, indicating the plant's effectiveness as antioxidant agent. Further findings on alpha amylase and alpha glucosidase inhibitory activity of *C. hebecarpa* was found to be in the good range of activity. In addition, mosquito larvicidal and pupicidal activities of *C. hebecarpa* supports the traditional utility of *C. hebecarpa* as an ethnomedicinal plant. Further studies on specific compounds isolation and their activities may support the utility of *C. hebecarpa* in the field of biomedical field.

Author contributions

A. Samiyappan: Conceptualization, Data curation, Investigation, Formal analysis, writing- original draft. R.

Sengottuvel: Supervision, Validation, Writing-review & editing. All authors approved the final manuscript.

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