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CORRELATION OF ANTIOXIDANT, ANTIBACTERIAL AND PHYTOCHEMICALS OF LEAVES AND ROOTS EXTRACTS OF *CLERODENDRUM INFORTUNATUM*

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Keywords:

Clerodendrum infortunatum, Phytochemicals, Antioxidant, Antibacterial, Correlation, Soxhlet extraction

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ABSTRACT: The leaves and roots extracts of *Clerodendrum infortunatum* was evaluated for its potential antioxidant and antibacterial properties. The correlation studies with phytochemicals were performed, which has not been widely reported yet. *Clerodendrum infortunatum* leaves and roots were screened for the presence of phytochemicals and were found positive for carbohydrates, saponins, alkaloids, tannins, phytosterols, coumarin glycoside, cardiac glycoside, phenols, flavonoids, and terpenoids. The phenols were quantified using the Folin-Ciocalteu method. The phenolics were found to be highest in ethyl acetate leaf (63.83 mg GAE/g) and ethyl acetate root extracts (87.21 mg GAE/g) at a concentration of 500 µl. Flavonoids were quantified by aluminium chloride colorimetric method, and it was found highest in ethyl acetate leaf extract (244.16 mg QE/g) and hexane root extracts (38.83 mg QE/g) at 500 µl. Aqueous leaf extracts (0.99 g) and hexane root extracts (0.99 g) had the highest total terpenoid content. The antioxidant activity was evaluated using DPPH assay. Aqueous leaves extract and ethyl acetate root extracts exhibited higher DPPH radical scavenging activity with an IC_{50} value of 223.6 µg/ml and 217.9 µg/ml. Antibacterial activity was determined by the disc diffusion method, and MIC was determined by broth dilution assay. Ethyl acetate root extracts exhibited significant activity against Escherichia coli (12 mm) and *Pseudomonas aeruginosa* (13 mm). Correlation analysis was determined using MS Excel software, version 2010. The correlation analysis revealed that the flavonoids in both leaves and root extracts contributed to the antioxidant activity and terpenoids for the antibacterial activity of *Clerodendrum infortunatum* root extracts.

INTRODUCTION: Plant resources have remained an integral part of human culture throughout history [1]. According to the World Health Organization (WHO), about three fourth of the world population depends mainly on herbs and traditional medicinal plants for treating various diseases [2].

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Medicinal plants produce a variety of bioactive molecules and have contributed to play a dominant role in the development of potent therapeutic agents and medicines due to their less toxicity and side effects since ancient times in different parts of the World [3].

Plants contain various natural products like vitamins, minerals, and other immune- modulators and have contributed to human welfare and plays a dominant role in curing disease caused by pathogenic microorganisms. *Clerodendrum infortunatum* is one among them [4]. Natural compounds present in herbal products, vegetables, fruits and grains could reduce oxidative damage by

acting as antioxidants. Most of the antioxidants are plant-based and the interest in natural antioxidants of plant origin has increased nowadays. Bacterial and fungal infection causes spoilage of food products. Also, it causes various diseases in plants, insects, and humans, which leads to a decrease in crop productivity and human health problems all over the world [5]. *Clerodendrum infortunatum* is a common shrub found in India and is commonly known as "Vattaparuvalam" in Malayalam, belonging to the family Verbenaceae and shows a spectrum of medicinal properties. This plant is rich in the characteristic medicinal terpenoid-clerodin, along with other important metabolites [6]. Clerodendrum possesses a wide array of secondary metabolites, including flavonoids and diterpenes, which are reported to possess antioxidant [7, 8] and antibacterial properties [4, 9]. The selection of this plant was based on its ethnopharmacological use and its wide application of the different plant parts. We extended our present research work with an aim to evaluate and explore the correlation between phytochemicals, antioxidant and antibacterial properties of the different solvent extracts of *Clerodendrum infortunatum*.

MATERIALS AND METHODS:

Leaf Sample Collection: The plant sample of *Clerodendrum infortunatum* was collected from K V M College of Science and Technology campus, Cherthala, Kerala, India, in the month of August 2019. The plant material was identified and authenticated as *Clerodendrum infortunatum* by Dr. P.K Shaji, Environmental Resources Research Centre (ERRC), Thiruvananthapuram-5 and the voucher specimen NO: 8977 of the sample was stored at Environmental Resources Research Centre (ERRC). The leaves and roots of the plant were washed under running tap water and shade dried for two weeks. The dried leaves and roots were then powdered and kept in an airtight container for analysis. The *in vitro* experimental analysis was performed at the Department of Biotechnology and Research Lab, KVM College of Science and Technology, Cherthala, Kerala, India.

Preparation of Plant Extract: The powdered leaves and roots were subjected to hot extraction using the Soxhlet apparatus for 24 h. 40 g of leaf sample and 200 g of root sample were extracted successively with 350 ml of hexane, ethyl acetate, methanol and water. The solvents from these extracts were removed under reduced pressure using a rotary vacuum evaporator (Heidolph, Germany) and stored in sterile pre-weighed screw-capped containers at 4 ºC until used.

Preliminary Phytochemical Analysis: The phytochemical screening of leaves and root extracts of *C. infortunatum* was performed using standard methods given by [10]. All the extracts of *C. infortunatum* prepared were subjected to a preliminary phytochemical screening for the presence of alkaloids, carbohydrates, saponins, flavonoids, phytosterols, phenols, tannins, terpenoids, coumarin glycoside and cardiac glycoside.

Quantification of Total Phenolic Content (TPC): Total natural phenolics present in the extracts were determined using Folin Ciocalteu reagent. The total phenolic content of hexane, ethyl acetate, methanol and aqueous extracts of the leaves and roots of C. infortunatum was evaluated according to the method given by [11]. An aliquot of 0.5 ml of the extract was treated with 2.5 ml of Folin-Ciocalteu's reagent (1:10 diluted with water), mixed thoroughly and after 5 min, 2 ml of 7.5 % Na_2CO_3 was added. The reaction mixture was kept for incubation at 45 ºC for 15 min. The blue colour developed was read at 760 nm using a UV-VIS spectrophotometer (UV-1700 Pharma spec, Shimadzu). Gallic acid was used as the reference standard, and the total phenolic content (TPC) was estimated from the calibration graph of gallic acid. The TPC in the extracts was calculated using the equation.

$$T = C \times V/M$$

Where, 'T' is the total phenolic content (mg/g) of the extracts, 'C' is the concentration of Gallic acid (mg/ml) obtained from the calibration graph, 'V' is the volume of the extract taken (ml) and 'M' the weight of the extract (g). The result was expressed as milligram gallic acid equivalents (mg GAE)/g of sample dry weight.

Quantification of Total Flavonoid Content (TFC): The total flavonoid content was estimated using the Aluminium chloride colorimetric method reported by [12]. To 0.5 ml extract, 1.5 ml of methanol, 0.1 ml of 10 % $AlCl_3$ (diluted in

methanol), 0.1 ml of 1 M potassium acetate (CH_3COOK) and 2.8 ml of distilled water were added. The mixture was then kept for incubation at room temperature (35 ºC) for 30 min. The absorbance was measured at 415 nm. In the blank aluminium chloride was substituted by the same amount of methanol, and quercetin served as the standard.

Total flavonoid content was calculated from the calibration graph plotted for quercetin using the formula.

$$T = C \times V/M$$

Where, 'T' is the total flavonoid content (mg/g) of the extracts, 'C' is the concentration of quercetin (mg/ml) obtained from the calibration graph, 'V' is the volume of the extract taken (ml) and 'M' is the weight of the extract (g). TFC is expressed as milligram quercetin equivalent (mg QE/g) sample dry weight.

Quantification of Total Terpenoid Content (TTC):

Dry Weight Method for Estimation of Terpenoid Yield: The terpenoid content present in *C. infortunatum* was determined according to the procedure reported by [13]. Dried plant extract 100 mg (wi) was taken and soaked in 9 ml of ethanol for 24 h. The extract, after filtration, was extracted with 10 ml of petroleum ether using a separating funnel. The ether extract was separated in pre-weighed glass vials and waited for its complete drying (wf). Ether was evaporated, and the yield (%) of total terpenoids content was measured by the formula.

$$(wi\text{-}wf/wi) \times 100$$

Total Antioxidant Activity:

DPPH (1-1-Diphenyl-2-Picryl Hydrazine) Free Radical Scavenging Assay: Quantitative measurement of radical scavenging property was carried according to the method reported by [14].

A methanolic solution of 1 ml of DPPH was added to 1 ml at different concentrations (50-1000 µg/ml) of the extracts and allowed to react at room temperature for 30 min in the dark. Absorbance was measured at 517 nm. Methanol served as blank, DPPH in methanol without the extract served as the control and ascorbic acid was taken as the reference standard. The lower absorbance of the reaction mixture indicated higher free radical scavenging activity.

The capacity to scavenge DPPH radical was calculated using the following equation:

% Radical scavenging activity = (Absorbance of control - Absorbance of sample) × 100 /Absorbance of Control

A graph was plotted with the percentage of inhibition against concentration. The IC_{50} values (concentration of the sample required to scavenge 50% of free radicals) were calculated from the graph's regression equation. The smaller the IC_{50} value higher will be the antioxidant activity of the extracts. The experiment was done in triplicate and presented in the form of mean ± standard deviation (SD) (n = 3).

Antibacterial Study:

Bacterial Strains and Culture Media: The studied microorganisms included reference (from the Microbial Type Culture Collection and Gene Bank, Chandigarh, India) strains of *Bacillus cereus* (MTCC 430), *Escherichia coli* (MTCC 729), *Salmonella typhi* (MTCC 3216) and *Pseudomonas aeruginosa* (MTCC 4676). A total of four bacteria strains were used in the experiment for the zone of inhibition and two bacteria were used in MIC assays. They were maintained on agar slant at 4 °C and sub-cultured on fresh appropriate agar plates 24 h prior to antimicrobial test. Nutrient Agar was used for the activation of bacteria.

Sample Preparation: Various concentrations of crude extracts were prepared in 1 ml each of methanol at the highest concentration possible depending on solubility. From this master dilution, 100 µl of extracts was used for testing. Cefotaxime at 5 mg/ml was used as the reference drug.

Inoculum Preparation: The tested microorganisms were separately cultured on a sterilized nutrient broth medium, and the culture was maintained at 28 ºC ± 2 ºC. The culture was swabbed over the nutrient agar plates at 37 ºC for 24 h by using streak plate method. Nutrient broth medium was prepared by adding the nutrient broth in distilled water and sterilized in an autoclave at a pressure of 151 lbs and temperature of 120 ºC. By

the standard method of inoculation, a loopful of the test organism was inoculated into 100 ml of nutrient broth and allowed to incubate at 37oC for 24 h until a slightly visible turbidity appeared. Inoculums were standardized to give a density of 106 colony-forming units / ml (CFU) / ml (corresponding to 0.5 McFarland standards). This was used as a starting inoculum for the assay.

Antibacterial Analysis by Agar Disc Diffusion Method: Antibacterial activity of the *C. infortunatum* extracts was carried out by the disc diffusion method described by [15]. First, the different extracts were dissolved in respective solvents at a concentration of 1 mg/ml. Then, 100 µl of bacterial inoculums containing 10^6 CFU/ml were spread over plates containing nutrient agar plates. The inoculum was spread uniformly over the entire agar surface using a sterile L-rod and allowed to dry for five minutes, and discs (6 mm in diameter) impregnated with 100 µl of the extract solutions were placed on the surface of the media. The control disc used contained Cifotaxime (5 mg/ml) as a positive control. The plates were incubated for 24 h at 37 °C and the experiments were performed in triplicates and the values were presented as mean ± SD (n=3). The diameters of inhibition zones were measured, and antibacterial activity was considered for diameters of inhibition zone greater than 10 mm.

Minimum Inhibitory Concentration (MIC): The MIC (minimal inhibitory concentration) was assessed for the effective plant samples tested during the disc diffusion assay. Bacterial tests were performed in nutrient agar broth and about 12 h-old bacterial cultures were used. The plant samples were first thoroughly dissolved in (10% DMSO) and then diluted to make a 1000 µg/ml concentration. The test was performed in sterile test tubes at 64, 128, 256 and 512 µg/ml concentrations with ethyl acetate root extract of *C. infortunatum*. The bacterial inoculum was spread uniformly over the entire agar surface using a sterile L-rod and allowed to dry for five minutes, and discs impregnated with 100 µl of the ethyl acetate root extracts solutions were placed on the surface of the media. The positive control was used containing Cefotaxime as a standard drug at a concentration of 5 mg/ml. The plate was covered with a sterile sealer and incubated for 24 h at 37 °C. The MIC was considered as the lowest concentration of the extract that completely inhibits bacterial growth. The lower the MIC, the higher the activity of the extract. MIC values of the plant samples were calculated against two bacterial strains isolates by broth dilution technique. Each test was replicated three times, and the MIC values of the extracts were determined as the lowest concentration that completely inhibited bacterial growth after 48th hr of incubation at 37 ºC.

Statistical Analysis: Analyses were done in triplicates, and the results were expressed as mean values with standard deviation and were calculated using MS Excel software, 2010. Correlation analysis was performed among IC_{50} value of DPPH and TPC and TFC and also a correlation among MIC values of antibacterial activity and TPC, TFC, and TTC. The comparison of the variable values of $P < 0.05$ was considered to indicate statistically significant differences.

RESULTS:

Extraction Yield: The percentage yield of leaves and roots crude extracts (hexane, ethyl acetate, methanol and aqueous) of *C. infortunatum*. is shown in **Table 1** and **Table 2.** Methanolic leaf extracts showed a higher percentage yield (8.02 %) and lowest was found in hexane leaf extracts (2.75 %). Methanolic root extracts exhibited a higher percentage yield (3.86 %), and the lowest was recorded in ethyl acetate (0.37 %) and hexane extracts (0.30 %).

This highlights that methanol is efficient in extracting phytochemicals from *C. infortunatum* leaf and root extracts than other extraction solvents; as methanol is highly polar, it can draw a variety of phytoconstituents when compared to other solvents.

TABLE 1: PERCENTAGE EXTRACTIVE AND CHARACTERISTICS OF *C. INFORTUNATUM* LEAVES EXTRACTS

Solvents	Appearance of leaf extract	Total weight (g) from 40 g	Percentage crude matter (%)
Hexane	Dark green colour, solid paste	1.1 g	2.75 %
Ethyl acetate	Dark green colour, semisolid paste	1.99 g	4.97 %
Methanol	Dark brown colour, semisolid paste	3.21 g	8.02 %
Aqueous	Brownish colour, solid paste	3.05 g	7.62 %

TABLE 2: PERCENTAGE EXTRACTIVE AND CHARACTERISTICS OF *C. INFORTUNATUM* ROOT EXTRACTS

Solvents	Appearance of root extract	Total weight (g) from 200 g	Percentage crude matter (%)
Hexane	Yellowish green, semisolid paste	0.8 g	0.37 %
Ethyl acetate	Yellowish green, semisolid paste	0.65 g	0.30 %
Methanol	Brownish, semisolid paste	8.32 g	3.86 %
Aqueous	Brownish, semisolid paste	3.3 g	1.53 %

Qualitative Phytochemical Screening: The results of qualitative analysis of phytochemicals in hexane, ethyl acetate, methanol and aqueous crude extracts of *C. infortunatum* leaves revealed the presence of bioactive components like alkaloids, carbohydrate, flavonoids, phytosterols, terpenoids, cardiac glycosides, phenols and tannins, and coumarin glycosides. Phytochemical analysis of *C. infortunatum* root extracts indicated the presence of alkaloids, carbohydrates, flavonoids, terpenoids, coumarin glycosides, phytosterols, phenols and tannins, and cardiac glycosides.

Carbohydrates, flavonoids, phenols, terpenoids, and coumarin glycoside were present in all the extracts. Phytosterols and alkaloids were present in hexane and ethyl acetate extracts and were absent in methanol and aqueous extracts. Cardiac glycoside was present in hexane, ethyl acetate, and methanol extracts but was absent in aqueous extract. Saponins were absent in hexane and ethyl acetate and present in methanol and aqueous extracts. Phenols, flavonoids and terpenoids are the major antioxidant and antibacterial compounds and the presence of these compounds was exhibited in all the solvent extracts, ranging from non-polar to polar.

Quantification of Total Phenolic Content (TPC): Phenolic compounds contain hydroxyl groups (-OH) that facilitate their free radical scavenging activity and serve as antioxidants; the total phenolic content could be used as a basis for the rapid determination of antioxidant activity [16]. The TPC was expressed in terms of gallic acid equivalents (mg of GAE/gm sample) using the following equation based on the calibration curve:

$$Y = 0.021 \times + 0.036, R^2 = 0.993$$

Where '×' is the absorbance and 'Y' is the mg GAE/gm sample. The values obtained for the concentrations of total phenolic content are expressed as mg of GAE/gm of a sample. From the obtained standard graph and evaluation, it was found that total phenolic content of *C. infortunatum* leaves extract in ethyl acetate, methanol, aqueous, and hexane was found to be 63.83 mg GAE/g, 60.90 mg GAE/g, 56.09 mg GAE/g and 16.52 mg GAE/g respectively at a concentration of 500 µl. The total phenolic content of *C. infortunatum* root extract was found to be 87.21 mg GAE/g, 29.59 mg GAE/g, 29.09 mg GAE/g, and 10.04 mg GAE/g in ethyl acetate, methanol, aqueous and hexane extracts, respectively. The total phenolic content of ethyl acetate root extract of *C. infortunatum* was observed to be maximum (87.21 mg GAE/gm) and that of aqueous extract was the lowest (10.04 mg GAE/gm) at a concentration of 500 µl. The total phenolic content of ethyl acetate leaves extract was found to be the highest (63.83 mg GAE/gm) and that of aqueous extract was the lowest (16.52 mg GAE/gm) at a concentration of 500 µl. *C. infortunatum* leaves and root extracts contained appreciable amounts of phenolic contents. **Fig. 1** shows the TPC of *C. infortunatum* leaves and root extracts.

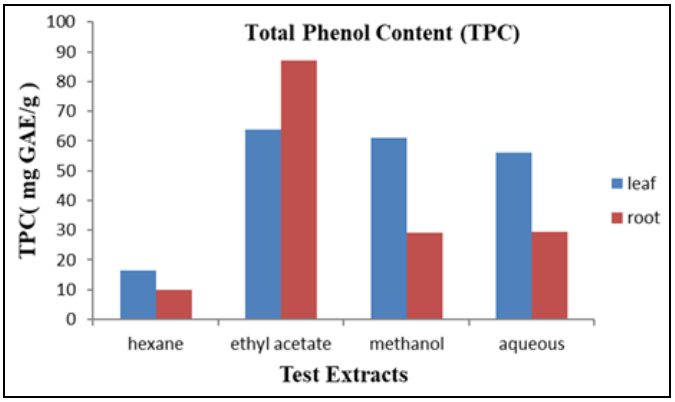


FIG. 1: DETERMINATION OF TOTAL PHENOL CONTENT (TPC)

Quantification of Total Flavonoid Content (TFC): Flavonoids are exceedingly effective antioxidants and are considered the most significant and broadly distributed single group of phenols present in plants. The TFC was expressed in terms of quercetin equivalents (mg of QE/gm sample) using the following equation based on the calibration curve

$$Y = 0.006 \times + 0.041, R^2 = 0.993$$

Where, 'x' is the absorbance and 'Y' is the mg QE/gm sample. The total flavonoid content of *C. infortunatum* leaves and root extracts was estimated using $AlCl_3$ method. The total flavonoid content of *C. infortunatum* leaves extract in ethyl acetate, hexane, methanol, and aqueous extract found to be 244.16 mg QE/g, 107.16 mg QE/g, 64.25 mg QE/g) and 27 mg QE/g respectively. The total flavonoid content of *C. infortunatum* root extract in hexane, methanol, ethyl acetate and aqueous extracts was found to be 38.83 mg QE/g, 35.5 mg QE/g, 32.41 mg QE/g) and 15.5 mg QE/g respectively.

The total flavonoid content of ethyl acetate leaves extract of *C. infortunatum* was observed to be maximum (244.16 mg QE/gm) and that of aqueous extract was the lowest (27 mg QE/gm) at a concentration of 500 µl. The total flavonoid content of hexane root extracts was found to be the highest (38.83 mg QE/gm) and that of aqueous extract was the lowest (15.5 mg QE/gm) at a concentration of 500 µl. The total flavonoid content was found to be the highest in ethyl acetate leaves extract and in hexane root extract. *C. infortunatum* leaves and root extracts contained appreciable amounts of flavonoid contents. **Fig. 2** shows the TFC of *C. infortunatum* leaves and root extracts.

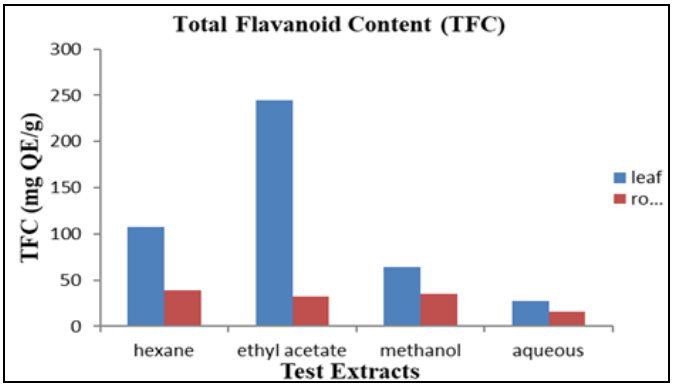


FIG. 2: DETERMINATION OF TOTAL FLAVONOID CONTENT (TFC)

Quantification of Total Terpenoid Content (TPC): The extracts obtained from *C. infortunatum* were evaluated for the total terpenoid content. The total terpenoid content of *C. infortunatum* leaves extract in hexane, ethyl acetate, methanol and aqueous was found to be 0.999 g, 0.999 g, 0.999 g, and 0.998 g, respectively. The total terpenoid content of root extracts in hexane, ethyl acetate, methanol and aqueous was found to be 0.999 g, 0.999 g, 0.999 g, and 0.999 g, respectively. The total terpenoid content was found to be the same in all the extracts.

Antioxidant Activity by DPPH Method: DPPH assay was used for measuring the scavenging activity of different extracts of *C. infortunatum* leaves and roots. The DPPH radical scavenging activity was evaluated using different concentrations (200, 400, 600, 800, 1000 µg/ml) of *C. infortunatum* leaves, root extracts, and ascorbic acid as standard for comparison.

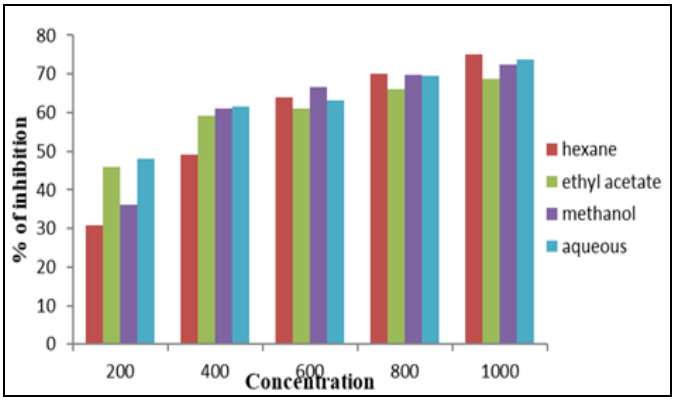


FIG. 3: DPPH RADICAL SCAVENGING BY *C. INFORTUNATUM* LEAF EXTRACTS

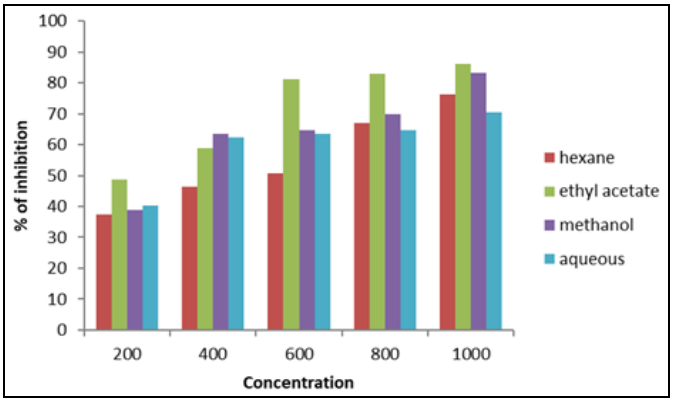


FIG. 4: DPPH RADICAL SCAVENGING BY *C. INFORTUNATUM* ROOT EXTRACTS

A 1000 µg/ml of hexane, ethyl acetate, methanolic and aqueous leaves extract exhibited 75.0%, 68.72%, 72.33% and 73.71% inhibition respectively and IC_{50} values of 394.37 µg/ml, 246.04 µg/ml, 294.04 µg/ml and 217.9 µg/ml respectively. A 1000 µg/ml of hexane, ethyl acetate, methanolic and aqueous root extract exhibited 76.11 %, 86.25 %, 83.16 % and 70.44 % inhibition respectively and IC_{50} values of 489.59 µg/ml, 223.63 µg/ml, 294.41 µg/ml and 285.71 µg/ml respectively. The standard ascorbic acid exhibited an IC_{50} value of 23.27 µg/ml. Aqueous leaves extract and ethyl acetate root extract has the

highest antioxidant activity with an IC_{50} value of 217.9 µg/ml and 223.63 µg/ml. **Fig. 3** and **4** show the radical scavenging activities of *C. infortunatum* leaves and root extracts at different concentrations. All values are expressed as mean ± SD of the three replicates.

Antibacterial Activity: The biochemical or physiological action of the plant cannot be determined only by the preliminary phytochemical analysis. Therefore, the *in-vitro* antibacterial activity was determined by measuring the zone of inhibition and MIC against pathogenic bacterial strains and the results are depicted in **Table 3**, **Table 4** and **Fig. 5.** *C. infortunatum* root extracts showed antibacterial activity against the tested bacterial strains. In the present investigation, *C. infortunatum* leaves extract did not exhibit antibacterial activity against the test bacteria. The ethyl acetate root extract of *C. infortunatum* exhibited antibacterial activity against the test bacteria. The maximum zone of inhibition was recorded in ethyl acetate root extract for *Pseudomonas aeruginosa* and *Escherichia coli* (16 mm) and minimum for *Salmonella typhi* (13 mm). *C. infortunatum* root extract was found to be active against *Pseudomonas aeruginosa* and *Escherichia coli* and *Salmonella typhi.*

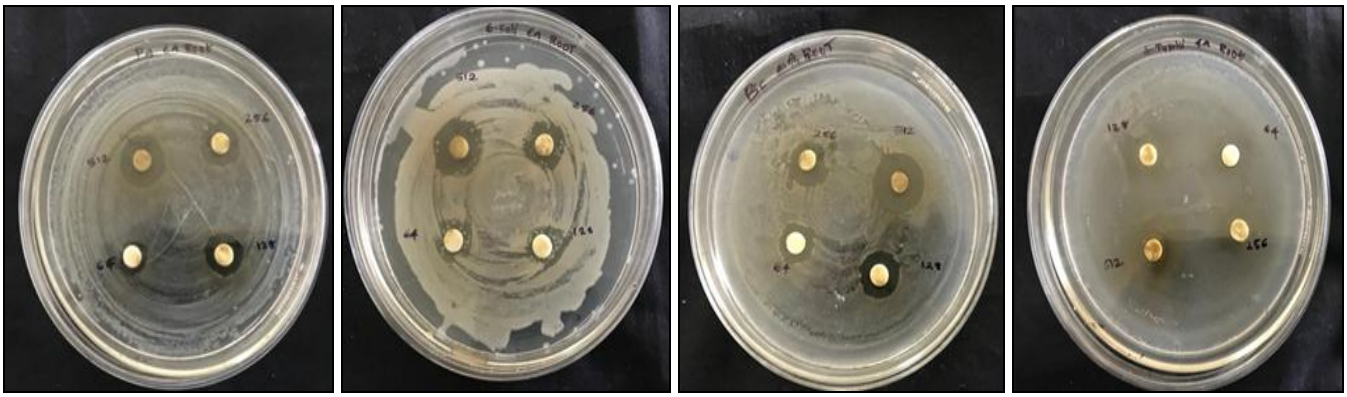
Diameter of Zone of Inhibition of Extracts of *C. Infortunatum* Against Bacteria in Disc Diffusion Method: In disc diffusion method, among the four root extracts, ethyl acetate root extract of *C. infortunatum* exhibited potent activity against all the test organisms, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Escherichia coli* with a zone of inhibition of 15 mm, 16 mm, 13 mm and 16 mm respectively. *C. infortunatum* leaves extract did not exhibit antibacterial activity against the test organisms. Ethyl acetate root extract was found to be most active against all the bacterial pathogens and exhibited significant activity when compared with the control antibiotic, Cefotaxime (5 mg) **Table 4.**

TABLE 3: ZONES OF INHIBITION OF MICROBIAL GROWTH BY *C. INFORTUNATUM* ROOT EXTRACTS

Tested Strains	Zone of Inhibition (mm diameter)			
	Hexane	Ethyl acetate	Methanol	Aqueous
B. cereus	Nil	15 mm	Nil	10 mm
P. aeruginosa	Nil	16 mm	10 mm	Nil
S. typhi	Nil	13 mm	Nil	Nil
E. coli	10 mm	16 mm	Nil	10 mm

Values obtained in triplicate and data expressed as mean ± SD

Minimum Inhibitory Concentration: MIC was determined for the four pathogenic bacterial organisms against the positive control, Cefotaxime, and the antibacterial activity was observed.



BC: *BACILLUS CEREUS*; PA: *PSEUDOMONAS AERUGINOSA*; *S TYPHI*: *SALMONELLA TYPHI*; *E. COLI: ESCHERICHIA COLI*; EA: ETHYL ACETATE

FIG. 5: MIC STUDY OF ETHYL ACETATE ROOT EXTRACTS AT 64, 128, 256 AND 512 µg/ml CONCENTRATIONS

The ethyl acetate root extracts showed significant activity when compared with other extracts. The ethyl acetate root extract at different concentrations (64, 128, 256 and 512 µg/ml) was tested against all test bacteria **Fig. 5.** The microorganisms that exhibited the highest activity when compared with

the control cefotaxime were *P. aeruginosa* (13 mm) and *E. coli* (12 mm) at 128 µg/ml. It was observed that on increasing the concentration of extracts, the anti-bacterial activity against tested pathogenic bacterial strains also increased, which indicated that antibacterial activity is concentration-dependent **Table 4.**

TABLE 4: ANTIBACTERIAL ACTIVITY OF ETHYL ACETATE ROOT EXTRACTS

Tested Strains	Zone of Inhibition (mm diameter)				
	Ethyl acetate root extract				Cifotaxime (5 mg/ml)
	64 µg/ml	128 µg/ml	256 µg/ml	512 µg/ml	
B. cereus	10 mm	13 mm	15 mm	18 mm	16 mm
P. aeruginosa	10 mm	13 mm	14 mm	15 mm	14 mm
S. typhi	10 mm	10 mm	11 mm	12 mm	17 mm
E. coli	10 mm	12 mm	14 mm	15 mm	12 mm

Values obtained in triplicate and data expressed as mean ± SD

Statistical Analysis: The correlation of total phenolic and flavonoid content with antioxidant activity is shown in **Tables 5** and **6.** There was a significant negative correlation between IC_{50} of DPPH and total flavonoids of leaf extracts (R^2 = -0.991, $p < 0.05$) and exhibited a weak positive correlation with the total phenolic content (R^2 = 0.272, $p > 0.05$), depicted in **Table 5.** There was a nonsignificant positive correlation between total phenols and IC_{50} value of DDPH with the root extracts (R^2 = 0.540, $p > 0.05$) and a significant negative correlation between total flavonoids and IC50 value of DPPH with the root extracts (R^2 = -0.925, $p < 0.05$), depicted in **Table 6**. The correlation study indicated that the total flavonoid content may have contributed to the antioxidant activities of *C. infortunatum* ethyl acetate leaves and root extracts.

TABLE 5: CORRELATION OF TOTAL PHENOL, TOTAL FLAVONOID AND IC_{50} VALUE OF DPPH OF LEAVES EXTRACT

Leaf	Phenol (R^2 value)	Flavonoid (R^2 value)	IC_{50} value of DPPH
TPC	1		
TFC	-0.14323	1	
IC_{50} value of DPPH	0.272686	-0.99124	1

TABLE 6: CORRELATION OF TOTAL PHENOL, TOTAL FLAVONOID AND IC_{50} VALUE OF DPPH OF ROOT EXTRACT

Root	Phenol (R^2 value)	Flavonoid (R^2 value)	IC_{50} value of DPPH
TPC	1		
TFC	-0.18073	1	
IC_{50} value of DPPH	0.540488	-0.92518	1

The Correlation analysis of total phenolic, total flavonoid and total terpenoid contents with minimum inhibitory concentration values of *E. coli* and *P. aeruginosa* were shown in **Table 7.** The correlation analysis between the total phenolics and MIC showed a nonsignificant negative correlation (R^2 = -0.814, $p > 0.05$) and (R^2 = -0.863, $p > 0.05$). The correlation between the total flavonoid and MIC showed a nonsignificant moderate positive correlation (R^2 = 0.433, $p > 0.05$) and (R^2 = 0.349, $p > 0.05$). The correlation analysis between the total terpenoid values and MIC showed a significant high positive correlation (R^2 = 0.970, $p < 0.05$) and (R^2 = 0.988, $p < 0.05$). It can be predicted that the terpenoid compounds in ethyl acetate root extracts are the main components contributing their antibacterial activities than phenol and flavonoid compounds.

TABLE 7: CORRELATION BETWEEN MIC AND TPC, MIC AND TFC AND MIC AND TTC FOR THE MICROORGANISMS STUDIED

Correlation	*E. coli* (R^2 value)	*P. aeruginosa* (R^2 value)
MIC x TPC	-0.814	- 0.863
MIC x TFC	0.433	0.349
MIC x TTC	0.970	0.988

DISCUSSION: Medicinal plants contain secondary metabolites, which could combat diseases and pathogens. To validate the ethnomedicinal claims associated with Clerodendrum genus, it is essential to elucidate their active principles since plants possess a wide array of chemical compounds that produce a definite physiologic action on the human body. Preliminary screening of leaf and root extracts of *C. infortunatum* revealed the presence of different active phytochemicals. Among them, aqueous extract possessed maximum types of phytochemicals such as alkaloids, carbohydrate,

flavonoids, phytosterols, terpenoids, cardiac glycosides, phenols and tannins and coumarin glycosides. Overall, the combination of phytochemicals found in *C. infortunatum* along with phenols and flavonoids make them a possible source of medicine as a curative remedy for oxidative stress-related diseases. The percentage yield studies go in accordance with the studies of [17]. The quantitative phytochemical screening of *C. infortunatum* revealed a higher quantity of total phenols in ethyl acetate leaves extract (63.83 mg GAE/g) and ethyl acetate root extract (87.21 mg GAE/g). The total flavonoid content was found to be higher in ethyl acetate leaves extract (244.16 mg QE/g) and ethyl acetate root extract (38.83 mg QE/g). A smaller quantity of total phenols was observed in aqueous leaves and root extract. It is evident that ethyl acetate solvents are effective in isolating the biological compounds based on polarity.

The radical scavenging activity was found to be higher in aqueous leaves extract with an IC_{50} value of 217.9 µg/ml and ethyl acetate root extract with an IC_{50} value of 223.63 µg/ml. The antioxidant activity of *C. infortunatum* root bark and leaves extract by DPPH method was studied. It was found that acetone extract exhibited the highest activity and was comparable with the standard, ascorbic acid 18. It is seen that ethyl acetate and acetone are both medium polar solvents. In the present work, leaf and root extracts of *C. infortunatum* showed promising results in the correlation analysis between total flavonoid content and antioxidant activity. The methanolic root extract of *C. infortunatum* L. contained flavonoids was reported by 7 and 8 and that flavonoids are responsible for the antioxidant property of *C. infortunatum*.

Among the four root extracts, ethyl acetate root extracts exhibited potent activity against all the test organisms with a maximum zone of inhibition against *P. aeruginosa* (16 mm) and *E. coli* (16 mm). The correlation analysis between the TTC and MIC values of *P. aeruginosa* and *E. coli* showed a significant positive correlation ($p > 0.05$). The leaves extract of *C. infortuatum* did not exhibit antibacterial activity against the test organisms. The antibacterial activity of *C. infortunatum* and *C. paniculatum* was evaluated by measuring the zone of inhibition and reported that ethanolic root extract at 100 µg was found to be more effective against *Klebsiella pneumoniae* and *E. coli* when compared with the standard [19].

In another study, the antibacterial activities of *C. infortunatum* were studied and reported that the ethyl acetate fraction of leaf and root showed the highest antibacterial activity against *Bacillus megaterium*, *K. pneumoniae,* and *Salmonella typhi* [20]. The presence of phytoconstituents in these extracts generally interferes with the growth and metabolism of microorganisms and it could be analyzed by determining the minimum inhibitory concentration. *P. aeruginosa* (16 mm) and *E. coli* (16 mm) exhibited the highest activity when compared with the control cefotaxime at 128 µg/ml. The correlation analysis between the total terpenoid and MIC values showed a significant positive correlation ($p < 0.05$). In the present work, ethyl acetate root extract of *C. infortunatum* showed promising results in total terpenoid content and antibacterial activity.

C. infortunatum leaves and roots were used in the present study due to its ease of availability and immense uses in traditional medicine. The results of the study revealed that *C. infortunatum* leaves and root extracts are a potential source of phytochemicals that contributes to its antioxidant and antibacterial properties. All the different extracts of *C. infortunatum* exhibited antioxidant activity using DPPH assay. TFC in leaves and root extracts had a significant negative correlation with their IC_{50} values of DPPH, and TTC in roots showed a positive correlation with the MIC values.

The results could be attributed to the flavonoid compounds in the leaves and roots extracts of *C. infortunatum* to their antioxidant activity by the DPPH method, and terpenoids contributed to its antibacterial activity. From the obtained results, when the efficacy of all the four extracts were compared, it was found that ethyl acetate leaves and root extract of *C. infortunatum* had remarkable antioxidant and antibacterial activity and this plant can be used to discover natural bioactive molecules that can provide enormous therapeutic potential in developing new pharmaceutical drugs with lesser side effects. *C. infortunatum* leaves and roots may have many benefits to prevent oxidative stress, and the roots possess antibacterial potential.

The active compounds responsible for antioxidant and antibacterial activity in *C. infortunatum* is not found out to date, and further research could be done to find out the new active metabolites.

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REFERENCES:

1. Jima TT and Megersa M: Ethnobotanical study of medicinal plants used to treat human diseases in Berbere district, bale zone of oromia regional state, south east ethiopia. Evidence Based Complementary and Alternative Medicine 2018; 2018(2): 1-16.
2. Gopinathan M and Balasubramanian M: Correlation analysis of phytochemicals and antioxidant activities of Heliotropium indicum L. leaves extract. International Journal of Pharmaceutical Sciences and Research 2021; 12(8): 4386-93.
3. Swargiarya A, Brahmab K, Boroa T, Daimaria M and Roya MK: Study of phytochemical content, antioxidant and larvicidal property of different solvent extracts of *Clerodendrum infortunatum* and *Citrus grandis*. Indian Journal of Traditional Knowledge 2020; 20(2): 329-24.
4. Sindhu TJ, Akhilesh KJ, Jose A, Binsiya KP, Thomas B and Wilson E: Antibacterial screening of *Clerodendrum infortunatum* leaves: experimental and molecular docking studies. Asian Jour of Resea in Chem 2020; 13(2): 128-32.
5. Gopinathan M, Manju S and Chandran RP: Phytochemical, antibacterial, antioxidant and cytotoxic activities of methanol and aqueous leaf extracts of *Pyrrosia heterophylla* (L.) M.G. Price. International Journal of Current Research in Biosciences and Plant Biology 2019; 6(5): 23-38.
6. Singh P, Goel A and Sehgal M: Phytochemical analysis and antimicrobial activity of *Madhuca longifolia* and *Clerodendrum infortunatum*: medicinal plants for application as textile finishes. International Journal of Chemical Studies 2018; 6(5): 2031-40.
7. Barman TK., Kalita P and Pal TK: Comparative evaluation of total flavonoid content and antioxidant activity of methanolic root extract of Clerodendrum infortunatum and methanolic whole plant extract of Biophytum sensitivum. International Journal of Pharmaceutical Sciences Review and Research 2013; 22(1): 62-66.
8. Modi AJ, Khadabadi SS, Farooqui IA and Ghorpade DS: Antioxidant effects of leaves of *Clerodendrum infortunatum*, *Argyreia nervosa* and *Vitex negundo*: A comparison. Der Pharmacia Lettre 2010; 2(1): 102-05.
9. Prasad MP, Sushant S and Chikkaswamy BK: Phytochemical analysis, antioxidant potential, antibacterial activity and molecular characterization of *Clerodendrum species*. International Journal of Molecular Biology 2012; 3(3): 71-76.
10. Harborne JB and Williams CA: Advances in flavonoid research since 1992. Phytochemistry 2000; 6: 481-04.
11. Singleton VL and Rossi JA: Colorimetry of total phenolics with phosphomolybdic acid reagents. American Journal of Enology and Viticulture 1965; 16(48): 144-58.
12. Woisky RG and Salatino A: Analysis of propolis: Some parameters and procedures for chemical quality control. Journal of Apicultural Research 1998; 37: 99-05.
13. Indumathi C, Durgadevi G, Nithyavani S and Gayathri PK: Estimation of terpenoid content and its antimicrobial property in *Enicostemma litorrale*. International Journal of ChemTech Research 2014; 6(9): 4264-67.
14. Mensor LL, Menezes FS, Leitao GG, Reis AS, dos Santos TC, Coube CS and Leitao SG: Screening of brazilian plant extracts for antioxidant activity by use of dpph free radical method. Phytotherapy Research 2011; 15: 127-30.
15. Bauer AW, Kirby WM, Sherris JC and Turck M: Antibiotic susceptibility testing by a standard single disk method. Amer Jour of Clin Pathology 1966; 45: 493-96.
16. Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R and Koirala N: Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from western nepal. Plants 2019; 8.
17. Haris M, Mahmood R, Rahman H and Rahman N: *In-vitro* cytotoxic activity of *Clerodendrum infortunatum* L against T47D, PC-3, A549 and HCT-116 human cancer cell lines and its phytochemical screening. International Journal of Pharmacy and Pharmaceul Sciences 2015; 8(1): 439-44.
18. Helen LR, Jyothilakshmi M and Latha MS: Phytochemical and antioxidant screening of various extracts of the root bark and leaf of Clerodendrum infortunatum Linn. Pharmacophore 2014; 5(2): 343-51.
19. Leena PN and Aleykutty NA: Comparative study on antibacterial activities of *Clerodendron infortunatum* Linn and *Clerodendron paniculatum* linn root extract. International Journal of Advances in Pharmacy Biology and Chemistry 2012; 1(3): 325-27.
20. Waliullah TM, Yeasmin AK, Alam A, Islam W and Hassan P: *In-vitro* antimicrobial study for biological evaluation of *Clerodendrum infortunatum* Linn. Recent Patents on Anti-Infective Drug Disc 2015; 10(2): 98-04.

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