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Phytochemical Screening, Antifungal and Antioxidant Activities of Ethanolic Leaf and Flower Extracts of *Clerodendrum Infortunatum* from Bhagalpur Region

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Abstract

Clerodendrum infortunatum is a well-known medicinal shrub with dark green leaves and whitish-pink flowers. Its extracts are used for the treatment of several diseases in traditional systems of medicine. It has phytomedicinal potential and is of great importance as it offers many promising health benefits. The objective of the study is to investigate the ethanolic extracts of leaves and flowers of *Clerodendrum infortunatum* for their antioxidant activity. The methods used include Ferric reducing power assay, DPPH, Hydrogen peroxide scavenging assay, and phosphomolybdate method. Total phenolic and flavonoid content are measured spectrophotometrically. The antifungal activity was evaluated using the food poison method, a technique that involves incorporating the test substance into a growth medium to observe its inhibitory effects on fungal growth. The screening of extracted phytochemicals is done by qualitative methods. The study shows the presence of various phytochemicals such as flavonoids, terpenoids, glycosides and phenolic components in the ethanolic extracts of leaf and flower of *Clerodendrum infortunatum*. The study also demonstrates that the ethanolic flower extract of *Clerodendrum infortunatum* exhibits the highest antioxidant activity. In contrast, the ethanolic leaf extract shows greater antifungal activity against *Aspergillus niger* and *Aspergillus flavus* compared to the flower extract.

Keywords: Antioxidant activity; Antifungal activity; *Clerodendrum infortunatum*; DPPH; phosphomolybdate.

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1. Introduction

Clerodendrum infortunatum, known as Bhat or Hill glory bower, belonging to family *Lamiaceae*, plays a significant role in Ayurveda. It is a flowering shrub having dark green colored, elliptically ovate leaves and whitish-pink flowers [1]. The leaf extract of this plant is used for dressing wounds, the root extract is used in the treatment of tumors and cirrhosis. The plant is also used in the treatment of jaundice, scorpion-sting, snake bites, post-natal care, asthma, fever, inflammation, in burning sensation of sores, ulcers, etc. *Clerodendrum*

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infortunatum has been reported to possess antioxidant [2], antimicrobial [3], wound healing [4], anti-inflammatory, diuretic [5], hepatoprotective [6], and antihyperglycemic activity [7]. Previous studies have analyzed the biochemical properties of *Clerodendrum infortunatum* but this plant from the Bhagalpur region is still to be explored for its biochemical properties. Recent studies have highlighted the importance of exploring natural sources as antioxidant and antifungal agents, due to the increasing concern about synthetic compound toxicity and the rising incidence of fungal infections. The chosen plant presents a promising exemplar for such an investigation.

Antioxidants are molecules that help prevent or reduce cell damage caused by free radicals or oxidants. Different types of oxidants cause oxidative stress leading to cell damage, aging, cancer, gastric ulcers, diabetes mellitus, and various diseases. These oxidants, also known as reactive oxygen species (ROS) are highly reactive molecules which include hydroxyl radicals ($^{\cdot}OH$), superoxide ions ($O_2^{\cdot-}$), nitric oxide radical ($NO^{\cdot}$), etc. Living cells generate highly reactive superoxide anion radicals after taking in oxygen [8]. Antioxidants neutralize these radicals or anion radicals either by transfer of electrons or by breaking the free radical chain. Synthetic antioxidants like butylated hydroxy toluene (BHT) and butylated hydroxy anisole (BHA) have been shown to have toxic effects [9]. Therefore, antioxidants of natural origin need to be developed that have either no or less toxic effects and are safer than synthetic antioxidants. The presence of various polyphenolic phytochemicals in plants makes them antioxidant-rich and a subject of research for safer antioxidants. Plants or plant parts have been used in either some formulations or in raw form in Indian medicinal systems like Ayurveda, Folk medicines, and Homeopathy to cure several diseases. Despite the great advances in allopathic (modern) medicines in recent times, plants still make important contribution to healthcare especially in Developing countries. The active biomolecules present in plants as secondary metabolites, such as known as Phytochemicals, can serve mankind in various ways by performing pharmacological functions. These phytochemicals have been found to show biochemical properties like antioxidant, antifungal, antimicrobial, and antidiabetic activities [10]. The phytochemicals present in plants are alkaloids, flavonoids, saponins, terpenoids, tannins, gums, resins, glycosides, oils, etc. [11,12].

Aspergillus flavus is a saprophytic, pathogenic soil fungus that grows on dead plant tissues. It belongs to the *Aspergillus* genus and is an opportunistic pathogen with a broad host range, particularly affecting crops. This fungus produces highly carcinogenic toxins called aflatoxins [13], which pose a significant health hazard to animals. In humans, *A. flavus* can cause aspergillosis, especially in immunocompromised individuals. The fungus forms powdery colonies and is thermotolerant, capable of growing in a wide temperature range of 18 °C to 48 °C. It contaminates pre-harvest and post-harvest oilseed crops through its secondary metabolites known as mycotoxins [14]. Among these, aflatoxin B1 is classified as a Group 1A carcinogen [15]. Preventing the production of mycotoxins by *Aspergillus flavus* can be achieved by inactivating or inhibiting its growth [16].

Aspergillus niger is a filamentous ascomycete fungus belonging to the *Aspergillus* genus and is ubiquitous in the environment. It is recognized as the primary agent responsible

for black mold formation on plant surfaces and is a major cause of seed deterioration [17]. This species is commonly associated with post-harvest decay of fresh fruits, including apples, citrus, grapes, strawberries, mangoes, and melons, though most of these diseases are sporadic. It also causes significant losses in crops such as tomatoes, breadfruit, onions, garlic and yams. Cereals and oilseeds are frequent hosts, particularly maize, corn snacks, barley, soybeans, rapeseed, rape oil, sorghum, stored and parboiled rice, blackgram, sunflower seeds, chickpeas, and pigeon peas [18]. Additionally, *A. niger* has been implicated in opportunistic human infections [19], as it is capable of producing toxins such as ochratoxin A and fumonisins B2 and B4 [20].

The antifungal activity of a substance refers to its ability to inhibit fungal growth, either by reducing mycelial growth or completely preventing spore formation. Chemical pesticides and synthetic fungicides are commonly used to protect fruits and vegetables from fungal spoilage. However, these synthetic chemicals often have toxic side effects, contributing to environmental pollution and posing serious health risks. In contrast, several studies have highlighted the potential of plant extracts as effective organic pesticides. These plant-based extracts offer a simple, environmentally safe alternative for use as botanical fungicides and could be harnessed for the effective management of pre- and post-harvest diseases in fruits and vegetables.

This study aims to evaluate the antioxidant activity and antifungal activity of ethanolic extract of *Clerodendrum infortunatum* leaf and flower collected from the Bhagalpur region of Bihar, India along with qualitative and quantitative phytochemical analysis.

2. Material and Methods

2.1. *Reagents and chemicals*

All the chemicals used are of analytical grade and bought from Loba Chemicals. Aluminium chloride was sourced from Merck and deionized X-tra pure water from Sigma chemicals. PDA (Potato Dextrose Agar RDM-PDA 01) was procured from Ready Med.

2.2. *Preparation of plant extract*

The leaves and flowers of *Clerodendrum infortunatum* were collected from the Sabour region of Bhagalpur, Bihar, India, in March 2023. Both plant parts were washed thoroughly with running water 5-10 times, followed by a final rinse with distilled water. After removing excess water, the plant material was refrigerated for complete drying over approximately 20 days. Thereafter, the leaves and flowers were powdered separately in the Usha Colt Mixer jar not letting the jar heat. The extract of *Clerodendrum infortunatum* leaves and flowers was prepared using maceration technique [21]. The powdered leaves and flowers were soaked in pure ethanol for 48 h, then filtered through normal filter paper. The filtrate was centrifuged and again filtered through Whatman's filter paper No. 41. The final filtrate was dried over a water bath to obtain semi-solid dry ethanolic extract. Two separate

ethanolic extracts were prepared: *Clerodendrum infortunatum* flower extract (CF) and *Clerodendrum infortunatum* leaf extract (CL).

2.3. *Antifungal activity*

The antifungal activity was tested against two fungi *Aspergillus niger* and *Aspergillus flavus*. The Food Poison Method was used which involves incorporating the test substance into a growth medium to observe its inhibitory effects on fungal growth [22]. The readymade PDA (potato dextrose agar) media is used for the preparation of media for the growth of fungus. 4 g of PDA and 100 mg of ethanolic extract was taken and the volume was made 100 ml by adding ultrapure distilled water. The mixture was autoclaved at 121°C and 15 psi. The autoclaved media was cooled at room temperature and poured into sterile and autoclaved Petri plates. The mycelia of respective fungi were inoculated in the center of media containing Petri plates using a sterilized inoculating needle (sterilized by dipping in absolute ethanol and flaming). The ethanolic plant extract was not added to the media, used as a negative control. The inoculated Petri plates were incubated at 37 °C for six days. The diameter of growth was recorded in mm every day at same time to check the daily fungal growth. Each treatment was performed in duplicates including control. The antifungal activity was calculated as follows:

Antifungal Activity (%) = $(D_C – D_S / D_C) \times 100$

Where, D_C = Diameter of Growth in Negative control plate and D_S = Diameter of growth in a plate containing ethanolic plant extract.

2.4. *Antioxidant activity assays*

The antioxidant activity of ethanolic extract of *Clerodendrum infortunatum* leaves and flowers was assessed by Ferric Reducing Power assay [23], DPPH [24], H_2O_2 scavenging assay [25], and Phosphomolybdate assay [26]. Each extract was dissolved in absolute ethanol to prepare a concentration of 1 mg/mL.

2.4.1. *Ferric reducing power assay*

Ethanolic extract samples of various concentrations were taken and the volume was diluted to 1 mL by adding respective solvent. Then 2.5 mL of phosphate buffer (0.2 M and pH 6.6) was added followed by the addition of 2.5 mL of 1 % potassium ferricyanide. The solution was incubated at 50 °C for 20 min. After adding 2.5 mL of 10 % trichloroacetic acid, the solution was centrifuged at 3000 rpm for 10 min. The supernatant liquid was diluted with deionized water followed by the addition of 0.5 mL of 0.1 % ferric chloride solution. The absorbance was recorded at 700 nm. The BHT was taken as standard. The results are expressed as mg BHT equivalent per mg of plant part extract. The assay was performed in triplicates with both the extracts and the standard.

2.4.2. *Phosphomolybdate assay*

Phosphomolybdate reagent was prepared by adding equal parts of 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. This reagent was added to different concentrations of ethanolic plant extracts and the solution was incubated at 90°C for 90 min and then cooled at room temperature. The absorbance was taken at 695 nm. BHT was used as standard and the results are expressed as mg BHT equivalent per mg of dry plant extract. Every experiment was performed in triplicates and the mean of absorbances was calculated and expressed as mean ± standard deviation.

2.4.3. *DPPH assay*

The solution of 10^{-4} dilution of DPPH was prepared in pure ethanol. 1 mL of this solution was added to 2 mL of plant extract solution in ethanol of different dilutions. The mixture was incubated at room temperature for 15 min and then the absorbance was noted at 517 nm. The control was prepared by mixing solution of DPPH with an equal volume of solvent without plant extract. The % of DPPH inhibition activity was calculated as follows:

$$\% \text{ of DPPH Inhibition Activity} = (A_C - A_S) / A_C \times 100$$

Where A_C = Absorbance of control and A_S = Absorbance of sample

2.4.4. *Hydrogen peroxide scavenging assay*

The 40 mM solution of hydrogen peroxide was prepared in a phosphate buffer of pH 7.4. 0.6 mL of this solution was added to different dilutions of plant extract solution. The mixture was allowed to rest for 10 min and then the absorbance was noted at 230 nm. The control was prepared by mixing equal volume of hydrogen peroxide and the solvent used. The experiment was repeated three times with each sample. The % of H_2O_2 scavenging activity was calculated as follows:

$$\% \text{ of } H_2O_2 \text{ Scavenging activity} = (A_C - A_S)/A_C \times 100$$

Where, A_C is absorbance of control and A_S is absorbance of sample, respectively.

2.5. *Total phenolic content*

Total phenolic content was estimated by using the Folin-Cio-Calteau Reagent [27,28]. To an aliquot of 500 µL of extract in ethanol, 500 µL of Folin-cio-calteau reagent is added then the solution is diluted by adding 6 mL of deionized distilled water followed by the addition of 1.5 mL of 20 % sodium carbonate solution and again 1.9 mL of deionized distilled water was added. After incubation for 2 h, the absorbance was recorded at 760 nm using Systronics-117-UV-Vis spectrophotometer. Gallic acid was used as a reference phenolic compound. The results were expressed as milligram gallic acid equivalents (GAE) per gram of extract.

2.6. *Total flavonoid content*

Total flavonoid content was estimated by the aluminium chloride method [29]. To an aliquot of 500 µL of extract, 4 mL of ethanol and 1 mL of 10 % aluminium chloride were added followed by the addition of 1ml of 1M sodium acetate solution. The absorbance was noted after incubation for 45 minutes in the dark at 420 nm using a Systronics 117 UV-VIS Spectrophotometer. Quercetin was used as standard and the results were expressed as milligram quercetin equivalent (QE) per gram of extract.

2.7. *Phytochemical screening*

The phytochemicals like alkaloids, glycosides, carbohydrates, flavonoids, etc. present in the ethanolic extract of *Clerodendrum infortunatum* leaves and flowers were detected by using qualitative analysis [30,31].

3. *Statistical analysis*

The experiments for antioxidant activity were done in triplicates. The values are calculated as mean with standard deviation using Microsoft Excel. TFC and TPC were analyzed twice and values are reported as mean with standard deviation. The significance of the results was analyzed using ANOVA: single factor ($p<0.05$).

4. Results

4.1. *Phytochemical screening*

The qualitative analysis of ethanolic extract of *Clerodendrum infortunatum* flower and leaf shows the presence of various phytochemicals as shown in Table 1.

Table 1. Qualitative phytochemical analysis.

Phytochemical Component	Ethanolic extract of *Clerodendrum infortunatum* flower	Ethanolic extract of *Clerodendrum infortunatum* leaf
Alkaloids	-	+
Carbohydrates	+	+
Flavonoids	+	+
Coumarins	+	+
Glycosides	+	+
Phlobatannins	-	-
Terpenoids	+	+
Phenols	+	+
Anthraquinones	+	+

Key: + means present and – means absent

The ethanolic extract of the leaf and flower of *Clerodendrum infortunatum* shows the presence of flavonoids, Coumarins, phenols, anthraquinones, glycosides, terpenoids, and carbohydrates. The qualitative test for alkaloids was found to be negative in the flower extract whereas it was positive for leaf extract of *Clerodendrum infortunatum*.

4.2. *Total phenolic content (TPC) and total flavonoid content (TFC)*

TPC of CF and CL is calculated using the linear regression equation y=0.0007x+0.0559, with coefficient of determination R^2=0.9676 from the calibration curve for Gallic acid and TFC is calculated using the equation y=0.0224x+0.1422, with coefficient of determination R2=0.965 from the calibration curve for quercetin. The high value of R^2 indicates the strong linear relationship between the concentration and the absorbance recorded. It shows the reliability of standard curve used for calculating TPC and TFC (Table 2). The TPC of CF is 242.214 ± 4.040 mg GAE/g of dry extract, which is higher than the TPC of CL, measured at 229.714 ± 19.698 mg GAE/g of dry extract. The TFC of CF is 3.774 ± 0.058 mg QE/g of dry extract, while the TFC of CL is 2.567 ± 0.153 mg QE/g of dry extract. The TFC of CF is also higher than that of CL. That means the flower of *Clerodendrum infortunatum* has more phenolic compounds than its leaves. Also, the flavonoids are present in larger amounts in flowers than in leaves of *Clerodendrum infortunatum*. The numerical values are shown in the Table 2 as means of duplicate experiments along with standard deviation as calculated. The values are also statistically significant at $P<0.05$ as determined through single-factor ANOVA.

Table 2. Total flavonoid content and total phenolic content.

Plant extract of *Clerodendrum infortunatum*	Total flavonoid Content as mg QE/g of dry extract	Total phenolic content as mg GAE/g of dry extract
Flower	3.774 ± 0.058	242.214 ± 4.040
Leaf	2.567 ± 0.153	229.714 ± 19.698

4.3. *Antioxidant activity*

The antioxidant activity of CF and CL was determined by using four Assays namely Phosphomolybdate, FRAP (Ferric Reducing Antioxidant Power), DPPH, and hydrogen peroxide Assay. The antioxidant activities for all four assays are expressed in terms of milligram equivalent of BHT (taken as standard) per milligram of dry extract as shown in Table 3. The milligram equivalent of BHT per milligram of dry extract for CF in DPPH scavenging, H_2O_2 scavenging, Phosphomolybdate, and FRAP assay are 1.209 ± 0.003, 1.344 ± 0.007, 1.637, and 5.657 ± 0.106, respectively, while for CL they are 1.108 ± 0.002, 1.257 ± 0.007, 1.148 ± 0.029, and 4.553 ± 0.008, respectively. The antioxidant activity values, in terms of mg BHT equivalent per mg of dry extract, are higher for CF as compared to CL.

Table 3. mg BHT eq/mg of dry extract.

Sample	DPPH scavenging assay	H_2O_2 scavenging assay	Phosphomolybdate assay	FRAP assay
CF	1.209 ± 0.003	1.344 ± 0.007	1.637	5.657 ± 0.106
CL	1.108 ± 0.002	1.257 ± 0.007	1.148 ± 0.029	4.553 ± 0.008

Each value represents mean ± SDs as calculated on MS Excel

The phosphomolybdate and FRAP Assay absorbances of CF, CL, and BHT are shown in Fig. 1a-b. CF exhibited the highest absorbance, followed by CL, while BHT showed the lowest absorbance. This indicates that CF reduced Mo(VI) to Mo(V) to a greater extent than CL and BHT, suggesting a larger number of antioxidants present in CF. Specific absorbance values for CF, CL, and BHT at concentration 0.6 mg/mL in phosphomolybdate assay are 0.756, 0.548, and 0.487 nm respectively. Higher absorbance shows higher antioxidant power in phosphomolybdate assay. The absorbance values indicates that CF has a higher antioxidant capacity than CL and BHT (used as standard antioxidant in the antioxidant assays).

The absorbances of CL and BHT are comparable to each other showing almost the same antioxidant activity in phosphomolybdate assay. In phosphomolybdate assay, the antioxidants reduce Mo (VI) to Mo(V) which forms green colored complex showing absorbance at 695 nm in the present study, CF has reduced Mo(VI) to Mo(V) to a greater extent, as compared to CL and BHT, showing a larger number of antioxidants present in it. When calculated, the antioxidants in terms of milligram equivalent of BHT, CF extract has shown a larger quantity of antioxidants than CL extract as shown in Table 3. CF was found to have 1.637 mg BHT eq/mg of dry extract and CL was found to have 1.148 ± 0.029 mg BHT eq/mg of dry extract. The value is larger for CF extract and smaller for CL extract showing a larger Mo(VI) Reducing power of CF than BHT and CL. Ferric reducing power antioxidant assay is one of the antioxidant assays used to determine the reducing power of various extracts or antioxidants by reducing Fe^{3+} to Fe^{2+}, forming Prussian blue complex showing absorbance at 700 nm. From Fig 1b, it is clear that CF has more absorbance than CL followed by BHT, suggesting higher reducing power of CF as compared to CL and BHT. The absorbance values of CF, CL and BHT at 0.3 mg/mL in FRAP assay are 1.887, 0.964, and 0.183 nm respectively. In the present study, the FRAP values are calculated as milligram BHT equivalent per mg of dry extract shown in Table 3 which clearly shows a larger reducing power of CF extract than CL and BHT.

DPPH is a synthetic stable radical that is used for the determination of the free radical scavenging activity of antioxidants. In the present study, the free radical scavenging activity of antioxidants, if present, in the ethanolic extract of *Clerodendrum infortunatum* flower (CF) and leaf (CL), was determined by using DPPH and hydrogen peroxide assay, taking BHT as standard. The percentage inhibition of DPPH and hydrogen peroxide is shown in Fig. 1c-d respectively. In this assay, the percentage free radical scavenging activity of CF, CL and BHT is in the order of CF > CL> BHT. The IC50 values of CF, CL, and BHT are shown in the form of a Bar chart in Fig. 2a. It is clear from Fig. 2a that the IC50 value of BHT is highest which is 581.927 µg/mL and is lowest for CF which is 482.014 µg/mL,

showing more free radical scavenging capacity of CF extract as compared to BHT and CL extract.

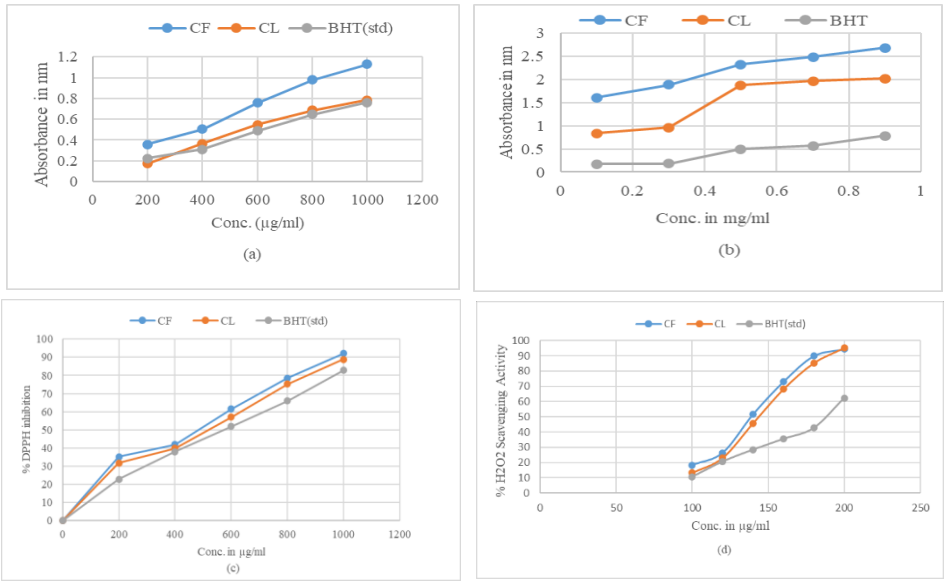

Fig. 1. (a) Phosphomolybdate assay showing absorbance of CL, CF, and BHT; (b) Ferric Reducing Antioxidant Power assay showing absorbance of CF, CL, and BHT; (c) DPPH assay showing percentage inhibition of DPPH by CL, CF and BHT; (d) H_2O_2 Scavenging Assay showing percentage scavenging activity by CL, CF and BHT.

Hydrogen peroxide scavenging assay of antioxidant activity shows that higher percentage of H_2O_2 being scavenged by CF, as compared to CL and BHT. BHT is a synthetic antioxidant which also decreases the concentration of hydrogen peroxide in the solution with its increasing concentration as shown in Fig. 1d in the hydrogen peroxide scavenging assay, while the scavenging activity of both CF and CL extracts is higher than BHT, showing larger antioxidant activity of CF and CL. The lower IC50 value for CF extract also suggests higher antioxidant activity of CF. The IC50 value of CF, CL and BHT is 139.43 µg/mL, 144.17 µg/mL and 185.05 µg/mL, respectively.

4.4. *Antifungal activity*

Ethanolic extracts of *Clerodendrum infortunatum* flower (CF) and leaf (CL) at concentration 1 mg/mL were tested for their antifungal activity against the common fungi *Aspergillus flavus* and *Aspergillus niger*, two different species of the same genus. CL extracts have shown some amount of antifungal activity but CF extracts lack any kind of antifungal activity as shown in the following Fig. 3a-c. The daily average growth of both fungi, in the presence of the CF extract, was comparable to that of the negative control, indicating minimal antifungal activity. In contrast, the CL extract demonstrated significant antifungal activity, resulting in a lower daily average growth of both *Aspergillus flavus* and

Aspergillus niger compared to the negative control. The antifungal effects of the extracts were further assessed by calculating the percentage of growth inhibition. As illustrated in Fig. 3a-b, the CL extract exhibited a higher percentage growth inhibition than the CF extract. Specifically, the CL extract showed the highest inhibition of *Aspergillus flavus* on the second day, while the inhibition decreased by the fifth day. For *Aspergillus niger*, the maximum inhibition was observed on day one, followed by a decline, reaching its lowest value on the fifth day, as shown in Fig. 3a-b.

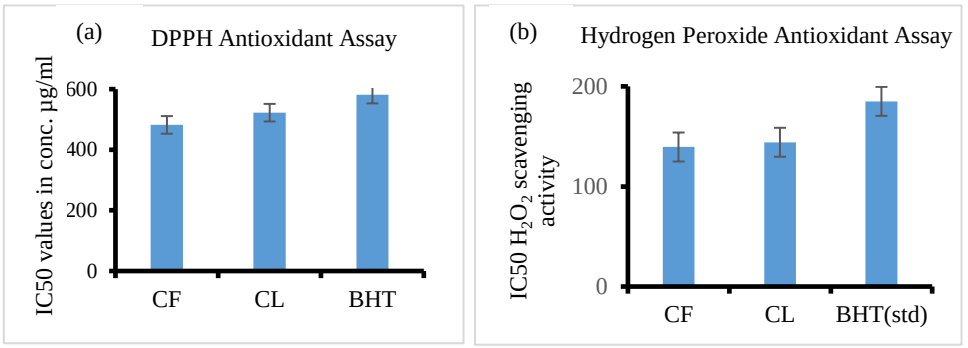

Fig. 2. (a) IC50 values for DPPH Inhibition Assay and (b) IC50 values for H_2O_2 Scavenging assay.

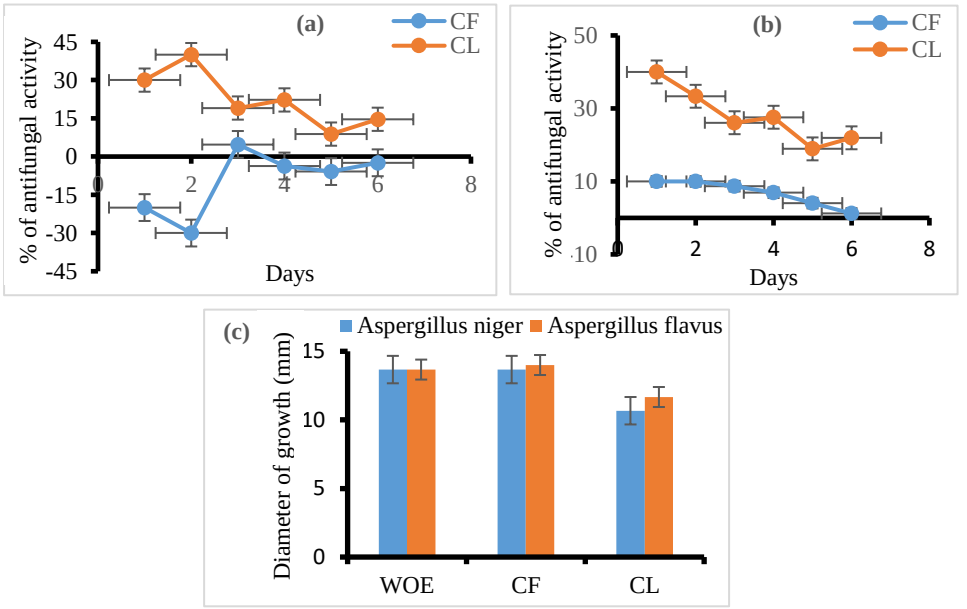

Fig. 3. (a) Percentage antifungal activity of CF and CL on *Aspergillus flavus*, (b) percentage antifungal activity of CF and CL on *Aspergillus niger*, and (c) average daily growth of fungi.

5. Discussion

The phytochemical screening of ethanolic extracts *of Clerodendrum infortunatum* flower and leaf shows the presence of secondary metabolites such as flavonoids, terpenoids, phenolics, glycosides, and anthraquinones which may be attributed to the ethnomedicinal importance of *Clerodendrum infortunatum* flower and leaf [32].

Phenolic compounds are very common phytochemicals and are secondary metabolites of the plant part, responsible for color, flavor and defense mechanism of the plant [33]. Phenolic compounds exhibit antioxidant and anti-microbial properties which help them to protect itself from pathogenic attack like fungi, bacteria etc. [34]. Phenolic compounds also protect major tissues of the plants from toxic oxidative stress [35] developed due to reactive free radicals or reactive oxygen species. Besides defensive mechanisms, phenolic compounds also play an important role in cross-talk and communications [36]. Flavonoids and phenolics also play an important role in the prevention of cancer and other cardiac diseases [37].

The values obtained for total phenolic content (TPC) and total flavonoid content (TFC) in the present study are not in agreement with other investigators where the value of TPC and TFC ranges as 0.12-48.25 mg GAE/g and 0.03-25.29 mg QE/g respectively [38]. In this study, the values of TFC of CF extract and CL extract are 3.774 ± 0.058 and 2.567 ± 0.153 mg QE/g of dry extract respectively and of TPC are 242.214 ± 4.040 and 229.714 ± 19.698 mg GAE/g of dry extract respectively. The variation in the values may be due to different places of collection, different agro-climatic conditions and variations in soil factors which significantly impact the synthesis of secondary metabolites in plants [39]. The variation may also arise due to different solvents used for extraction and preparation of solutions.

The antioxidant activities of CF and CL extract are determined using more than one assay. The DPPH, hydrogen peroxide, ferric reducing power and phosphomolybdate assay are used for determining antioxidant activities because it is always better to assess the activities by more than one method to confirm the results [40]. Antioxidants are substances that prevent lipid peroxidation, scavenge free radicals and help the biological systems fight any kind of oxidative stress developed due to the presence of reactive oxygen species within the biological systems. All assays showed that CF extracts had higher antioxidant activities compared to CL extracts and the standard. Different researchers have shown the antioxidant activity of CL [41] which is in agreement with the results of the present study. There was a strong correlation between the different methods of antioxidant assays, as well as between total phenolic and flavonoid content and antioxidant activity, suggesting that the antioxidant activity may be related to the concentration of phenolics and flavonoids present in the extract. The findings are similar to the results of other investigators [42]. The antioxidant potential increases with an increase in concentration suggesting that higher concentration of extracts will have greater antioxidant activity. The IC50 value of BHT in DPPH assay is highest which is 581.927 µg/mL, followed by CL, 522.296 µg/mL and is lowest for CF which is 482.014 µg/mL. The IC50 values of CF, CL and BHT in hydrogen peroxide assay are 139.43 µg/mL, 144.17 µg/mL and 185.05 µg/mL respectively. The IC50 values of

DPPH and H_2O_2 scavenging assays suggest that the ethanolic extract of CF has higher antioxidant activity as compared to BHT and ethanolic extract of CL. The compounds present in the flower of *Clerodendrum infortunatum* such as Apigenin, acacetin [43] Antisal, β-cubebene, tyranton, 2-trans-β-ocimene, 3-allyl methoxy phenol, 4H-1, 3 oxazin and others [44] may be responsible for its biochemical activities. Oleanolic acid, Clerodinin A [45] gallic acid [46], and other phenolics, flavonoids, and terpenoids [47] found in extracts of CL may be responsible for its biochemical activities.

The antifungal activity of *Clerodendrum infortunatum* leaf and flowers against *Aspergillus niger* and *Aspergillus flavus* were studied. The ethanolic extract of the flower of *Clerodendrum infortunatum* has almost negligible effect on the growth of *Aspergillus flavus* and *Aspergillus niger*. In contrast, the ethanolic extract of *Clerodendrum infortunatum* leaf shows inhibitory effect on the growth of both the fungi, *Aspergillus flavus* and *Aspergillus niger*. Several investigations have revealed the antifungal activity of various solvent extracts of *Clerodendrum infortunatum* leaves and other plant extracts against pathogenic fungi. Anthocyanin reported from leaves [48] may be responsible for the antifungal effect against the fungi considered in the present study. The antifungal effect of ethanolic extract of *Clerodendrum infortunatum* leaf is maximum on *Aspergillus sp.* [49]. It has been reported that secondary plant metabolites are responsible for the antifungal effect of plant extracts [50]. The presence of phenolics in the extract may be responsible for the antifungal activity of the given extract.

6. Conclusion

In the present study, the ethanolic extracts of *Clerodendrum infortunatum* leaf and flower collected from the Bhagalpur region demonstrated significant biochemical properties, such as antioxidant and antifungal activities. Qualitative phytochemical screening revealed the presence of bioactive compounds such as flavonoids, phenols, and terpenoids, which are likely contributors to these activities. Different antioxidant assays, including DPPH, hydrogen peroxide scavenging, FRAP and phosphomolybdate assay, yielded positive results, which were further compared with well-established standard butylated hydroxytoluene (BHT). The extracts showed comparable or superior activity in some assays, indicating their potential efficacy. For instance, in the DPPH assay, at a concentration of 0.2 mg/mL, the scavenging activity of the leaf extract was 31.7 % compared to 22.8 % for the standard (BHT), while the flower extract demonstrated a 35.1 % inhibition, suggesting that both extracts possess robust free radical scavenging capabilities and has a potential to be a powerful antioxidant. In antifungal activity assays, the extracts were tested against common fungal strains such as *Aspergillus flavus* and *Aspergillus niger* and compared with negative control without any extract. The percentage of inhibition for the leaf extract was found to be 40 % on day 2, indicating notable antifungal potential. The average growth diameter of both fungi, *Aspergillus flavus* and *Aspergillus niger* was nearly the same, measuring 13.6 mm for the flower extract and 14.0 mm for the negative control, indicating negligible antifungal property of the flower extract. These

results highlight the potential use of *Clerodendrum infortunatum* leaf and flower extracts as natural antioxidants and antifungal agents. The extracts showed promising bioactivity as compared to conventional standards. Further systematic pharmacological investigations and clinical studies are recommended to explore their potential for therapeutic drug development.

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References

1. A. K. Gupta and M. Sharma, Rev. Indian Med. Plants **7**, 104 (2008).
2. K. G. Chandra, R. Mahmood, and H. Manjunatha, Environ. Toxicol. Pharmacol. **30**, 11 (2010).
3. A. J. Modi, S. S. Khadabadi, I. A. Farooqui, and D. S. Ghorpade, Sch. Res. Libr. Pharm. Lett. **2**, 229 (2010). https://doi.org/10.1016/S0975-3575(10)80098-7
4. G. Kuluvar, R. Mahmood, B. M. K. Ahamed, P. S. Babu, and V. Krishna, Int. J. Biomed. Pharm. Sci. **3**, 21 (2009).
5. N. Khatry, J. Kundu, S. C. Bachar, M. N. Uddin, and J. K. Kundu, Dhaka Univ. Pharm. Sci. **5**, 63 (2005).
6. S. Sannigrahi, U. K. Mazumder, D. Pal, and S. L. Mishra, Phcog. Mag. **5**, 394 (2009). https://doi.org/10.1177/1934578X1000500818
7. S. Das, S. Bhattacharya, A. Prasanna, S. R. B. Kumar, G. Pramanik, and P. K. Haldar, Diabetes Ther. **2**, 92 (2011). https://doi.org/10.1007/s13300-010-0019-z
8. A. M. Mohammad, Y. Koji, M. Toshiki, N. Yumi, S. Katsumi, S. Hiroaki, and T. Yoshifumi, Int. J. Bio. Sci. **3**, 349 (2007).
9. M. F. A. Ghafar, K. N. Prasad, K. K. Weng, and A. Ismail, Afr. J. Biotechnol. **9**, 326 (2010).
10. H. Tlili, N. Hanen, A. Ben Arfa, M. Neffati, A. Boubakri, D. Buonocore et al., PLoS ONE **14**, 9 (2019). https://doi.org/10.1371/journal.pone.0213049
11. K. S. Banu and L. Catherine, Int. J. Adv. Res. Chem. Sci. **2**, 25 (2015).
12. S. Pagare, M. Bhatia, N. Tripathi, and Y. K. Bansal, Curr. Trends Biotechnol. Pharm. **9**, 293 (2015).
13. M. A. Klich, Mol. Plant Pathol. **8**, 713 (2007). https://doi.org/10.1111/j.1364-3703.2007.00436.x
14. S. Amaika and N. P. Keller, Annu. Rev. Phytopathol. **49**, 107 (2011). https://doi.org/10.1146/annurev-phyto-072910-095221
15. D. Gizachew, C. -H. Chang, B. Zonyi, S. D. L. Torre, and W. -t. E. Ting, Int. J. Food Microbiol. **296**, 8 (2019). https://doi.org/10.1016/j.ijfoodmicro.2019.02.017
16. Y. Liu, J. H. Galani Yamdeu, Y. Y. Gong, and C. Orfila, Compr. Rev. Food Sci. Food Saf. **19**, 4 (2020).
17. K. Ziani, I. Fernandez-Pan, M. Royo, and J. I. Mate, Food Hydrocolloids **23**, 2309 (2009). https://doi.org/10.1016/j.foodhyd.2009.06.005
18. J. I. Pitt and A. D. Hocking, Fungi and Food Spoilage, 2nd Edition (Aspen Publication, Gaithersburg, 1997).
19. S. Nondi, B. Talukder, M. Abony, A. Banik, and Z. Ahmed, Int. J. Dev. Res. **10**, 08 (1999).
20. K. F. Nielsen, J. M. Mogensen, M. Johansen, T. O. Larsen, and J. C. Frisvad, Anal. Bioanal. Chem. **395**, 1225 (2009). https://doi.org/10.1007/s00216-009-3081-5
21. K. S. Banu and L. Catherine, Int. J. Adv. Res. Chem. Sci. **2**, 25 (2015).

22. M. S. Ali-Shtayeh and I. S. A. Ghdeib, Mycoses **42**, 665 (1999). https://doi.org/10.1046/j.1439-0507.1999.00499.x
23. K. I. Berker, B. Demirata, and R. Apak, Food Anal. Methods **5**, 1150 (2012). https://doi.org/10.1007/s12161-011-9358-2
24. W. Brand-Williams, M. E. Cuvelier, and C. Berset, LWT Food Sci. Technol. **28**, 25 (1995). https://doi.org/10.1016/S0023-6438(95)80008-5
25. A. A. Al-Amiery, Y. K. Al-Majedy, A. A. Kadhum, and A. B. Mohamad, PLoS ONE **10**, ID e0132175 (2015). https://doi.org/10.1371/journal.pone.0132175
26. P. Prieto, M. Pineda, and M. Aguilar, Anal. Biochem. **269**, 337 (1999). https://doi.org/10.1006/abio.1999.4019
27. V. L. Singleton and J. A. Rossi, Am. J. Enol. Viticult. **16**, 144 (1965). https://doi.org/10.5344/ajev.1965.16.3.144
28. S. Adeniyi, G. A. Olatunji, and O. S. Oguntoye, J. Sci. Res. **14**, 617 (2022). https://doi.org/10.3329/jsr.v14i2.56287
29. C. C. Chang, M. H. Yang, H. M. Wen, and J. C. Chern, J. Food Drug Anal. **10**, 178 (2002).
30. J. R. Shaikh and M. K. Patil, Int. J. Chem. Stud. **8**, 603 (2020). https://doi.org/10.22271/chemi.2020.v8.i2i.8834
31. K. Manna, B. A. Debnath, A. M. Ikbal, K. Paul, A. Rajkhowa, and R. Majumder, J. Sci. Res. **15**, 547 (2023). https://doi.org/10.3329/jsr.v15i2.62193
32. T. P. Kekuda, V. D. Shree, G. S. Noorain, B. Sahana, and H. Raghavendra, J. Drug Deliv. Ther. **9**, 547 (2019). https://doi.org/10.22270/jddt.v9i2.2433
33. S. Kumar, M. M. Abedin, A. K. Singh, and S. Das, in Plant Pathogens and Food Security, R. Lone (ed) et al., (Springer, Singapore, 2020). https://doi.org/10.1007/978-981-15-4890-1_22
34. K. Kulbat, Biotechnol. Food Sci. **80**, 97 (2016).
35. H. Bergmann, V. Leinhos, and B. Machelett, Acta Hortic. **381**, 390 (1994). https://doi.org/10.17660/ActaHortic.1994.381.50
36. A. Bhattacharya, P. Sood, and V. Citovsky, Mol. Plant Pathol. **11**, 705 (2010). https://doi.org/10.1111/j.1364-3703.2010.00625.x
37. M. P. Kahkonen, A. I. Hopia, H. J. Vuorela, J. P. Rauha, K. Pihlaja et al., J. Agric. Food Chem. **47**, 3954 (1999). https://doi.org/10.1021/jf990146l
38. S. Saha, A. Mukherjee, S. Biswas, D. Choudhury, J. Saha, S. Pal, M. Sarkar, and R. K. Kole, Heliyon **4**, ID e01047 (2018). https://doi.org/10.1016/j.heliyon.2018.e01047
39. N. Bibi, M. H. Shah, N. Khan, A. Al-Hashimi, M. S. Elshikh, A. Iqbal, S. Ahmad, and A. M. Abbasi, Plants (Basel) **11**, 950 (2022). https://doi.org/10.3390/plants11070950
40. C. Milan, C. Hana, D. Petko, K. Maria, S. Anton, and L. Antonín, Food Control **21**, 518 (2010).
41. L. R. Helen, M. Jyothilakshmi, and M. S. Latha, Pharmacogn. J. **12**, 630 (2020). https://doi.org/10.5530/pj.2020.12.94
42. A. Kokate, R. P. Limsay, and S. A. Dubey, Pharma Innov. J. **SP-11**, 661 (2022).
43. N. K. Sinha, K. K. Seth, V. B. Pandey, B. Dasgupta, and A. H. Shah, Planta Med. **42**, 296 (1981). https://doi.org/10.1055/s-2007-971645
44. R. Islam and A. Rahman, J. Essent. Oil-Bear Plants **18**, 1271 (2015). https://doi.org/10.1080/0972060X.2015.1024448
45. S. Sannigrahi, U. K. Mazumder, D. Pal, and S. L. Mishra, Pharm. Biol. **50**, 304 (2012). https://doi.org/10.3109/13880209.2011.604089
46. R. Gupta and H. K. Singh, Int. J. Pharm. Pharmacol. **4**, 467 (2016). https://doi.org/10.15406/ppij.2016.04.00097
47. S. Verma, R. Gupta, AYU **35**, 416 (2014).
48. V. G. M. Krishnan, M. Greeshma, J. M. Aswathy, L. Bosco, and K. Murugan, Int. J. Pharm. Pharmaceutical Sci. **10**, 68 (2018).
49. P. Janarthani and A. G. B. Aruggoda, J. Eng. Technol. (JET-OUSL) **9**, 12 (2021).
50. C. Stracquadanio, J. M. Quiles, G. Meca, and S. O. Cacciola, J. Fungi. **6**, 263 (2020). https://doi.org/10.3390/jof6040263