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Extraction of Bioactive Compounds from *Cardiospermumhalicacabum* Using Various Solvents and Potential Applications in Medicine

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Abstract:

Cardiospermum halicacabum plant is a medicinally important plant based on the reviews of other research findings. This study was carried out to find the bioactive compounds, antioxidant properties of the plant extract using different solvents. The best extract was selected (methanol) and these were used for anticancer activity. Column chromatography was done to purify the sample, UV-Visible and FTIR was done to find out the compounds, followed by TLC was made and the colored spot which formed on TLC were used against *E.coli* bacteria.

Keywords: Phytochemical, UV- Visible, FTIR, Chromatography, antibacterial, anticancer

1. INTRODUCTION

Cardiospermum halicacabum is a climbing shrub. It is a small genus of ornamental, herbaceous or shrubby vines. It is annual or sometimes perennial climber. It is commonly found as a weed. *Cardiospermum halicacabum* is known as balloon plant or love in a puff. Its general names include: balloon vine, heart vine, heart pea, love-in-a-puff, heart seed [1]**.** It is found along the roads and rivers. It grows about 3 metres tall and with many branches from the base **[**2]**.** *Cardiospermum halicacabum* (Sapindaceae) is an herbaceous climber of about 2–4 m long, evergreen, branched, with inflated fruits bearing a white heart-shaped pattern on the seed [3].

The plant is a dioecious, hairy, climbing vine with clusters of white flowers, finely dissected, delicate foliage and balloon like fruits.The root of the plant is considered as diaphoretic, diuretic and aperient. The tender, young shoots are used as a vegetable, fodder, diuretic, stomachic, and rubefacient [4]**.** The plant *Cardiospermum halicacabum*, was traditionally used as anxiolytic and as anticonvulsant. The whole plant *Cardiospermum halicacabum* contains saponins, traces of alkaloids, flavonoids, apigenin and phytosterols. Although medicinal plants are rarely used as antioxidants in traditional medicine, their claimed therapeutic properties could be due, in part to their capacity for scavenging oxygen free radicals, which may be involved in many diseases

Cardiospermum halicacabum L. was used to treat various diseases such as skin diseases (rashes, itching, skin irritation, etc.), dandruff, rheumatoid arthritis, gastrointestinal diseases, respiratory tract diseases, urogenital diseases, etc. [5]**.**

The stem is scrambled over the ground, climbing the surrounding vegetation supported by tendrils. It is harvested from the wild and used as medicine, food and source of various commodities. It is also used as an ornamental plant. It is found in tropical and subtropical areas of Africa, Asia, America. It prefers moist soil and a sunny sheltered positions. It is a noxious weed. The size and shape of the weed is similar to that of soyabeenseeds. The leaves are Ternately compound, deltoid, biternate, 3-8 cm long. Flowers are white in umbellate cymer with a pair if peduncles modified into tendrils. Capsules are covered with bladder like calyx, winged at the angles. The seeds are globose, black, smooth, 4-6 mm, with small white heart shaped aril. And the branches are slender, liming by means of tendrillar hooks [2]**.** Flowers are unisexual, obliquely zygomorphic, having straight pedicel. Fruits are membranous, depressed, pyriform casing branched at the angles [6]**.**

Edible use :

The leaves and the young shoots of the plant is cooked and eaten as vegetables [7]. Oil can be obtained from the seed.

Medicinal use :

The whole plant is used in the treatment of rheumatism, nervous disease, stiffness of the limbs and snake bite. The tea made from the leaves is used for itchy skin. The root is used in the treatment of catarrh of the bladder and urinary tract [8] **.** The plant extract decreases body ache [9]**.** Plant is reported to possess anti-inflammatory activity by reducing PLA2 activity that is concerned with inflammatory process [10]**.**

Other uses : For treatment of infections associated with the nervous system, the root alone has been employed [11]**.** Seed oil has insect repellent properties and anti feedant action on insects. Leaves are used in washing clothes and hair. Seeds are used as beads. Stem is used in basket making. The whole plant is applied to reduce swellings and hardened tumours. It is also one of the ingredients in “Allergy Relief Liquid” [12]**.** The plant based herbal products like gel, cream, shampoo, spray etc. are present in the market and are helpful in dry itchy skin and scalp [13]**.**

2. MATERIALS AND METHODS

2.1 Collection of the plant

The leaves, stem and the aerial parts of *Cardiospermum halicacabum* is collected from the farmland of Coimbatore, Tamilnadu, India.

2.2 Extraction of the plant material

The leaves,stem and the aerial parts of the plant was cut into pieces and then dried in the shade. The dried sample powdered to get a coarse powder. The plant material was extracted by four solvents, out of which two are polar solvents and other two are non- polar solvents. The four solvents are methanol, water, chloroform and petroleum ether. About 0.5 to 1.0 gram of the dried powder of the plant is extracted with 15 ml each of the four solvents separately and is shaken well in the orbital shaker at 40 to 60 rpm at 40^0Cand then filtered.

2.3 Phytochemical studies

Phytochemical screening was carried out to assess the qualitative chemical composition of the crude extracts [14] with slight modifications.

2.3.1 Test for alkaloids -1ml of the plant extracts was taken and added 1 ml of Mayer's reagent. Added four drops of iodine solution. Shaken well and the formation of yellow colour indicates the presence of alkaloids.

2.3.2 Test for terpenoids - 1 ml of the crude extracts and 1 ml of concentrated sulphuric acid was Boiled in a water bath for 2 to 4 minutes. Formation of greyish colour indicates the presence of terpenoids.

2.3.3 Test for phenols - 1 ml of the crude extracts and 1 ml of ferric chloride solution was mixed and the formation of blue green or black colour indicates the presence of Tannins.

2.3.4 Test for sugars - 1 ml of the crude extracts was taken and added 1 ml of Fehling's solution A and 1 ml of Fehling's solution B. Boiled in water bath for 2 to 4 minutes. The formation of red colour indicates the presence of sugars.

2.3.5 Test for saponins - 1 ml of the extracts was taken in the test tube and added 1 or 2 ml of distilled water. Shake well for few seconds. The formation of 1 cm foam layer indicates the presence of saponins.

2.3.6 Test for flovonoids - 1 ml of the crude plant sample was taken in a test tube and added few fragment of Magnesium ribbon. Then added few drops of concentrated hydrochloric acid and mix it well. The formation of pink scarlet colour indicates the presence of flavonoids.

2.3.7 Test for quinines –equal amount of the plant extract and 2% NaOH solution was mixed, observed formation of blue green or red colour indicates the presence of quinines.

2.3.8 Test for proteins -1 ml of the crude extracts was taken in a test tube and added 2 drops of mercuric chloric acid or nitric acid. The formation of yellow colour indicates the presence of proteins.

2.3.9 Test for steroids - 1 ml of the crude plant extracts was taken and added 1 ml of chloroform and 1 ml of concentrated sulphuric acid along the sides of the test tube. A red colour produced at the lower chloroform layer indicates the presence of steroids.

2.4 Antioxidant studies [15,16]

2.4.1 DPPH (2,2-Diphenyl-1-picryl hydrazyl assay)

100 µl of the plant extract (sample) was added to 0.1 molar 0.5 ml of DPPH solution and 0.4 ml of 50 millimolar Tris hydrochloric acid, after shaking make up to 2 ml with distilled water. The tubes were incubated in room temperature for 30 minutes and the OD value was measured at 517 nm using spectrophotometer (ELICO SL 159). Ascorbic acid was used as a standard to calculate the mg/g (20, 40,60,80, 100 mg/ml) of DPPH activity.

2.4.2 FRAP (Ferric Reducing Antioxidant Power Assay)

To the 1 ml of the plant extract added 1 ml of phosphate buffer solution and 1 ml of potassium ferric cyanide (0.1 %), these were incubated at 50 ˚C for 20 minutes. After incubation with the addition of 1 ml of 10% trichloro acetic acid , 1 ml of distilled water and 0.5 ml of 0.1% ferric chloride solution the sample was again incubated for 5 to 10 minutes and the reducing power activity was measured by taking OD value at 700 nm using spectrophotometer (Labtronics LT 291). The results were reported in mg/g (20, 40,60,80, 100 mg/ml)by using ascorbic acid as standard.

2.4.3 H_2O_2 (peroxide assay)

0.5 ml of the plant extract was added to 0.5 ml of methanol and 2 ml of 20 millimolar H_2O_2 in phosphate buffer solution. Mix it well and incubated at room temperature for 10 to 15 minutes. The OD value was determined at 230 nm using spectrophotometer (ELICO SL 159). The results were reported in mg/g (20, 40,60,80, 100 mg/ml) by using ascorbic acid as a standard.

2.4.4 SOD (superoxide assay)

To the 1ml of reaction mixture I (1 ml of 50 milli molar phosphate buffer solution, 0.075 ml of 20 millimolar L-methionine, 0.04 ml of 10 millimolar hydroxyl amide hydrochloride and 0.1 ml of 50 millimolar ethylene diamine tetra acetic acid), added 0.1 ml of the plant extract and Incubated at 30˚C for 5 to 10 minutes. 80 µl of 50 millimolar Riboflavin was added and the mixture and exposed under 200 V of fluorescent light for 1 to 2 minutes. 1 ml of the reaction mixture II(Reaction mixture II- 1 % sulphonyl amide in 5 % phosphoric acid)was added and OD value at 543 nm, measured by using spectrophotometer (Labtronics LT 291).

$$\textbf{Percentage of inhibition} = \frac{\textbf{ControlOD} - \textbf{SampleOD}}{\textbf{ControlOD}} \textbf{X100}$$

2.5 Purificationand identification of compounds

2.5.1 Column chromatography

The stationery phase is solid and the mobile phase is liquid. 20 ml of the methanol extract was chromatographed over silica gel column, using the protocol of [17]**.** The eluted solutions are collected separately to the tubes and these fractions are measured in three different wavelengths of 260 nm, 280 nm and 400 nm using spectrophotometer to identify the compounds based upon the resulted higher OD values.

2.5.2 UV-Visible spectroscopy and FTIR [18]

Extract was examined using the UV-VIS spectrometer under 200 to 800 nm and the characteristic peaks were analysed for the detection of proximate analysis. Followed by plant sample was analysed in infrared spectra

(Shimadzu) in the range of about 4000 – 400 cm^{-1}, to identify the functional group which is present in the plant extract.

2.5.3 Paper chromatography and TLC

To run chromatography plate, the following procedure is carried out using a capillary; a small spot of solution containing the methanol extract is applied to a plate, about 1.5 cm from the bottom edge. The solvent is allowed to completely evaporate to prevent it from interfering with sample's interactions with the mobile phase in the next step. A spot of each of these is applied and allowed to dry. Then a second spot of the same material is applied directly on top of the first now dry spot and is called as respotting [17], after loading the paper was tested with two different solutions, Solvent A consists of water (1 ml), methanol (2 ml), acetic acid (1 ml) and chloroform (1 ml). Solvent B consists of water (1 ml), methanol (1 ml), n-hexane (1 ml), formic acid (1 ml) and n-butanol (1 ml). After elution the distance travelled by the solvent and the substance is noted separately. Followed by TLC also done to get clear results about the plant sample, the Retardation factor,R_f value was measured by using the formula,

$$\textbf{Retardation factor} = \frac{\textbf{Distance travelled by the slute}}{\textbf{Distance travelled by the solvent}}$$

2.6 Antibacterial activity

Using well diffusion method antibacterial activity against *E.coli* was done by using the protocol of [19].

2.7 Anticancer studies (MTT Assay)

For the anticancer activity He LaCell Line was used to study the anticancer activity of *Cardiospermum halicacabum*. The cell Line were purchased from National centre for cell Science, Pune, India and has been maintained further in Centre for Bioscience and Nanoscience Research, Eachanari, Coimbatore, Tamil Nadu, India. After obtaining the Cell Line was Sub-cultured into DMEM medium with the addition of sodium carbonate, glucose and BSA (10%). After adding all the chemicals in T flask, the cells were incubated in CO_2 incubator in the pH of 7 to 7.5, temperature 37℃, humidity 70-80% for 24-72 hrs. After incubation the growth of the cell line was confirmed by viewing under inverted microscope and used for further study.Cell line with sample in different concentration 5µl,10µl,15µl and 20µl along with blank (DMSO) and control (Cell Line) was done and the purple colour were read at 570 nm using 96 well plate ELISA reader (Robonik, India). % of cell death were calculated by following formula [19].

$$\textbf{Percentage of cell death} = \frac{\textbf{ControlOD} - \textbf{SampleOD}}{\textbf{ControlOD}} \textbf{X100}$$

3. RESULTS AND DISCUSSION

3.1 Phytochemical analysis

Phytochemical screening of four solvent extracts of *Cardiospermum halicacabum* are tabulated in Table.1. The four different solvent extracts were analysed for compounds such as alkaloids, terpenoids, phenols, sugar, saponins, flavonoids, quinines, proteins, steroids.

Table1: Phytochemicalanalysisof *Cardiospermumhalicacabum*

Phytochemicals	Water	Methanol	Petroleumether	Chloroform
Alkaloids	P	P	A	A
Terpenoids	A	A	A	A
Phenols	A	A	A	A
Sugars	A	A	A	A
Saponins	P	A	A	P
Flavanoids	A	A	A	A
Quinines	P	P	A	A
Proteins	A	P	A	P
Steroids	A	P	P	P

P – Presence, A - Absence

Alkaloids are present in the water and methanol extracts of *Cardiospermum halicacabum*, whereas it is absent in the petroleum ether and chloroform extracts. Terpenoids are absent in all the four solvent extracts of *Cardiospermum halicacabum.* The role of terpenoids in plants are used for growth and development, defence against herbivores. Phenols present in methanol and the remaining shown absence in other extracts. The role of phenols is protection against stress, plant development, pigment biosynthesis and kills microorganism. Sugars and flavonoids are absent in all the four extracts. Saponins are present in water and chloroform extracts of *Cardiospermum halicacabum* and are absent in methanol and petroleum ether extracts. Therole of saponins in plants are defence against diseases and herbivores, development, as surface active agents or surfactants. Quinines are fairly present in water and methanol extracts of *Cardiospermum halicacabum* and are absent in petroleum ether and chloroform extracts. The role of quinines in plants is drug in the treatment of malaria. Proteins are present in chloroform and methanol extracts of *Cardiospermum halicacabum* and are absent in petroleum ether and water extracts. The role of proteins in plants is in biosynthesis of hormones, enzymes and membrane channels and in plant development. Steroids are present in chloroform, methanol and petroleum ether and is absent in water extracts. The role of steroids in plants are: an important component of cell membrane which alters membrane fluidity, as a signalling molecule, growth and development. Form the result the best extracts was selected for further studies.

3.2 Antioxidant studies

3.2.1 DPPH

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant component. The DPPH assay is used to predict antioxidant activities by mechanism in which antioxidants act to inhibit lipid oxidation, so scavenging of DPPH radical and therefore determinate free radical scavenging capacity.

The method is based on the scavenging of DPPH by antioxidants, which upon a reduction reaction decolorizes the DPPH solution. The assay measures the reducing ability of antioxidants toward the DPPH radical. From the results it is found that the scavenging of DPPH is very

high for the chloroform extract (24mg/g) of *Cardiospermum halicacabum*. For methanol extract (12mg/g) of *Cardiospermum halicacabum*, scavenging of DPPH is relatively lower than that of the chloroform extracts. In case of water extract (3mg/g), the scavenging of DPPH by antioxidants is very lower compared to methanol and chloroform extracts of *Cardiospermum halicacabum.*

3.2.2 FRAP

The FRAP determination (ferric reducing antioxidant power) is used to measure antioxidant power of plant extracts in their ability to reduce Fe3 +-tripyridyltriazine to Fe2 +-tripyridyltriazine. The assay is based on electron-transfer reactions.

The reaction mechanism involves the reduction of ferric 2,4,6-tripyridyl-s-triazine to the coloured ferrous form. The absorption wavelength is 593 nm. Among water, methanol and chloroform extracts, chloroform extract of *Cardiospermum halicacabum* has higher (276mg/g) ferric reducing antioxidant power. Methanol extract is having ferric reducing power lower than that of the chloroform extract (186mg/g). Water is having very lower (58mg/g) ferric reducing antioxidant power.

3.3.3 H_2O_2

The ability of the water, methanol and chloroform extracts of *Cardiospermum halicacabum* to scavenge hydrogen peroxide was determined by H_2O_2 assay. The scavenging ability of water, chloroform and methanol extracts of *Cardiospermum halicacabum* on hydrogen peroxide is shown the above figure. Methanol extract of *Cardiospermum halicacabum* is having greater scavenging (106mg/g) ability on hydrogen peroxide. Water extract is having slightly lower scavenging (96mg/g) ability on hydrogen peroxide. Whereas, chloroform is having very low scavenging (13mg/g) ability than methanol and chloroform extracts of *Cardiospermum halicacabum.*

3.3.4 SOD

Superoxide radical is known to be very harmful to cellular components as a precursor of more reactive oxidative species such as singlet oxygen and hydroxyl radicals. From this study which was observed that the methanol (57.3%) extract showing high inhibition compared with water (37.7%) and chloroform (18 %).

3.4 Purification and identification of compounds

Purification and identification of the compound was done with the solvent extract of methanol. Compare with other extracts, methanol shown higher activity in the covered tests. further methanol was used to screen the other findings.

3.4.1 Column chromatography

In this study, column chromatography was done with the methanol extracts of *Cardiospermum halicacabum.* *Cardiospermum halicacabum* plant upon column chromatographic analysis yield number of fractions of components. These higher OD value was denoting the presence of bioactive compounds. Based upon the higher OD value obtained, 3 tubes was used to pool, and this was used for further studies.

3.4.2 UV-Visible spectroscopy and FTIR

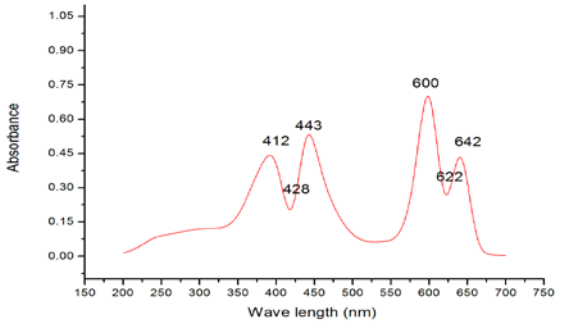


Fig. 1: UV-Visible analysis result

The UV – Visible spectrum is obtained between 200 to 800 nm. The presence of peak at 412 to 642 nm confirmed various bioactive compounds in the sample. Peak locations or the peak wavelengths were specific for different bioactive compound groups and the peak height depends on the concentration of the compound. Flavonoids have absorption maximum around 240 – 290 nm, which was affected by the conjugation of ring structure and its substitution pattern. The spectra for phenolic compounds (tannins) typically lie in the range of 230 – 290 nm.

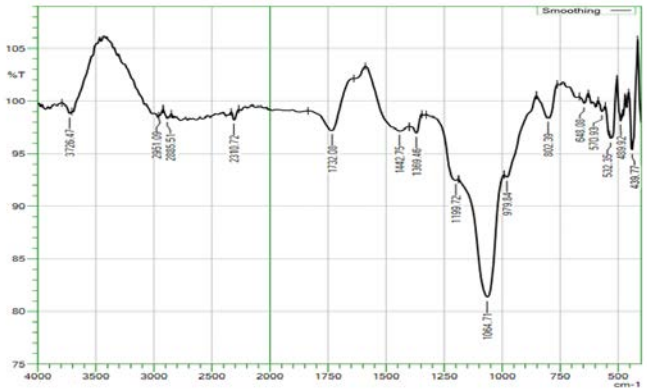


Fig. 2: FTIR results

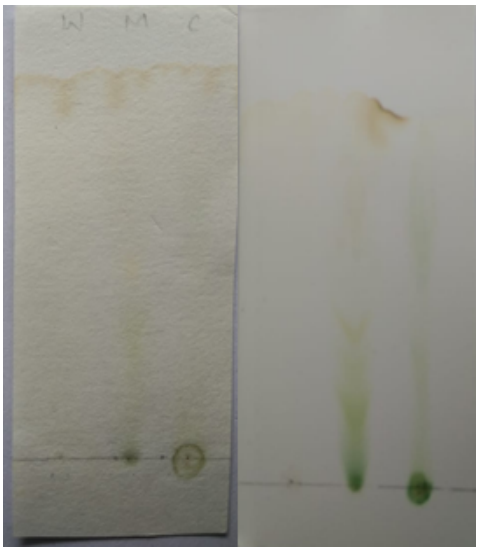
Table 2: Result of column chromatography

Wavelength	Fractions							
	1	2	3	4	5	6	7	8
260 nm	0.023	0.027	0.033	0.048	0.056	0.076	0.034	0.048
280nm	0.026	0.012	0.019	0.045	0.051	0.038	0.028	0.064
400nm	0.029	0.025	0.041	0.061	0.067	0.074	0.064	0.091

The interpretation of infrared spectra involves the correlation of absorption bands in the spectrum with that of the desired sample. The plant sample is analysed in infrared spectra in the range of about 4000 – 400 cm^{-1}. The frequency range of about 3726.47 cm^{-1}peaks represents OH stretching may be alcohols or phenols. The peaks at 2951.09 cm^{-1} and 2885.51 cm^{-1} represents the C-H overlapping, mainly the presence of carboxylic acids and derivatives. The peak at 2370.72 cm^{-1} represents the C ≡ C stretching, mainly alkynes. The peak at 1732.08 cm^{-1} represents C=O stretching, mainly aldehydes and ketones. The frequency ranges from 1442.75 cm^{-1}peaks represents CH_2 and CH_3 deformation. The peaks at about 1369.46 cm^{-1} represents C – H stretching, mainly alkanes. The peaks at 1199.72 cm^{-1} represents C-O stretching mainly aldehydes and phenols. The peaks at 1064.71 cm^{-1} represents C – N stretching vibration. The peaks at 979.84 cm^{-1} and 802.39 cm-1 indicates the C − H stretching vibration. The peaks at 648.08 cm^{-1} represents the –R−Cl stretching. The peaks at 570.93 cm^{-1} and 532.35 cm^{-1} represents-$R-Br$ stretching i.e, alkyl halides. The peaks at 489.92 cm^{-1} and 439.77 cm^{-1} is of strong intensity, represents −R−I stretching.

3.4.3Paper chromatography and TLC

The water, methanol and chloroform extracts of *Cardiospermum halicacabum* is used in chromatography studies. The distance travelled by the solute and the solvent are measured and the resultant R_f value was calculated.



Paper chromatography TLC

Fig.3: Results of Paper and Thin Layer Chromatography

Thin layer chromatography process is similar to paper chromatography with the advantage of faster runs, better separations, and the choice between different stationary phases. Because of its simplicity and speed TLC is often used for monitoring chemical reactions and for the qualitative analysis of reaction products. The methanol, chloroform and water extract of *Cardiospermum helicacbum* is used in the study of thin layer chromatography, compare with the Solvent A and B, Solvent B showing clear spot and different spots in paper and TLC. The R_f value is calculated for the spots identified in TLC plate.

Table 3: Result of TLC

Extract name	R_f value on Paper chromatography	R_f value on TLC
Water	Spot 1- 0.8823	Spot 1- 0.8766
	Spot 1 - 0.9117	Spot 1- 0.8695
Methanol	Spot 2- 0.3235	Spot 2- 0.386
		Spot 3- 0.1739
Chloroform	Spot 1 - 0.9264	Spot 1 - 0.6086
		Spot 2- 0.486

3.5 Antibacterial studies

Anything that destroys bacteria or suppresses their growth or their ability to reproduce is called as antibacterial activity. In the antibacterial studies of *Cardiospermum halicacabum*, the two compounds obtained from the Thin Layer Chromatography were used. The organism used was *E.coli* bacteria. The zone of inhibition was measured and tabulated.

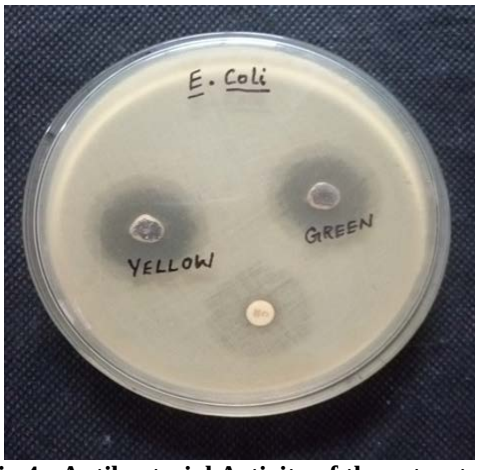


Fig.4 : Antibacterial Activity of the extracted compound

Table 4 : Antibacterial activity

Samples from TLC sheet	Zone of inhibition
Yellow spot	7 mm
Green spot	6 mm
Antibiotic (Chloramphenicol- C30)	2 mm

Yellow coloured component of the methanol extract of *Cardiospermum halicacabum* is found to kill the bacteria *E.coli* for about 7 mm, whereas the green coloured component kills the bacteria for about 6 mm. Thus, the inhibitory zone for yellow coloured component of *Cardiospermum halicacabum* is maximum. Therefore, the plant *Cardiospermum halicacabum* has the ability to kill the bacteria. Hence, they are found to be antibacterial in nature.

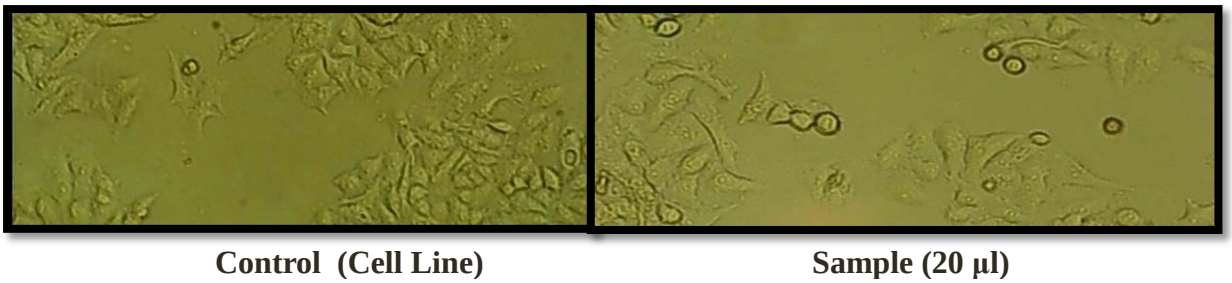


Fig. 5: Microscopic view of Cell Line (Control and Treated sample)

3.6 Anticancer studies (MTT Assay)

Cancer results from a series of molecular events that fundamentally alter the normal properties of cells. Currently, a few plant products are being used to treat cancer effectively.

The MTT assay showed the anticancer activity of the He La cell lines using the plant extract (methanol). When compared with the control the extracted cells showed the maximum cell death (26.7 %)

From the above figure, it is found that the percentage of cell death for 5 µl of the sample against HeLa Cells is 5.55 %. For 10 µl of the extract, 13.58 % of cell death occurred. For 15 µl of the sample, 16.45 % of cell death has been occurred. For 20 µl of the plant extract, 26.79 % of cell death has been occurred. Thus, increase in the concentration of the plant extract increases the percentage of cell death.

4. DISCUSSION

The results of phytochemical analysis of *Cardiospermum halicacabum* were similar to the results of [20**]** investigations on phytochemical analysis of *Cardiospermum halicacabum.* The extracts on Preliminary phytochemical analysis revealed the presence of alkaloids, carbohydrates proteins and saponins. The other secondary metabolites like steroids, glycosides, lignin,etc. were present in trace amounts. Secondary plant metabolites can disturb development, lead to malformations or malfunctions, extend the duration of developmental stages or act as repellents [21].

Upon phytochemical analysis of extracts of areal parts, the major constituents like alkaloids, carbohydrates, cardiac glycosides, phytosterol, saponins, tannins, flavonoids and triterpenoids were found in ethanol and chloroform extract. N-hexane extract contained only phytosterol and saponins [22]. The phytochemical analysis of methanolicstem extract of*Cardiospermumhalicacabum*was analysed for the compounds such as Alkaloids, Cardiac Glycosides, Flavonoids, Glycosides, Saponins, Steroids and Tannins. The preliminary phytochemical analysis revealed the presence of six compounds i.e. Alkaloids, Cardiac Glycosides, Flavonoids, Saponins, Steroids and Tannins and absence of glycosides [23].

DPPH radical scavenging and Nitric oxide radical scavenging activity, Superoxide radical scavenging activity, ferric ion scavenging activity, were compared with standard antioxidant like ascorbic acid. In the FRAP test, the total extract and methanol fraction of NP showed the highest activity with values of 10.1 and 12.8 µM Fe(II)/g, respectively [24]. Fe (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of antioxidant activity. In the reducing power assay, the presence of antioxidant in the test drugs converts Fe3+ to Fe2+ by donating electron. Ti is found from the results that the reducing power of the extracts increases with increase in their concentration [25].

SOD results were relevant to mention here that the results of [26] investigated and reported the antioxidant activity of the plant *C. halicacabum* extracts. Superoxide scavenging activity of the methanol extract as measured by the in vitro riboflavin–NBT–light system. Superoxide radical is known to be very harmful to cellular components as a precursor of more reactive oxygen species.

The results of UV - VIS spectroscopic analysis confirms the presence of tannins and flavonoids in the ethanolic extract of *I. Aspalathoides* [26]. FTIR spectroscopic analysis showed the presence of phyto constituents in the garlic extract. Fourier transform infrared spectroscopy (FTIR) can provide fundamental information on the molecular structure of organic and inorganic components and is one of the most versatile analytical techniques for the non-destructive, chemical characterization of samples [27].

The Thin Layer chromatographic results were same as the results of isolation of flavonoids from methanol extracts of *Cardiospermum halicacabum* [28]. Fractions of methanol extracts of *Cardiospermum halicacabum* showed a single spot with the R_f value of about 0.70 on TLC. Fractions of methanol extracts of *Cardiospermum halicacabum* showed a single spot with the R_f value of about 0.67 on TLC.[17] Investigations showed that the TLC profiling of 5 extracts gives an impressive result that directing towards the presence of number of phytochemicals. This variation in R_f values of the phytochemicals provides a very important clue in understanding of their polarity and also helps in selection of appropriate sol-vent system for separation of pure compounds by column chromatography.

The results of antibacterial studies of *Cardiospermum halicacabum* were similar to the results of antimicrobial activities of *Cardiospermum halicacabum* by [8]. The leaf and stem extracts of *C.halicacabum* were tested for their antimicrobial activity against *S. aureus, B.Subtilis, C. freundii, E. coli, P. aeroginosa, S. typhi, K. Pneumoniae,*

Streptococcus aureus was found to be more susceptible towards the ethanol cxtracts of leaf and stem with a maximum inhibitory zone followed by benzene, acetone, Chloroform and aqueous. The results obtained are encouraging as the benzene, ethanolic and chloroform extracts have shown considerable antibacterial activity against the tested organisms [4].

The cytotoxic potential of plant is related to their different phytochemicals. The seeds of *Cardiospermum halicacabum* were selected because of their traditional use and high medicinal value. Five different extracts of *Cardiospermum halicacabum* seeds are used in the anticancer studies. The results revealed that the n – hexane extract of seeds has a very good yield of about 24.18 % of cell death [29]**.** The Anticancer activity areal parts of *Cardiospermum halicacabum* was studied by using MTT assay. The results showed that *Cardiospermum halicacabum* was a potent material for the treatment of cancer [23].

5. CONCLUSION

In this study, *Cardiospermum halicacabum* was extracted with four solvents namely water, methanol, chloroform and petroleum ether. The extracts on preliminary phytochemical analysis showed the presence of phytochemical constituents of alkaloids, saponins, sugars, steroids, proteins and quinines. The extracts are subjected to antioxidant studies. It was found that the plant has very good antioxidant properties. Further, the compounds present in the plant were identified by UV-Visible spectroscopy, FTIR spectroscopy also recorded to identify the various functional groups of the extract. Column and TLC chromatography were also performed to evaluate the bioactive compounds. R_f values were calculated and confirmed the presence of alkaloids and flavonoids. Finally, the plant extracts are tested for its medicinal property, for which it is subjected to antibacterial and anticancer studies. The results showed that the methanol extracts of *Cardiospermum halicacabum* has the antibacterial property against *E.coli*. Also, the methanol extracts has very good cytotoxic activity to kill cancer cells (HeLa Cells). From the results the plants have good medicinal use.

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Conflict Of Interest

This research work has no conflict of interest

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