INTRODUCTION

Medicinal plants are natural properties of bioactive phytochemical constituents which, for the physiological activities delivered on the human organism, can be employed against various ailments. Therefore, it is essential for medicinal plants to be evaluated for their phytochemical attributes by qualitative methods, Fourier transform infrared (FTIR), antioxidant, antibacterial, and bioactive compound properties. The bactericidal activity of the in vivo stem and in vitro callus extract has been evaluated in both Gram +ve and Gram -ve microorganisms using the disk diffusion method.

RESULTS

The highest frequency (78%) of well-developed, dark green organogenic callus was induced from stem explant on Murashige and Skoog (MS) medium supplemented with 0.7 mg/l 2,4-Dichlorophenoxyacetic acid (2, 4-D) and 0.5 mg/l benzylationdine (BA). The results of FTIR spectra confirmed the presence of functional groups in wild stem and in vitro callus extract of *C. halicacabum* with various peaks. The total phenolic content in ethanolic extract of in vivo plant and in vitro callus was 80.46 mg gallic acid equivalent (GAE)/g dry weight and 76.4 mg GAE/g dry weight, respectively. The highest percentage of tannins was measured at 78.03 in wild stem ethanol extracts followed by 75.22 in callus extract. The antioxidant activity of 2,2-diphenyl-2-pircylhydrazyl (DPPH) ethanol extract was found to be 206.54 µg/ml IC50 values of the stem extracts of *C. halicacabum* are 306 µg/ml and 286 µg/ml in callus extract, respectively. Antimicrobial activity of the ethanol extract was higher for *Staphylococcus aureus* (S. aureus) with a 17 mm zone of inhibition.

CONCLUSION

The present investigation recommended that the callus ethanolic extract function as a good source of biologically active compounds and natural antioxidants.

Keywords: *Cardiospermum halicacabum*, Anti-bacterial activity, Phytochemicals, Antioxidant activity.

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Preparation of extracts
The in vivo grown plant was collected and shade dried for 2–3 weeks. The dried plant materials were then ground into a coarse powder in a mechanical blender and kept in an airtight container. The coarsely powdered sample was weighed (50 g) and the extract was prepared and kept on a rotator shaker with 100 ml of ethanol, acetone, petroleum ether, and water solvents each alone for 2 days. The stem extracts were filtered through Whatman No.1 filter paper and collected in a 1 l beaker and they were covered with aluminum foil to avert evaporation. The stem extract of C. halicacabum was obtained by using three types of solvents (Petroleum ether, Ethanol, and Aqueous) and stored in an airtight container at 4°C. Further, the dried residue was preserved in an airtight container or glassware for further analysis. The in vitro grown callus was dried at room temperature for 3 days. The dried callus was macerated using a mortar and pestle. Ten grams of coarsely macerated callus were subjected separately for the extraction using 100 ml distilled water and solvent such as petroleum ether, chloroform, and ethanol.

Preliminary phytochemical studies
The ethanol extract was evaluated for the following phytochemical components such as reducing sugars, terpenoids, alkaloids, tannins, steroids, flavonoids, saponins, phenolic compounds, amino acids, and anthraquinones.

Test for alkaloids
Mayer's test
Two milliliters plant sample extract, two drops of Mayer’s reagent are included in a test tube. The form of white creamy precipitate specified the existence of alkaloids [6].

Test for phenols
Ferric chloride test
Fifty milligrams extract was dissolved with distilled water and added few drops of neutral 5% ferric chloride solution. The form of a dark green color specified the existence of the phenolic compound [7].

Test for flavonoids
Lead acetate test
One milliliters of the crude extract added with two to three drops of dilute sodium hydroxide. A strong yellow color exhibited in the crude extract, which turns colorless with the inclusion of a few drops of dilute acid which signify the existence of flavonoid [8].

Test for reducing sugars
Plant extract (1 ml) was added with 1 ml of each Fehling’s solution A and B and boiled in a water bath. The presence of red precipitate specified the existence of reducing sugars [9].

Test for tannin
Gelatin test
One milliliters of plant extract was added with 5 ml of distilled water and 1% of gelatine was mixed into the test tube. Afterward, 10% Sodium chloride (NaCl) solution was added. The appearance of a white precipitate displayed the existence of tannin [10].

Test for anthraquinones
Ammonium hydroxide test
The plant extract (10 mg) was dissolved in isopropyl alcohol and a few drops of concentrated ammonium hydroxide solution were added. After 2 min a form of red color indicated the existence of anthraquinones [11].

Test for terpenoids
The plant extract (5 ml) added with chloroform (1 ml) and then placed in a water bath and 3 ml of concentrated Sulphuric acid (H_2SO_4) was included ultimately boiled on a water bath. The presence of grey color specified the existence of terpenoids [11].

Test for amino acids
Biuret test
The plant extract (2 ml) was added with one drop of 2% copper sulphate solution. Afterward, 1 ml of 95% ethanol and few Potassium hydroxide (KOHI) pellets were added. The presence of pink color formation confirms the existence of amino acids [12].

Test for steroids
Salkowski test
Plant extract (2 ml) was added with 2 ml of chloroform and 2 ml of H_2SO_4. The presence of a reddish-brown ring junction confirms the existence of steroids [13].

Determination of total phenolic content
Folin-Ciocalteu assay was used for the determination of the total phenol content [14]. Briefly, in a volumetric flask (25 ml), 1 ml of the extract was added with 9 ml of distilled water then 1 ml of Folin-Ciocalteu phenol reagent was added and shaken well. Ten milliliter of 7% sodium carbonate (Na_2CO_3) was added to the reaction mixture after 10 min. Afterward, the solution was incubated for 60 min at room temperature and the absorbance was measured at 550 nm with a UV visible spectrophotometer: Gallic acid at different concentrations (0.2, 0.4, 0.6, 0.8, and 1.0 mg/ml) was used as a standard. In the same manner, as delineated earlier and the results were expressed as mg of gallic acid equivalent (GAE)/g of extract.

Determination of tannin content
The total tannin content was firm by Folin-Ciocalteu method with slight variations [15]. Approximately, 0.1 ml of the plant extract was added to a volumetric flask (10 ml) comprising 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent. One milliliters of 35% Na_2CO_3 solution diluted with 10 ml of distilled water. The reaction mixture was stirred well and kept at room temperature for 15 min. The different concentrations (0.2, 0.4, 0.6, 0.8, and 1.0 mg/ml) of gallic acid standard solutions were prepared in the same manner as described earlier. The absorbance was determined against the blank at 725 nm. The content of tannin was calculated as mg of tannic acid equivalents (TAE)/g of plant extract.

Antioxidant activity
Hydroxyl radical scavenging assay
Hydroxyl radical scavenging activity of the extracts was examined as stated by the earlier described technique [16]. The reaction mixture enclosed 1 ml of various concentration of extracts (2–10 mg/ml), 1.0 ml of iron-ethylenediamine tetra acetate acid (EDTA) solution (1.5 mM ferrous ammonium sulfate and 0.1 mM EDTA), 0.018% EDTA 0.5 ml, DMSO 1.0 ml (0.85% in 0.1 mol/l phosphate buffer pH 7.4), and 0.22% ascorbic acid 0.5 ml. The firmly enclosed reaction contained test tubes were heated in a water bath at 70–80°C for 20 min, this process was ended by the addition of ice-cold trichloroacetic acid (TCA) (1.0 ml) (17.5%). Eventually, Nash reagent 3.0 ml (75.0 g of ammonium acetate, 3.0 ml of glacial acetic acid, and 2.0 ml of acetic acid) and distilled water was added to a total volume of 1 l) was added and kept room temperature for 15 min for color change. The formation of yellow color developed was measured at 412 nm and gallic acid was used as a standard. The IC50 value for the extract and standard preparation was assessed using the following formula,

% scavenging/Inhibition = |[Absorbance of control–Absorbance of test sample]/Absorbance of control| × 100
**Ferric reducing antioxidant potential (FRAP) assay**

The reducing power capability of the extracts was investigated as stated by the earlier described method [17]. In brief, 1.0 ml of different concentrations of extracts (20 µg/ml–100 µg/ml), 2.5 ml of 1% potassium ferricyanide, and 2.5 ml of 0.2 mol/L sodium phosphate buffer were mixed well and incubated at 60°C for 20 min. Afterward, the reaction was ended by the addition of 2.5 ml of 10% trichloroacetic acid, followed by centrifugation at 2000 rpm/min for 15 min. Ultimately, the upper layer (2.5 ml) was mixed with deionized water (2.5 ml) and 0.5 ml of 0.1% ferric chloride. Ascorbic acid was used as standard and the absorbance was measured at 700 nm.

**2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay**

The capability of the ethanolic extract of *C. halicacabum* to scavenge the DPPH free radical was determined according to the previously described method [18] with minor modification. The extract at different concentrations (20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, and 100 µg/ml) was prepared. Each sample (0.5 ml) was prepared and mixed with 0.5 ml of 1 mM DPPH solution in ethanol. The reaction mixture was kept at room temperature for 20 min. The reduction ability of DPPH radicals was measured by decreased induction of antioxidants in their absorbance at 517 nm. The ascorbic acid was used as a standard and similar concentrations were prepared as the test solutions. The difference in absorbance between the test and the control (DPPH in ethanol) was determined and expressed as % scavenging of DPPH radical. The ability to scavenge the DPPH radical was measured using the following equation.

\[
\% \text{ DPPH radical scavenging activity} = \left( \frac{(A_0 - A_1)}{A_0} \right) \times 100
\]

where *A*<sub>0</sub> is the absorbance of the control, and *A*<sub>1</sub> is the absorbance of the extract/standard. Afterward, the % of inhibition was calculated against concentration, and from the graph IC<sub>50</sub> was determined.

**Fourier transform infrared (FT-IR) analysis**

FTIR spectrophotometer is possibly the most efficient tool to find the types of chemical bonds and functional groups existing in compounds. Dried powder of *C. halicacabum* ethanolic extract was used for FTIR analysis. The powdered ethanolic extract (10 mg) was placed in 100 mg of potassium bromide (KBr) pellet, to prepare a translucent sample disc. The powdered sample was loaded on to FTIR spectroscope and the spectroscopy results were documented on an FTIR spectrometer alpha II Compact Bruker, with a scan range from 400 to 4000 cm<sup>-1</sup>.

**Antibacterial activity**

**Test microorganism**

The antibacterial properties of *C. halicacabum* extracts were examined against Gram-positive bacteria: *Staphylococcus aureus, Enterococcus faecalis*, and Gram-Negative bacteria: *Moraxella sp., Escherichia coli*, and *Proteus mirabilis*. The bacterial strains were obtained from the Department of microbiology, Alagappa University, Karaikudi.

**Antibacterial assay**

The disk diffusion method was used to examine the antibacterial activity of the *C. halicacabum* extract. The bacterial strains were inoculated in the nutrient broth under aseptic conditions and incubated at 37°C for 24 h. After the incubation period, the test bacteria were inoculated on the nutrient agar (HiMedia) plate using a sterile cotton swab. The different concentrations of petroleum ether, chloroform, acetone, ethanol, and aqueous extracts (200, 400, 600, and 800 µg/disc) were loaded on the 6 mm sterilized discs (HiMedia) to measure the dose-dependent activity of the extracts. Cefepime (30 mcg/disc) was used as a positive control and the individual solvents were used as a negative control. Afterward, the Petri plates were kept at incubator (37°C) for 18–24 h. The zones of inhibition and the mean diameter were documented.

**Statistical analysis**

Each experiment of quantitative phytochemical analysis and antibacterial activity was conducted in three replicates. Statistical analysis was performed by SPSS version 20.0 (SPSS Inc. Chicago, IL). Differences between means were verified using one-way ANOVA and the least significant difference test. The significance levels were considered at p<0.05.

**RESULTS AND DISCUSSION**

**Callus induction**

Callus induction was detected from cut margins of stem explants of *C. halicacabum* after 2 weeks of incubation, cultured on MS medium supplemented with auxins, namely, 2, 4-D, IAA, indolebutyric acid (IBA), and NAA (0.5–2.0 mg/l) along with BA (0.5 mg/l), under a partial incubation in dark (Table 1). Initial responses such as stem expand subsequently swelling of explant were observed from the 6<sup>th</sup> day of the culture period (Fig. 1a-d). Among various treatments, 2, 4-D along with BA gave the best callus initiation and proliferation, followed by NAA, IAA, and IBA (Table 1, Fig. 1b and e). Contingent on the concentration and combination of plant growth regulators (PGR) benefited, an extensive range of variation in the frequency of callus formation and nature of callus was observed. The fresh weight and dry weight of callus biomass for stem explants of *C. halicacabum* are shown in Table 1. The highest fresh weight was achieved on 0.5 mg/l IBA and 0.7 mg/l 2, 4-D. NAA at lower and higher concentrations produced light yellow to brown friable or nodular callus from stem explants, which were failed to respond further (Table 1). In contrast, NAA was reported to develop higher callus induction [19]. Medium containing 0.7 mg/l IAA and 0.5 mg/l BA produced yellowish green compact callus was obtained from stem explants, whereas IBA (0.7 mg/l) with BA (0.5 mg/l) produced dark brown callus with rooting. Likewise, Thaniarasu et al. [20] proposed that the modified callus did not form adventitious roots on medium with auxins but only with cytokinins; therefore, it is suggested that cytokinin has a stimulating effect on root formation from callus. The highest frequency (78%) of well developed, healthy, and uniform roots were obtained on MS medium supplemented with 2, 4-D (0.7 mg/l) + BA (0.5 mg/l), and 6 µM NAA. The best callus initiation and proliferation, followed by NAA, IAA, and IBA (Table 1, Fig. 1b and e).

**Fig. 1**: Callus induction and proliferation of *Cardiospermum halicacabum* L. (a) Stem explant, (b–d) Callus induction on MS + 2, 4-D (0.7 mg/l) + BA (0.5 mg/l), (e) Callus fresh weight (407 mg), (f) Callus dry weight (99 mg)
Table 1: Callus induction from leaf and internode explants of C. halicacabum cultured on MS medium supplemented with different concentrations of auxins with BA, after 4 weeks of culture

| PGR’s (mg/l) | % of response | Callus fresh weight mg/explant | Callus dry weight mg/explant | Texture of callus |
|--------------|---------------|--------------------------------|-----------------------------|-------------------|
| Control      | 0.0±0.0       | 0.0±0.0                         | 0.0±0.0                     | 0.0±0.0           |
| 2.4+ BA (0.5) | 0.5           | 63.0±1.5                        | 335.4±2.45                  | GC                |
|              | 0.7           | 78.0±1.6                        | 407.6±1.20                  | GC                |
|              | 1.0           | 58.0±1.3                        | 326.6±0.90                  | GC                |
|              | 2.0           | 44.0±1.6                        | 278.2±1.40                  | GC                |
| NAA+ BA (0.5) | 0.5           | 35.0±1.6                        | 268.1±0.64                  | YF                |
|              | 0.7           | 55.0±2.2                        | 349.3±1.33                  | YF                |
|              | 1.0           | 43.0±1.5                        | 308.9±0.38                  | YG                |
|              | 2.0           | 32.0±1.3                        | 230.2±0.41                  | YG                |
| IAA+ BA (0.5) | 0.5           | 32.0±1.3                        | 160.7±0.68                  | YF                |
|              | 0.7           | 34.0±1.6                        | 228.4±0.49                  | YG                |
|              | 1.0           | -                             | -                            | -                 |
|              | 2.0           | -                             | -                            | -                 |
| IBA+BA (0.5)  | 0.5           | 33.0±1.5                        | 140.4±0.21                  | BCC               |
|              | 0.7           | 35.0±1.6                        | 124.4±0.39                  | BCC               |
|              | 1.0           | -                             | -                            | -                 |
|              | 2.0           | -                             | -                            | -                 |

Table 2: Qualitative analysis of ethanolic extracts of stem and callus of C. halicacabum

| Phytochemicals | Callus extract | Plant extract |
|----------------|----------------|---------------|
| Steroids       | -              | +             |
| Terpenoids     | +              | -             |
| Reducing sugar | -              | -             |
| Alkaloids       | +              | +             |
| Tannins        | +              | +             |
| Flavonoids     | +              | +             |
| Saponins       | -              | +             |
| Phenolic compounds | +     | +             |
| Amino acids    | -              | +             |
| Anthraquinones | -              | -             |

Values are mean±S.E. from seven replicates per treatment and all the experiments were repeated twice. Means followed by the same letters in each column are not significantly different (p=0.05) using Duncan’s multiple range test. GC: Green Compact, BCC: Brownish Compact, YF: Yellowish Yellow Friable, YF: Yellow friable, GF: Green friable, YGC: Yellowish Green Compact, -: no response, PGR: Plant growth regulators, NAA: Naphthalene acetic acid, IAA: Indole-3 acetic acid, BA: Benzyl adenine, IBA: Indolebutyric acid.

Screening and qualitative comparison of phytochemicals

Both the crude and callus extracts were subjected to preliminary chemical tests to detect the presence and absence of various phytochemicals such as alkaloids, flavonoids, steroids, triterpenes, saponins, phenolics, and tannins (Table 2). The phytochemical constituents greatly differed among the tested solvents in both wild stem extract and in vitro callus extract. This is a sign of solvents varied extracting abilities for phytochemicals [24].

FT-IR analysis

The results of FTIR spectra confirmed the presence of functional groups in wild stem extract of C. halicacabum with peaks at 3329.72 cm⁻¹ (alkohols, phenols), 2973.28 cm⁻¹ (alkanes, amine), 1450.96 cm⁻¹ ([Alkanes], 1417.42 cm⁻¹ (alcohols), 1379.87 cm⁻¹ (phenols, nitrogen groups, alcohols, carboxylic acids, esters, and ethers), 1275.39 cm⁻¹ (alkyl esters), 1086.75 cm⁻¹ (anhydrides), and 1661.42 cm⁻¹ (acid chlorides and alkanes) (Fig. 2 and Table 3), followed by callus ethanolic extracts with peaks at 3388.01 cm⁻¹ (alkohols, phenols), 2974.38 cm⁻¹ (Carboxylic acid), 2924.01 cm⁻¹ (carboxylic acid and amine salts), 2138.50 cm⁻¹ (alkanes and amine), 1706.76 cm⁻¹ (alkanes and amides), 1362.76 cm⁻¹ (alkohol), 1274.01 cm⁻¹ (amines, phenols, nitrogen groups, alcohols, carboxylic acids, esters, and ethers), 1087.08 cm⁻¹ (alkyl esters), 880.01 cm⁻¹ (anhydride), and 670.66 cm⁻¹ (acid chlorides and alkanes) (Fig. 3 and Table 4). The FT-IR spectrum of wild stem and callus ethanolic extracts confirms the presence of functional groups for phenolics and flavonoids, which are widely reported for their antioxidant potential. Major groups are present in the callus extract. Flavonoids and phenolic acids have antibacterial, antifungal, antiviral, hepatoprotective, immunomodulating, and anti-inflammatory properties [25]. Major peaks were observed at 3388.01 cm⁻¹ that possibly designated to the O-H stretching vibrations of O-H Alcohol. Consequently, the present investigation results show that the primary functional group presents in C. halicacabum is O-H Alcohol. O-H group possibly specifies the higher potential toward inhibitory activity against microorganisms. Like a higher antimicrobial activity of ethanol extracts of the leaf has been previously established by some authors [26]. Through the FT-IR spectrum, we identified the functional groups from the given extract. Many researchers employed the FT-IR spectrum as a tool for observing closely associated plants and other organisms. The outcomes of the present investigation improved an innovative phytochemical marker to find the medicinally significant plant.

Determination of total phenol

The total phenolic content for C. halicacabum (1.0 mg/ml) was calculated from the standard graph of gallic acid with the standard curve equation, Y=0.97×+0.176, R²=0.999 (Fig. 4). The total phenolic content in aqueous extract of in vivo plant and in vitro callus was 80.46 mg GAE/g dry weight and 76.4 mg GAE/g dry weight, respectively (Fig. 5). At present, plant materials rich in phenolics are used in the
food industry because they decrease the oxidative degradation of lipids and maintain the quality and nutritional value of food. We determined that the total phenolic content was slightly more in the aqueous extract compared to the methanol and ethyl acetate extract [27].

Determination of total tannin content
The concentration of total tannin in *C. halicacabum* samples was derived from standard curve of tannic acid ranging from 0.2 to 1.0 mg/ml (y=0.97x−0.086; R=0.9932) (Fig. 6). The obtained values for the concentration of tannin contents are measured as mg of TAE/g of extract. The maximum percentage of tannins was determined at 78.03 in wild stem ethanol extracts followed by 75.22 in callus extract (Fig. 7). Tannins possess high radical scavenging capability can be extensively found in plants and have several positive results on human health.

### Antioxidant activity

**Hydrogen peroxide radical scavenging activity**

The hydroxyl radical is an extremely reactive free radical formed in the biological organization. It is as main active oxygen gathering radicals developed from the reaction of several hydroperoxides with transition metal ions and it is proficient in damaging about every molecule found in the living system causing lipid peroxidation and harmful to the biological system [28]. The higher hydroxyl radical scavenging activity was delivered at the concentration of 1000 µg/ml. The *C. halicacabum* showed higher scavenging activity shown in Fig. 8. Each concentration of the extracts display hydroxyl radical scavenging activity was increased with the increasing concentration of the extracts. Significantly lower IC50 values were observed in comparison to in vivo grown stem and in vitro raised callus. It is found that IC50 values of the stem extracts of *C. halicacabum* are 306 µg/ml and 286 µg/ml in callus extract respectively (Fig. 8).

### FT-IR: Fourier transform infrared.

**Table 3: FT-IR analysis of in vivo stem ethanolic extract of *C. halicacabum* L.**

| Frequency range (cm⁻¹) | Bond | Functional group name |
|------------------------|------|-----------------------|
| ~3400–3300             | O-H Stretch | Aromatics            |
| ~2950–2800             | C-H Stretch | Alkanes              |
| ~3400–2400             | O-H Stretch | Carboxylic acids     |
| ~2950–2800             | C-H Stretch | Alkanes              |
| 1690–1640              | C=N-R Stretch | Imines              |
| 1550–1490              | -NO₂ (Aromatic) | Nitro groups        |
| 1440–1400              | O-H Bend | Carboxylic acids      |
| 1400–1000              | C-F Stretch | Alkyl halides        |
| 1360–1250              | C-N Stretch (Aryl) | Amines            |
| 1300–1100              | C-C Stretch | Ketones              |
| 1090–810               | PH Bend | Phosphines            |
| ~1400–1000             | C-F Stretch | Alkyl halides        |
| ~880                   | C-H Bend (meta) | Aromatics      |
| 850–800                | C-H Bend (para) | Aromatics     |
| 730–550                | C=O Stretch | Acid chlorides       |

**Table 4: FT-IR analysis of ethanolic callus extract of *C. halicacabum* L.**

| Frequency range (cm⁻¹) | Bond | Functional group Name |
|------------------------|------|-----------------------|
| ~3400–3300             | O-H stretch | Alcohols             |
| ~3400–2400             | O-H stretch | Carboxylic acid      |
| ~3400–2400             | O-H stretch | Carboxylic acid      |
| ~2150–1800             | C=C triple bond stretch | Alkenes         |
| 1730–1700              | C=O stretch | Carboxylic acid      |
| 1680–1630              | C=O stretch | Amides              |
| 1440–1400              | O-H bend | Carboxylic acid       |
| 1390–1300              | -NO₂ (aliphatic) | Nitro group      |
| 1360–1250              | C-N stretch (aryl) | Amines          |
| 1260–1000              | C=O stretch | Alcohols            |
| 1260–1000              | C=O stretch | Alcohols            |
| 1075.08                | C=O stretch | Alcohols            |
| 1046.28                | C=O stretch | Alcohols            |
| 880                    | C-H bend (meta) | Aromatics   |
| 850–800                | C-H bend (para) | Aromatics   |
| 715–685                | C-H bend (mono) | Aromatics  |
Fig. 3: Fourier transform infrared Spectrum of ethanolic callus extract of *Cardiospermum halicacabum*

**STANDARD GRAPPH OF GALIC ACID**

![Graph showing standard graph of Gallic Acid with linear regression line](image)

- $y = 0.97x - 0.176$
- $R^2 = 0.999$

**STANDARD GRAPH OF TANNIC ACID**

![Graph showing standard graph of Tannic Acid with linear regression line](image)

- $y = 0.97x - 0.086$
- $R^2 = 0.9932$

Fig. 4: Total phenolic content standard graph.

Fig. 5: Total phenolic content of *Cardiospermum halicacabum*

- Wild stem extract: 78 mg GAE/g
- Callus extract: 65 mg GAE/g

Fig. 6: Total tannin content standard graph.

Fig. 7: Total tannin content of *Cardiospermum halicacabum*

- Wild stem extract: 72 mg TAE/g
- Callus extract: 69 mg TAE/g
FRAP Assay
The reducing potential of the ethanolic extract of *C. halicacabum* wild stem and callus extracts was examined by the FRAP method to determine the direct electron transfer capability of the extract. The FRAP results were measured from a calibration graph which was linear over the calibration range with an $R^2$ value of 0.9794 (Fig. 9) and the antioxidant ability of callus extract was found to be 225.29 μmoles/mg followed by wild stem extract 424 μmoles/mg. The reducing capacity of the *C. halicacabum* callus extract was similar to earlier studies on medicinal plants such as *Albizia odoratissima* [29] and *Salvia officinalis* [30].

DPPH radical scavenging activity
DPPH radical scavenging assay is a simple and extensively employed method to determine the quenching potential of antioxidants. DPPH is an unfirm radical forming purple in color and gets reduced in the presence of antioxidant to a non-radical firm form. The absorbance is measured at 517 nm by a UV spectrophotometer [31]. Ascorbic acid was used as a standard. It was noted, that the DPPH radical scavenging activity increased with the increasing concentration of the extract. The IC50 value was estimated with the help of a graph and the lower IC50 gives better radical scavenging capability [32]. The IC50 value was calculated from a formerly developed standard curve (Fig. 10) and was found to be 206.54 μg/ml. This least value of IC50 of the current study suggests that *C. halicacabum* callus extract has strong hydrogen donating capability. The examines performed on ethanolic plant extracts disclose that *C. halicacabum* displays DPPH radical scavenging capability in dose-dependent manner. The standard ascorbic acid exhibits higher radical scavenging compare to plant extract. The results were measured and expressed as ascorbic acid equivalent.

Antibacterial activity
The antibacterial activity of the various solvent extracts of *C. halicacabum* in vivo stem and in vitro callus against human pathogenic bacteria such as *S. aureus*, *E. faecalis*, Moraxella sp., *P. mirabilis*, and *E. coli* was examined and assessed by the zone of inhibition.
in disc diffusion method. The ethanol extract exhibits significant antibacterial activity against all the tested bacteria. The action of different solvent extracts was comparable to the standard Cefepime. Among the different solvent extracts, petroleum ether and aqueous extracts showed lower antibacterial activity compared to other solvent extracts. The callus chloroform extracts displayed maximum activities against three of the strains; P. mirabilis (10 mm), Moraxella sp (10 mm), and S. aureus (12 mm), followed by stem extract P. mirabilis (8 mm), Moraxella sp (10 mm), and S. aureus (10 mm). The acetone callus extracts of maximum activities against two of the strains; S. aureus (10 mm) and E. faecalis (8 mm). The result of antibacterial activities is presented in Tables 5 and 6. Among the various solvent extracts tested, the least antibacterial activity exhibited by aqueous extracts against all the tested strains. Among the different solvent extract tested, callus ethanolic extracts exhibited the highest activity of 17 mm (400 µl) inhibition zone against S. aureus this was followed by 13 mm E. coli, 14 mm E. faecalis, 8 mm P. mirabilis, and 10 mm inhibition zone against Moraxella sp. The in vivo stem extract showed the highest activity of 14 mm (400 µl) inhibition zone against S. aureus this was followed by 12 mm E. coli, 12 mm E. faecalis, 9 mm P. mirabilis, and 10 mm inhibition zone against Moraxella sp (Tables 5 and 6). Among the different tested strains, S. aureus showed the most resistance to various solvents of extracts and E. coli showed moderate activity. The ethanolic extract was most effective when compared to the standard antibiotic Cefepime. The lowest activity of the extract is 3 mm against E. coli and P. mirabilis. The extracts at the lower concentrations displayed a maximum zone of inhibition compared to higher concentrations. In particular, S. aureus showed the fact that C. halicacabum ethanolic extract could be effective in chronic lung disorders and skin disorders, which are often induced by S. aureus. Based on the results, Gram-positive strains exhibited more effectiveness than Gram-negative strains, for the reason that

![Fig. 10: Percentage of 2,2-diphenyl-2-picrylhydrazyl inhibition in vivo and in vitro callus extract with respect to ascorbic acid standard](image)

Table 5: Zone of inhibition (mm) of Gram-ve and bacteria Gram+ve agents at various concentrations of different in vivo stem extracts of C. halicacabum and standard cefepime

| Extracts    | Concentration µg/ml | Zone of inhibition (in mm) | Gram negative | Gram positive | Cefepime 30 mcg/Disc |
|-------------|---------------------|---------------------------|---------------|---------------|----------------------|
|             |                     |                           | P. mirabilis | Moraxella sp. | E. coli             | S. aureus | E. faecalis | S. aureus | E. coli | E. faecalis | S. aureus | E. coli | E. faecalis | S. aureus | E. coli | E. faecalis | S. aureus | E. coli | E. faecalis | S. aureus | E. coli | E. faecalis | S. aureus | E. coli | E. faecalis | S. aureus |
| Petroleum ether | 200                | 3.0                       | 6.0           | 5.0           | 4.0                | 22.5       |               |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |
| Petroleum ether | 400                | 5.0                       | 8.0           | 6.0           | 6.0                | 7.0        |               |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |
| Petroleum ether | 600                | 6.0                       | 9.0           | –            | –                  | –          |               |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |
| Petroleum ether | 800                | –                         | –             | –            | –                  | 3.0        |               |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |
| chloroform     | 200                | 6.0                       | –             | 5.0           | 8.0                | 18.4       |               |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |
| chloroform     | 400                | 8.0                       | 10.0          | 6.0           | 7.0                |           |               |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |
| chloroform     | 600                | 9.0                       | 6.0           | 7.0           | 9.0                |           |               |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |
| chloroform     | 800                | –                         | 8.0           | 9.0           | 10.0               |           |               |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |
| Acetone        | 200                | 5.0                       | 4.0           | –             | –                  | 17.0       |               |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |
| Acetone        | 400                | 7.0                       | 5.0           | 5.0           | –                  |           |               |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |
| Acetone        | 600                | 5.0                       | –             | 7.0           | –                  |           |               |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |
| Acetone        | 800                | 3.0                       | –             | 8.0           | –                  |           |               |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |
| Ethanol        | 200                | 6.0                       | –             | 7.0           | 8.0                | 10.0       |               |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |
| Ethanol        | 400                | 9.0                       | 10.0          | 7.0           | 6.0                | 8.0        |               |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |
| Ethanol        | 600                | 5.0                       | 10.0          | 11.0          | 10.0               |           |               |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |
| Ethanol        | 800                | 6.0                       | 8.0           | 8.0           | 9.0                |           |               |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |
| Aqueous        | 200                | –                         | –             | –             | –                  | 18.0       |               |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |
| Aqueous        | 400                | –                         | –             | –             | –                  |           |               |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |
| Aqueous        | 600                | 5.0                       | 7.0           | 4.0           | 6.0                |           |               |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |
| Aqueous        | 800                | 6.0                       | 5.0           | 5.0           | 4.0                |           |               |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |           |             |           |

(-): No response.
Table 6: Zone of inhibition (mm) of Gram-ve and bacteria Gram+ve agents at various concentrations of different in vitro callus extracts of C. halicacabum and standard cefepime

| Extracts | Concentration µg/ml | Zone of inhibition (in mm) | Cefepime 30 mcg/Disc |
|----------|---------------------|-----------------------------|----------------------|
|          |                     | Gram negative | Gram positive |                     |
|          |                     | P. mirabilis | Moraxella sp. | E. coli | E. faecalis | S. aureus |
| Petroleum ether | 200 | 3.0 | – | – | 6.0 | 8.0 | 20.5 |
|                 | 400 | 5.0 | – | – | 6.0 | 9.0 | –  |
|                 | 600 | 6.0 | 9.0 | 5.0 | 3.0 | 5.0 | –  |
|                 | 800 | –  | 7.0 | 4.0 | 7.0 | 9.0 | 6.0 |
| chloroform      | 200 | 6.0 | –  | –  | 5.0 | 8.0 | 6.0 |
|                 | 400 | 8.0 | 4.0 | 6.0 | 7.0 | 12.0 | 10.0 |
|                 | 600 | 10.0 | 6.0 | 7.0 | 9.0 | 6.0 | 14.0 |
|                 | 800 | –  | 8.0 | 9.0 | 10.0 | 5.0 | 10.0 |
| Acetone        | 200 | 4.0 | 4.0 | –  | –  | –  | 21.0 |
|                 | 400 | 5.0 | 5.0 | 5.0 | –  | –  | –  |
|                 | 600 | 6.0 | –  | 7.0 | –  | –  | –  |
|                 | 800 | 3.0 | –  | 5.0 | 8.0 | 10.0 | 15.0 |
| Ethanol        | 200 | 5.0 | –  | 7.0 | 6.0 | 8.0 | 15.0 |
|                 | 400 | 8.0 | 10.0 | 13.0 | 14.0 | 17.0 | –  |
|                 | 600 | 7.0 | 9.0 | 11.0 | 12.0 | 13.0 | –  |
|                 | 800 | 6.0 | 6.0 | 8.0 | 10.0 | 12.0 | –  |
| Aqueous        | 200 | –  | –  | –  | –  | –  | –  |
|                 | 400 | –  | 5.0 | –  | –  | –  | 20.5 |
|                 | 600 | 5.0 | 4.0 | 4.0 | 6.0 | 6.0 | –  |
|                 | 800 | 6.0 | 3.0 | 5.0 | 4.0 | 5.0 | –  |

(-): No response.

the disrupt membrane of Gram-negative bacteria surrounding the cell wall, which prevents the diffusion of hydrophobic compounds across its lipopolysaccharide protection [33,34].

CONCLUSION
Well-developed green compact callus and maximum (78%) callus fresh weight was derived from stem explant on MS medium supplemented with 0.7 mg/l 2, 4-D and 0.5 mg/l BA. C. halicacabum exhibited the potential antibacterial activity against S. aureus in both the wild stem and in vitro callus extracts. The FT-IR spectrum of wild stem and callus ethanolic extracts confirm the presence of amino acids, which are widely reported for their antioxidant potential and major functional groups are present in the callus extract. The results confirmed the occurrence of conceivable phytoconstituents, promise effectiveness in the C. halicacabum callus extract also.

ACKNOWLEDGMENT
The authors acknowledge the Department of Botany, Alagappa University, Karaikudi, for providing lab facilities, infrastructure support to conduct the research work.

AUTHORS’ CONTRIBUTIONS
Both authors are equally contributed in performing the experiments and preparation of the manuscript.

CONFLICT OF INTERESTS
Both authors declare that they have no competing interests.

AUTHOR’S FUNDING
The authors are also grateful to the RUSA-2.1, for providing financial support.

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