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Antioxidant and anti-proliferative potential of *Cardiospermum halicacabum* stem extracts against human breast cancer (MCF-7) cells

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ABSTRACT

Cancer is the most important health care problem worldwide. An estimated 10 million new cases worldwide are recorded annually, of which 46% are in developed countries. Breast cancer is one of the second most common malignant tumors in the world. All forms of cancer treatment, including surgical treatment, chemotherapy and/or radiotherapy are often poor and often toxic to the normal cell. Therefore, there is still an urgent need for new cancer treatment options. More research is now being done to determine which compounds occur naturally. The current study aims to extract *Cardiospermum halicacabum* L. for the prevention and treatment of cancer. A few methods may account for the perceived therapeutic effects, most importantly direct cytotoxicity. These herbal medicines when combined with regular antioxidant and anticancer drugs may be helpful in combining the antioxidant and anticancer effects and reducing the side effects associated with common drugs.

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INTRODUCTION

Cancer as the second most leading cause of death following cardiovascular disease has posed a main challenge in the field of immunology and medicine (Hemalatha *et al.*, 2020). Cancer is a complex, multifaceted and multimechanistic disease that requires a variety of treatments, controls and prevention (Manimekalai *et al.*, 2016; Rajesh *et al.*, 2016b). Conventional cancer treatment has many methods all aimed at kill the cancer cells or controlling their growth. Chemotherapy is the most common form, not to select a cancer cells, but damage to normal cells; Immune stress is a major consequence of chemotherapy (Hersh & Freiriech, 1968). Herbal medicines have been alternate by chemical remedy, and developed countries have skilled a decline in the reputation of medicinal plants (Rajesh *et al.*, 2020; Rajesh & Sivakumari, 2020; Mohamad Sitheek *et al.*, 2020; Angalammal *et al.*, 2021; Padmavathy *et al.*, 2021).

According to the WHO above 80% of the population in global suffered from traditional medicine for their health care needs. Traditional medicines are considered safe and good, inexpensive and readily available without any side effects due to this fact universal demand for traditional medicines is growing steadily and then in Indian markets are growing at 20 percent an annual rate (Chau *et al.*, 2006). Herbs are used in various countries for medicinal purposes and are the goal of many powerful and effective herbs. India is gifted with the riches of herbal remedies that are widely used by all classes of people directly as traditional medicine or in many traditional medicine systems or indirectly in the preparation of novel medicine medicines (Alagesaboopathi, 2011).

Cardiospermum halicacabum L. is the most essential medicinal plant used in the ayurvedic medicinal system in India to treat arthritis. It is used to treat neurological disorders; reduce hardy plants, asthma, such as demulcent in orchids and dropsy. The leaf is etic, invigorating and their decoctions are given to treat acne, diarrhea, as a way to treat common ulcers and reduce obesity. The whole plant of *C. halicacabum* has been used for centuries to treat rheumatism, paralysis, snake bites, etc.; its neurological roots, such as diaphoretic, diuretic, emetic, laxative, refrigerant, stomachic and sudorific; its leaves and stems are

used in the treatment of diarrhea, diarrhea, and headaches and as an ingredient in the treatment of inflammation, the herbal extract is used as a treatment for ear pain (Rajesh *et al.*, 2016a,b,c; Rajesh *et al.*, 2020; Rajesh & Sivakumari, 2020). The current study was intended to analyze the antioxidant and anti-proliferative effects against human breast cancer (MCF-7) cells and its phytocontiuents present in the stem extract of *C. halicacabum*.

MATERIALS AND METHODS

Preparation of Stem Extracts

Aqueous extraction

After collection stems were separated and shade dried in the dark room and dried stems are ground into a powder form using a mixer-grinder. 5% suspension (w/v) was set in a flask by adding boiled water and kept in a shaker at 200 rpm for 4 hours at room temperature. Followed by stirring, the suspension was brought to room temperature. The suspension was filtered via four layers of No.1 Whatman filter paper and finally passed through a 0.22 µm filter (Millipore, Billerica). The filter was dried and stored in a refrigerator for future uses (Rajesh *et al.*, 2016b).

Chloroform, Ethyl Acetate, Hexane and Methanol Extractions

Similar to the preparation of chloroform, ethyl acetate, hexane and methanol, 20 grams of dried powder (5% w/v) dipped in chloroform, ethyl acetate, hexane and methanol, stored for 4 hours in the shaker, and then filtered. Suspensions were evaporated at room temperature. Dried extracts were collected and stored at -20°C until further uses (Rajesh *et al.*, 2016b).

Determination of Free Radical Scavenging Activity

DPPH free radical scavenging activity is measured by the modified version of (Rajesh *et al.*, 2020).

The Anti-Proliferative Effect Using the MTT Method

The anti-proliferative effect was assessed by MTT (3- (4, 5-dimethylthiazol- 2yl) -2, 5- diphenyltetrazolium bromide) by the method of Mosmann (1983).

Phytochemical Examination

All five of these extracts were included in the phytochemical examination for its phytoconstituents according to Kokate (1988).

Numerical Analysis

Data of DPPH and MTT were induced to statistical examination, and the average and standard error of each five data were calculated and relevant tables and figures presented in the text. Significance of samples mean between the different extracts and the concentration of extracts were assessed by two Way ANOVA using the Prism Grappad software (Rajesh *et al.*, 2020).

RESULTS

Percentage inhibition of free radical scavenge of aqueous, chloroform, ethyl acetate, hexane and methanol extracts of the *C. halicacabum* stem and L-Ascorbic acid (standard) are shown in Table 1. Significant inhibition of free radical scavenge was detected at 60.15 µg/mL consecutively emitted methanol showing higher disposal performance compared to aqueous, chloroform, ethyl acetate and hexane extracted only as shown in Figure 1 and the exact IC_{50} values shown in Table 2.

Percentage of MCF-7 cells activity was observed 24 and 48 hours across all five extracts with various concentrations such as 0, 25, 50, 75, 100 and 125 µg/mL. Control cells were 100% effective, in the form of aqueous, chloroform, ethyl acetate, hexane and methanol extract, performance decreased significantly with increased concentration; the decrease in percentage is indirectly related to the concentration of the output. All the data show that methanol extract has shown high activity leading to a decrease in cellular activity. When the data were under two-way ANOVA, all values were significantly different between concentrations (Table 3 & 4).

The amount of inhibiting concentration (IC_{50}) was 50% of active cells in all five extracts such as aqueous, chloroform, ethyl acetate, hexane and methanol extracts, even at 125 µg/ml; IC_{50} value could not be reached. Similarly, at the end of 48 hours, the IC_{50} value was observed in all five extracts; trend is methanol extract (34.57 µg/µg/mL) > ethyl acetate extract (57.84 µg/mL) > chloroform extract (58.48 µg/µg/mL) > hexane extract (71.03 µg/mL) > i -aqueous extract (83.26 µg/µg/mL) (Table 5). All the data show that methanol extract significantly controls the proliferation of MCF-7 cells even at low concentrations (Figure 2 & 3).

To detect morphological observations of 48 hrs IC_{50} concentration treated with MCF-7 cells. Untreated MCF-7 cells showed normal cell morphology. MCF-7 cells treated with all the five extracts of *C. halicacabum* have shown a steady increase in cell proliferation; the increase depends on the concentration and time. Cell shrinkage was higher in methanol extract than that of other extracts (Figure 4).

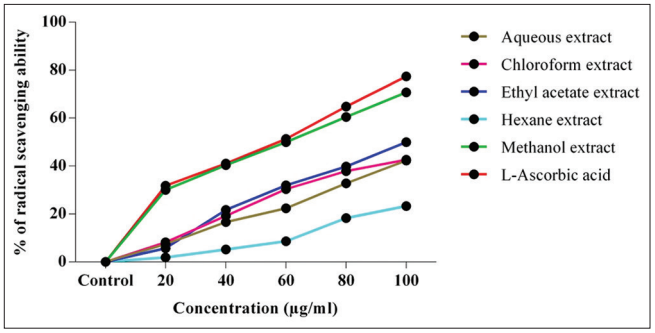

Figure 1: Free radical scavenging activity of *C. halicacabum* stem extracts

Table 1: Free radical scavenge of various stem extracts of *C. halicacabum*

Concentration	Aqueous	Chloroform	Ethyl acetate	Hexane	Methanol	L-Ascorbic acid
Control (0 µg/mL)	1.212±0 (0)	1.212±0 (0)	1.212±0 (0)	1.212±0 (0)	1.212±0 (0)	1.212±0 (0)
20 µg/mL	1.123±0.002 (-7.34)*	1.113±0.002 (-8.17)*	1.142±0.002 (-5.75)*	1.189±0.003 (-1.89)*	0.848±0.006 (-30.01)*	0.827±0.003 (-31.77)*
40 µg/mL	1.010±0.005 (-16.64)*	0.979±0.001 (-19.22)*	0.949±0.004 (-21.69)*	1.149±0.003 (-5.23)*	0.723±0.002 (-40.35)*	0.714±0.003 (-41.03)*
60 µg/mL	0.941±0.004 (-22.36)*	0.844±0.018 (-30.34)*	0.824±0.003 (-31.99)*	1.107±0.002 (-8.66)*	0.607±0.004 (-49.92)*	0.591±0.001 (-51.27)*
80 µg/mL	0.815±0.005 (-32.78)	0.753±0.010 (-37.87)*	0.730±0.007 (-39.79)*	0.991±0.001 (-18.26)*	0.479±0.008 (-60.48)*	0.427±0.009 (-64.77)*
100 µg/mL	0.699±0.004 (-42.33)*	0.695±0.007 (-42.63)*	0.607±0.003 (-49.95)*	0.93±0.007 (-23.27)*	0.355±0.004 (-70.71)*	0.275±0.004 (-77.34)*

Values are mean±SE of five observations.
- Denote per cent decrease opposition to control.
*Indicates, values are significant at P<0.001

Table 2: IC_{50} Value of aqueous, chloroform, ethyl acetate, hexane and methanol stem extracts of *C. halicacabum*

Sl. No	Samples	IC_{50} values
1	Aqueous	116.06 µg/mL
2	Chloroform	130.96 µg/mL
3	Ethyl acetate	100.09 µg/mL
4	Hexane	206.71 µg/mL
5	Methanol	60.15 µg/mL
6	L-Ascorbic acid	57.52 µg/mL

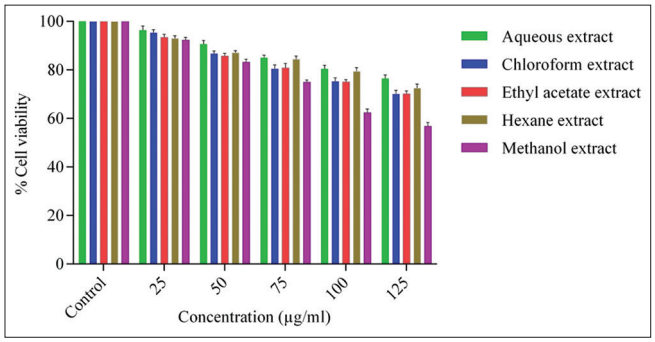

Figure 2: Per cent cell viability of MCF-7 cells for 24 hours when treated with aqueous, chloroform, ethyl acetate, hexane and methanol stem extracts of *C. halicacabum*

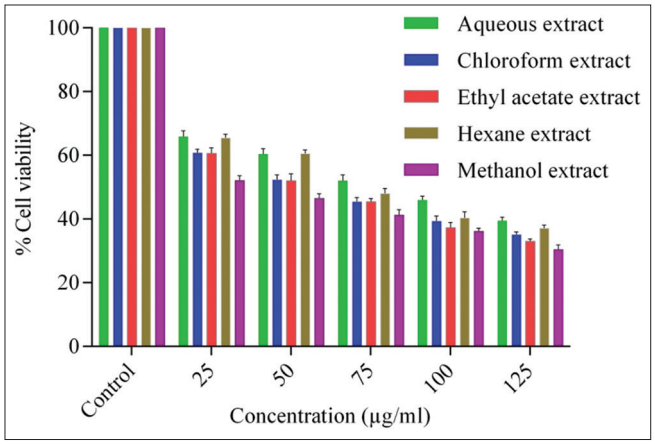

Figure 3: Per cent cell viability of MCF-7 cells for 48 hours when treated with aqueous, chloroform, ethyl acetate, hexane and methanol stem extracts of *C. halicacabum*

The present investigations of all the five extracts of *C. halicacabum* stem resembled a dark brown coloured paste, and the paste is highly soluble in respective solvents. Then samples are induced to preliminary phytochemical screening, carbohydrate, saponin, tannins, acid and protein present in aqueous extract, triterpenoid, carbohydrate, glycosides, alkaloids and protein present in chloroform extract, coumarin, glycosides and acid present in ethyl acetate extract, triterpenoid, saponin and phenols present in hexane extract and triterpenoid, flavonoids, saponins, tannins, phenols and protein present in methanol extract (Table 6).

DISCUSSION

The plants have been used as medicines for more-then thousand years, and used today true and processed in several medicinal plants forgotten by modern peoples, because of dependence on the immediate effects of allopathic treatments. Plants are always a source of drugs and have many functions in humanity (Abu-Rabia, 2005). They are also used as dietary supplements by nutraceuticals, pharmaceutical industries and chemical organizations in the preparation of synthetic drugs derived from these plants (Ncube *et al.*, 2008) Modern medicine has evolved into traditional medicine after a thorough examination of chemicals medicines (Boopathi & Sivakumar, 2011; Savithramma *et al.*, 2011).

Herbal medicines have become very popular in the treatment of various diseases because of the common faith; that green medicines are safe, readily available, and have no side effects. Certainly, the market and social demands have been growing so much, so that there is a high risk that many medicinal plants today may face extinction or loss of genetic diversity (Misra, 2009). Therefore, current research was conducted to conclude the antioxidant and anti-proliferative effect of aqueous, chloroform, ethyl acetate, hexane and methanol stem extracts of *C. halicacabum* against human breast cancer (MCF-7) cells.

In our study, the half-maximum inhibitory concentration of methanol stem extract was IC_{50} 60.15 µg/mL and was similar to that of ascorbic acid with IC_{50} 57.52 µg/mL. These results have shown that the release of methanol has significant antioxidant activity

Table 3: Cell viability percentage of Mcf-7 cells for 24 hrs when treated with aqueous, chloroform, ethyl acetate, hexane and methanol stem extracts of *C. halicacabum*

Concentration	Aqueous	Chloroform	Ethyl acetate	Hexane	Methanol
Control (0 µg/µg/mL)	100±0	100±0	100±0	100±0	100±0
25 µg/µg/mL	96.34±0.803 (-3.66)*	95.39±0.541 (-4.61)*	93.47±0.561 (-6.53)*	93.01±0.512 (-6.99)*	92.38±0.485 (-7.62)*
50 µg/mL	90.59±0.704 (-9.41)*	86.80±0.481 (-13.20)*	85.75±0.506 (-14.25)*	87.13±0.391 (-12.87)*	83.28±0.523 (-16.72)*
75 µg/µg/mL	85.05±0.495 (-14.95)*	80.51±0.72 (-19.49*	80.85±0.843 (-19.15)*	84.43±0.621 (-15.57)*	75.05±0.383 (-24.95)*
100 µg/mL	80.46±0.677 (-19.54)*	75.29±0.672 (-24.71)*	75.11±0.419 (-24.89)*	79.49±0.638 (-20.51)*	62.48±0.66 (-37.52)*
125 µg/mL	76.48±0.673 (-23.52)*	70.15±0.674 (-29.85)*	70.24±0.525 (-29.76)*	72.52±0.742 (-27.48)*	56.86±0.692 (-43.14)*

Values are mean±SE of five observations.
- Denote per cent decrease opposition to control.
*Indicates, values are significant at P<0.001

Table 4: Cell viability percentage of MCF-7 cells for 48 hrs when treated with aqueous, chloroform, ethyl acetate, hexane and methanol stem extracts of *C. halicacabum*

Concentration	Aqueous	Chloroform	Ethyl acetate	Hexane	Methanol
Control (0 µg/µg/mL)	100±0	100±0	100±0	100±0	100±0
25 µg/µg/mL	65.89±0.803 (-34.11)*	60.79±0.529 (-39.21)*	60.64±0.792 (39.36)*	65.45±0.526 (-34.55)*	52.16±0.669 (-47.84)*
50 µg/µg/mL	60.37±0.773 (-39.63)*	52.34±0.699 (-47.66)*	52.07±0.939 (-47.93)*	60.53±0.544 (-39.47)*	46.52±0.651 (53.48)*
75 µg/µg/mL	52.01±0.817 (-47.99)*	45.44±0.591 (-54.56)*	45.47±0.454 (-54.53)*	48.01±0.716 (-51.99)*	41.27±0.768 (-58.73)*
100 µg/µg/mL	45.93±0.571 (-54.07)*	39.33±0.739 (-60.67)*	37.31±0.732 (-62.70)*	40.35±0.859 (-59.65)*	36.21±0.431 (-63.80)*
125 µg/µg/mL	39.47±0.517 (-60.53)*	35.19±0.383 (-64.88)*	33.04±0.325 (-66.96)*	37.13±0.431 (-62.87)*	30.45±0.646 (-69.55)*

Values are mean±SE of five observations.
- Denote per cent decrease opposition to control.
*Indicates, values are significant at P<0.001

Table 5: Exact IC_{50} Value of aqueous, chloroform, ethyl acetate, hexane and methanol stem extracts of *C. halicacabum* when treated with MCF-7 cells

Sl. No	Samples	IC_{50} values	
		24 hrs	48 hrs
1	Aqueous	292.30 µg/mL	83.26 µg/mL
2	Chloroform	223.01 µg/mL	58.48 µg/mL
3	Ethyl acetate	228.91 µg/mL	57.84 µg/mL
4	Hexane	205.77 µg/mL	71.03 µg/mL
5	Methanol	155.52 µg/mL	34.57 µg/mL

against free radicals of DPPH. This antioxidant activity of our study is consistent with the findings of Rajesh *et al.*, (2020) in the leaf phase of methanol *C. halicacabum*. The present study showed that methanol extraction of *C. halicacabum* is rich in secondary metabolites. However, these phytocompounds are known to have a high antioxidant effect against various forms of active oxygen and nitrogen. Therefore, the antioxidant activity of our extract may be due to the presence of high content of phytocompounds.

IC_{50} value of active cells in all the five extracts at the end of 48 hours, had been trending to extract methanol (34.57 µg/mL) > ethyl acetate extract (57.84 µg/mL)> chloroform extract (58.48 µg/mL)> hexane extract (71.03 µg/mL) > aqueous extract (83.26 µg/mL) was observed. A similar study by Rajesh *et al.* (2016b, c) reported that *C. halicacabum* leaf extract against liver cancer and breast cancer cells, and Rajesh and Sivakumari (2020) also reported a fractions from methanol leaf extract of *C. halicacabum* against HepG-2 cells, this finding is based on our results. Similarly, morphological analysis of cells showed a decrease in all three quotes and a high degree of shrinking was reported in the release of methanol. This decrease may be due to the effect of inhibiting the growth of phytoconstituents present in the stem of *C. halicacabum*. We have observed an excellent effect on inhibition of MCF-7 cells in the release of methanol at 34.57 µg/mL 48 hours later than in aqueous, chloroform, ethyl acetate and hexane extract. Our *in vitro* study of MCF-7 cells treated with aqueous, chloroform, ethyl acetate, hexane and methanol extracted from *C. halicacabum* stem and the effects of inhibiting the growth of this plant prove its anti-cancer effects.

In the present study, phytochemical investigations of stem extracts showed the presence of carbohydrates, saponin, tannins, acids and proteins found in aqueous extract, triterpenoid, carbohydrate, glycosides, -alkaloids and proteins found in chloroform extract, coumarin, glycosides and acids present in ethyl acetate extract, triterpenoid, saponin and phenols present in hexane extract and triterpenoid, flavonoids, saponins, tannins, phenols and proteins present in methanol extract. Similarly alkaloids, saponins, flavonoids, apigenin, proanthocyanidin and

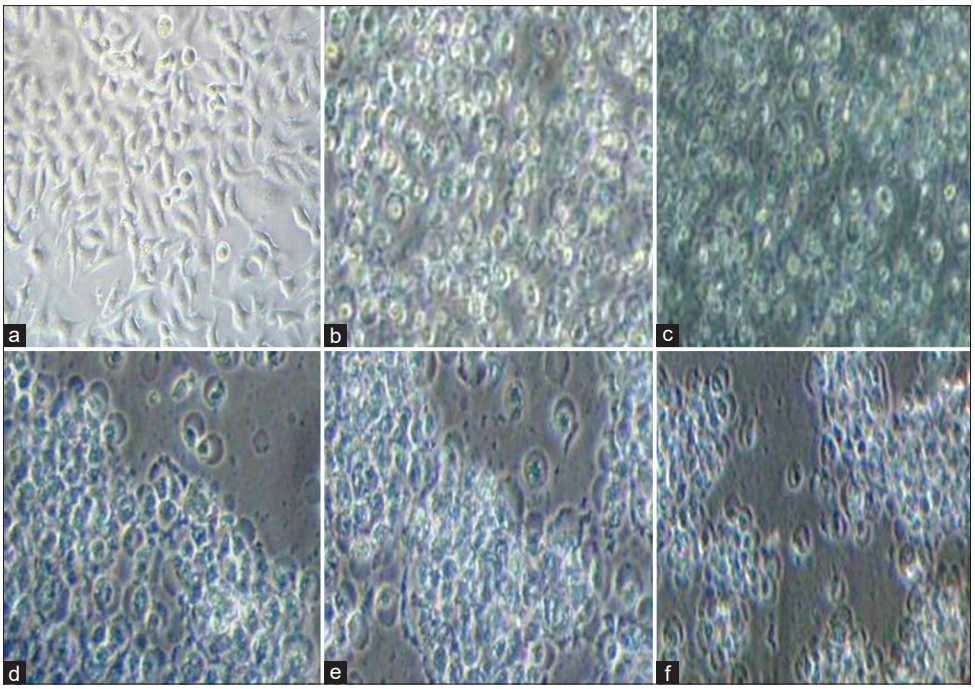

Figure 4: Cytomorphological observation of MCF-7 cells. a: Control, b: Aqueous, c: Chloroform. d: Ethyl acetate, e: Hexne, f: Methanol

Table 6: Phytochemical screening of various stem extracts of *C. halicacabum*

Phytochemical Parameters	Aqueous extract	Chloroform extract	Ethyl Acetate extract	Hexane extract	Methanol extract
Triterpenoid	-	+	-	+	+
Flavonoids	-	-	-	-	+
Carbohydrate	+	+	-	-	-
Coumarin	-	-	+	-	-
Glycosides	-	+	+	-	-
Quinone	-	-	-	-	-
Saponin	+	-	-	+	+
Alkaloid	-	+	-	-	-
Tannin	+	-	-	-	+
Phenol	-	-	-	+	+
Acid	+	-	+	-	-
Protein	+	+	-	-	+

"+"–Present; "-"–Absent

phytosterols present in *C. halicacabum*, Its leaves containing D-glucose, β-sitosterol, oxalic acid, 7-o-glucuronides of apigenin, (+) pinitol, chrysoeriol and luteolin, root contains phlobaphene, phloba-tanin, β-sitosterol and pro anthocyanidine, seeds contains fatty acid and fixed fat, and extracts of this plant are reported to contains various triterpenoids, glycosides, and a range of fatty acids (Ahmed *et al.*, 1993; Ferrara *et al.*, 1996; Srinivas *et al.*, 1998; Rajesh *et al.*, 2016 b,c).

India is the birthplace of a new system of traditional medicine such as Siddha, Ayurvedha and Unani. Traditional methods of medicine are prepared from a single plant or combination of plant species (Savithramma, 2011). Efficacy depends on the use of the right part of the plant and its biological strength, which also depends on the presence of the required amount and the nature of the secondary metabolites in the raw plant (Savithramma *et al.*, 2010; Vinoth *et al.*, 2011). The results of current research point out that the presence of the above mentioned phytochemical is potent in the treatment of different diseases. The effectiveness also may be due to the presence of secondary metabolites either individually or in combinations.

In the present study, we could not pinpoint the biological properties observed between aqueous, chloroform, ethyl acetate, hexane and methanol stem extracts of *C. halicacabum*. Our results revealed that these stem extracts may show greater results due to the presence of secondary metabolites in *C. halicacabum*. Based on the previous findings, we are conducting to explore the free radical scavenging, anti-proliferative effect on MCF-7 cells and its phytochemical analysis. These may lead to the possibility that a synergetic compound of biological agents may occur naturally in plants and that these compounds may be chemically beneficial to anti-cancer agents.

CONCLUSION

While human breast cancer is known as most risky disease in the global, several researches have been focused on it. This attempt has led to the finding of a cancer-fighting movement for *Cardiospermum helicacabum* stem extracts. This will help to develop pharmaceutical standards for universal. Finally, it should be noted that herbal medicines have been used by up to 80% of people in budding countries. Now, this is the time to lead taking herbal products for the advantage of the beneficiary so that the idea "Herbal plants save human life" will fit.

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