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An In-Vitro Study of the Antioxidant and Anticancer Potential of Cardiospermum Halicacabum (Linn.) on Human Hepatocellular Carcinoma (Hepg2) Cells

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KEYWORDS

Anticancer, Antioxidant, Cardiospermum halicacabum, phytochemicals, HepG2-Cell Line.

ABSTRACT:

Background: The medicinal plants are rapidly gaining reputation due to the numerous pharmacologic effects. The aim of this study was to investigate the different solvent extracts of *Cardiospermum halicacabum* on phytochemical constituents of total phenolic, flavonoid content, antioxidant and antiproliferation activity against HepG2 Cell Line.

Objectives: The objective of this study was to examine the *in-vitro* antioxidant and anticancer as well as the phytochemical constituents of the different crude extracts of *C. halicacabum* L. leaves.

Methods & Materials: The total phenolic and flavonoid contents were analyzed by Folin Ceocalteu method and Aluminium Colorimetric method respectively. The antioxidant activity of the extracts was determined by using DPPH and Nitric oxide radical scavenging assay. MTT assay was used to estimate the antiproliferative activity of the extracts against HepG2 Cell Line.

Results: The ethanol extract of *C. halicacabum* exhibited the highest total phenolic (72.58±0.63 mg of GAE/g extract) and total flavonoid content (50.65±0.88 mg of QE/g extract). The hexane and ethanol extracts showed significant and dose-dependent anti-oxidant effects with the IC_{50} values of 48.43µg/mL and 51.73µg/mL by DPPH and 46.34 µg/mL and 48.95µg/mL respectively by nitric oxide scavenging assay. The ethanol and ethyl acetate leaf extracts were found to be cytotoxic *in-vitro* to HepG2 Cell Line with IC_{50} values of 49.70 µg/mL and 51.12 µg/mL, respectively.

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Conclusion: The present study concluded that the ethanolic and hexane extracts of *C. halicacabum* have significant antioxidant and anticancer activities. The potent antioxidant and anticancer activity of *C. halicacabum* might be due to the presence of phytochemicals.

1. Introduction

Plants extracts have been used for therapeutic purposes long before recorded history. Several drugs listed as conventional medications were originally derived from plant sources [1]. Mankind has been dependent on nature for their vital needs since time immemorial. The oldest report dates back to the 26th century BC, documenting around ten thousand plants and their derivatives in Mesopotamia. The Egyptian traditional medicine system dates from about 2900 BC. The documentation of the Indian Ayurvedic system of medicine dates from before the 10th century BC [2]. Most of the world's population relies on herbal medicine for their medicinal needs [3]. The World Health Organization (WHO) has estimated that 80% of the world's population depends primarily on traditional medicine [4]. In most developing countries, the indigenous and traditional modes of herbal treatment are a part of the culture, customs, and religious importance and are the dominant method of healing therapy. These remedies are socially accepted because of their efficacy, low toxicity, safety, cost-effectiveness, and easy availability. Notably, the Nobel Prize of 2015 in Physiology and Medicine was half shared by Youyou Tu for the discovery of the antimalarial molecule of plant origin, artemisinin (qinghao) from *Artemisia annua* [5-6].Cancer is one of the most fatal diseases, characterized by asymmetrical cell proliferation that causes morbidity and mortality in several millions of people worldwide. Due to its prevalence, there is undoubtedly an unmet need to discover novel anticancer drugs [7]. The most common reason behind cancer is lifestyle changes, highlighting the urgent need to find better treatment methods for the disease. According to the World Health Organization [8], more than 14 million people were diagnosed with cancer, and 8 million died in 2012 (www.who.int). High mortality and incidence rates make it an important public health and economic issue, requiring effective prevention [9]. Medicinal plants have various advantages over chemical products because plant-derived compounds are more tolerant and non-toxic to normal human cells. Currently available conventional therapies for cancer treatment, such as radiotherapy and chemotherapy, have various side effects like neurological, cardiac, renal, and pulmonary toxicity, seriously affecting the health of patients. Therefore, an alternative method is required to develop less toxic and more potent anticancer drugs compared to those available in the market. Several studies have been made on naturally occurring compounds known to possess cytotoxic effects, as they display potential to destroy cancer cells. Due to these advantages, medicinal herbs are in high demand, and several species of medicinal plants have been documented and selected for the preparation of cancer drugs. Recently, there has been increased scientific interest in studying materials from plant sources as anticancer compounds. Several studies have found the role of medicinal plants in the prevention and treatment of cancer [10]. The National Cancer Institute has screened approximately 35,000 plant species for their potential anticancer activities and found that about 3,000 plant species have shown reproducible anticancer properties [11]. The emergence of important anticancer agents from natural sources requires more research to develop more drugs to treat cancer. Medicinal plants contain a wide range of secondary metabolites, including flavonoids, flavones, anthocyanins, lignans, coumarins, isocatechins, and catechins [12]. These bioactive compounds are mainly responsible for the antioxidant properties of medicinal plants. The increasing side effects and expensive medications have tilted the focus of researchers towards herbal medicines. Therefore, this review aims to provide information about medicinal plants that possess anticancer activity.Free radicals or reactive oxygen species are generally defined as excess formation or incomplete removal of ROS, which cause oxidative damage to biomolecules, resulting in oxidative stress [13]. These damages lead to many diseases in humans, such as atherosclerosis, cancer, diabetes, aging, and other degenerative syndromes [14]. Medicinal plants have garnered great attention for their antioxidant properties due to the presence of a wide variety of free radical scavenging molecules, such as phenols, flavonoids, alkaloids, terpenoids, tannins, etc. [15]. The aim of this study was to identify the

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phytochemical constituents present in the ethanol extract of *K. Pinnata* leaves by GC-MS analysis and assess its antimicrobial activity against human pathogenic bacteria. Furthermore, this study aimed to evaluate the in vitro enzymatic inhibitory effects of leaf extract against free radicals such as DPPH, nitric oxide, and hydroxyl.

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are free radicals formed in the body as a consequence of normal metabolic reactions, exposure to ionizing radiation, and the influence of many xenobiotics. They have been implicated as the cause of several diseases, including cancer, heart diseases, and aging. In this context, antioxidants play an important role in biological systems. They are emerging as prophylactic and therapeutic agents that scavenge free radicals and prevent the damage caused by them. Several antioxidants of plant origin have been experimentally proven and used as effective protective agents against free radical-mediated toxicity.The use of natural products has been considered of exceptional value in the control of cancer [16]. Furthermore, the search for new sources of biologically active compounds is important for discovering new drugs for cancer treatment. Currently, there is considerable scientific and commercial interest in the ongoing discovery of newer anticancer agents from natural product sources [17]. A myriad of plant products exists that have shown very promising anti-cancer properties in vitro but are yet to be evaluated. Further intensive investigations are required to determine the efficacy of these plant products in treating cancers. It was proposed to screen the successive extracts and the isolated compounds for their in vitro antioxidant and anticancer activities using standard procedures. The active extracts and compounds isolated were also proposed to be screened for their in vivo anticancer activity. The aim of the present study is to investigate the phytochemical and bio-medicinal properties of *CL*-LE.

2. Materialsand Methods

2.1Collection, identification and preparation of extracts

The fresh and healthy leaves of *C. halicacabum* were collected from in and around Theni District, Tamil Nadu, India during the period of June, 2018. Plant specimen was identified by authentic plant taxonomist. The leaves were cleaned and shade dried at room temperature in clean and hygienic conditions. The dried leaves were powdered using an electrical grinder and was extracted sequentially with hexane, ethanol, and ethyl acetate according to increasing polarity using a Soxhlet apparatus. The liquid extracts were filtered and concentrated by evaporating them to dryness under reduced pressure. The concentrated extracts were stored at -80°C until use [18] (Figure. 1).

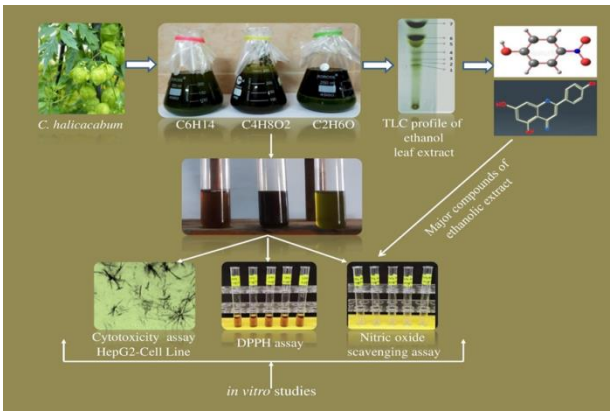

Figure1: The schematic illustration of research study design.

2.2Anticancer activity of different solvent extracts against lung cancer cell line (HepG2)

The cytotoxicity of *CL*-DSLE (hexane, ethyl acetate and ethanol leaf extract of *C. halicacabum*) was tested against Human Hepatocellular Carcinoma (HepG2) Cell Line [19]. Cell lines were obtained from the National Centre for Cell Science, Pune, India. The cell lines were maintained at 5% CO_2 in a CO_2 incubator at 37 °C. Cell lines were transferred to 96 well plates at a concentration of 1×10^3 cells per well and incubated for 24 h. Cells were later washed with 100 µL of serum-free medium and were starved for one hour in a CO_2 incubator at 37 °C. Cells were then treated with different concentrations of selected plant extracts (10µg/mL, 25µg/mL and 50µg/mL) and incubated for 24 more hours in a CO_2 incubator. The 96 well plates of cells were wrapped in aluminum foil to avoid light exposure. After the incubation period was over, MTT reagent (0.05 mg / mL) was added to each well and incubated for 4 h in a CO_2 incubator at 37 °C. After the incubation, MTT reagent was discarded and cell lines were washed with 200 µL of phosphate buffer saline (PBS). 100 µL of DMSO was used to dissolve the crystals. The absorbance value was recorded at 570nm. The absorbance value was plotted against cell density concentration. The experiment was performed in triplicate.

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Absorbance for treated cellsCell viability(%) = $\frac{\text{Mean OD}}{\text{Control OD}}$ x 100

2.3Antioxidant DPPH assay

The experiment was carried out using DPPH activity [20]. The deep violet color of DPPH turns yellow in the presence of an antioxidant compound. When DPPH is mixed with a hydrogen donor substance, free radicles are reduced and a color change occurs. The different volume of plant extract was added to 1 mL of 0.1 mM DPPH solution in methanol. The solution mixture was incubated for 30 min at room temperature in the dark. The absorbance was measured at 517 nm after the incubation period to estimate the reduction in DPPH free radical number. Methanol solution mixed with DPPH was used as a control, vitamin C was used as the standard and methanol plus plant extract solution was used as a blank. All the experiments were performed in triplicate. DPPH free radical scavenging activity was calculated by the following formula:

2.4Nitric oxide radical scavenging assay

Sodium nitroprusside (SNP) was utilized for the generation of nitric oxide (NO), which was subsequently measured using the Griess reagent, composed of 1% sulphanilamide, 0.1% naphthylethylenediamine dichloride (NED), and 3% phosphoric acid. SNP spontaneously produces NO in an aqueous solution at physiological pH, resulting in the formation of nitrite ions through interaction with oxygen. The Griess reagent is used to estimate these nitrite ions. NO scavengers compete with oxygen, leading to a reduced production of NO.

Various concentrations (10, 25 and 50µg/mL) of hexane, ethyl acetate and ethanol extract of *C. halicacabum* leaves dissolved in ethanol and water, were mixed with 10 mM SNP in phosphate-buffered saline (PBS) and incubated at 25°C for 3 hours. The samples were then reacted with the Griess reagent, and the absorbance of the chromophore formed through the diazotization of nitrite with sulphanilamide and subsequent coupling with NED was recorded at 546 nm using a microplate reader. These readings were compared to a positive control, which in this case was ascorbic acid treated in the same way with the Griess reagent. Ethanol was used as a control. The following formula was applied to calculate the results:

$$\% \text{ of inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of tests samples}}{\text{Absorbance of control} - \text{Absorbance of test samples}} \times 100$$

2.5Determination of total polyphenol content using Folin Ciocaltue reagent

The concentration of phenolic in plant extracts was determined using spectrophotometric method [21-22]. Aliquot of (20 µl) gallic acid calibration standards were mixed with 1.58 ml of distilled water and 100 µl of Folin-Ciocalteu's reagent. The reaction mixture was incubated at room temperature for 8 minutes (not exceeding 8 minutes) and then 300 µl of 200mg/mL sodium carbonate was added, re-incubated for another 2 hours at room temperature and absorbance was measured at 765 nm. Analysis was performed in triplicate and the average absorbance was used to plot the calibration graph. The same procedure was repeated for the plant extracts. Based on the measured absorbance, the concentration of phenolics were read (mg/ml) from the calibration graph and the content of phenols was expressed in terms of gallicacid equivalents per gram of extract (mg of GAE/g). Data are expressed as mean± SD of three replicates.

2.6Determination of flavonoid content by Aluminium Chloride Colorimetric assay

Total flavonoid content was determined by Aluminium colorimetric method [23, 24]. Quercetin (QE) calibration standards (500µl) were pipetted in to 5ml volumetric flasks with 2mL of distilled water and 0.15ml of 5% $NaNO_2$, vortex thoroughly, and incubated at room temperature for 5 minutes. After incubation, 0.15ml of 10 % $AlCl_3$ was added to each mixture, except for the blank to which same volume of distilled water was added, and incubated again at room temperature for 6 minutes. 1ml of 4% NaOH was added to the mixture, vortexed and made the volume up to 5ml with distilled water. Absorbance was measured at 430 nm after 15minutes. All dilutions were analyzed in triplicate and average absorbance was used to plot the calibration graph. The standard graph was drawn with absorbance against the concentration of QE dilutions. Data are expressed as mean± SD of three replicates. Same

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procedure was repeated with the plant extract and the content of the flavonoids was expressed in terms of QE equivalents per gram of plant extract.

$$\% \text{ of inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of tests samples}}{\text{Absorbance of control}} \times 100$$

2.7Statistical analysis

All experiments were performed thrice and the results averaged Data were expressed as mean ± SD. Linear regression analysis was used to calculate IC_{50} for each plant extract.Origin pro 8.5 software was used for statistical analysis.

3. Results

3.1 Total phenolic and flavonoid content evaluation

The total phenolic content of the crude n-hexane, ethyl acetate and methanolic extract of *C. halicacabum* was estimated by Folin–Ciocalteu reagent and expressed in gallic acid equivalents (GAE) and it was calculated from the linear regression equation of standard curve ($y = 14.715x + 26.74$, $R^2 = 0.9617$). The results showed that the crude methanolic extract was found to contain a high amount of phenols (72.58±0.63µg GAE/mg).

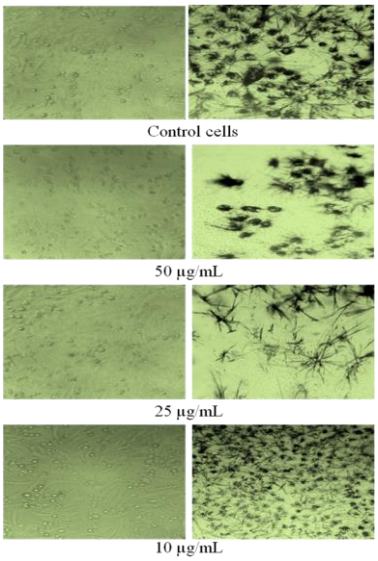
The total flavonoid content of crude methanolic extract was determined via aluminum chloride colorimetric method and it was calculated from the linear regression equation of standard curve of quercetin ($y= 9.965x+23.36$, $R^2= 0.8299$) and expressed as quercetin equivalent per gram of plant extract. The tested extract was found to contain high amounts of flavonoids (44.09 ± 2.13 µg QE/mg) **(Figure 2)**.

3.2 Anti-cancer activity of plant extract with different solvents

To investigate the cytotoxicity effect of hexane, ethyl acetate and ethanolic leaf extracts of *C. halicacabum* were determined by MTT assay. The anticancer activity was carried out against human hepatocellular carcinoma (HepG2) cell linesand the results showed a decreased viability (%) with increased concentration of the plant extracts. The results have shown that different solvent extracts of leaves inhibited HepG2 cancer cells in a dose-dependent manner. The ethanol and ethyl acetate extracts exhibited a good cytotoxic activity against HepG2cell lines with IC_{50} values of 49.70 µg/mL and 51.12µg/mL respectively, followed by hexane extract withIC_{50} values of 56.32µg/mL compared with the positive control Cyclophosphamide **(Table 1 and Figure3).**

Table 1: Anticancer activity of different solvent extracts of *C. halicacabum* leaves against the HepG2 cell line.

Con. of Drugs	MTT Assay			Cpd (Standard)
	Hn	El	Ml	
10 µg/mL	84.50±0.69	83.42±0.56	71.57±0.89	87.44±0.57
25µg/mL	71.72±1.01	65.36±0.50	62.28±0.96	72.77±0.76
50 µg/mL	36.77±1.12	42.15±0.60	45.25±0.60	66.23±0.48
IC_{50}	56.32	51.12	49.7	62.48



Before MTT treatment After MTT treatment

Figure3: Formation of formazan crystals in control cells and hexane treated cells, and cell morphology of Hep-G2 cells when treated with a hexane extract of *C. halicacabum* leaves.

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3.3 Antioxidant activity

This study to investigate the ability of the plant extracts to scavenge free radicals was evaluated using the DPPH radical scavenging method, with ascorbic acid (vitamin C) as the positive control. Substances capable of donating electrons/hydrogen atoms convert DPPH radical to its non-radical form 1,1′-diphenyl-2-picrylhydrazine, a reaction which can be measured at 517 nm. The n-hexane and ethanol extracts of *C.halicacabum* had the best antioxidant activities with IC_{50} values of 48.43µg/mL and 51.73µg/mL by DPPH compared with the positive controls **(Figure 4).**

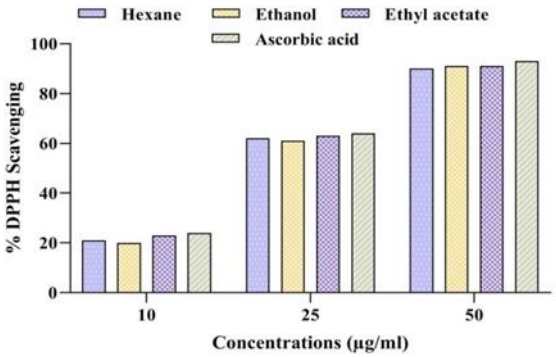

Figure4: DPPH-scavenging antioxidant power assay for different extracts of *C. halicacabum* leaves

3.4 Nitric oxide radical inhibition assay

NO is an important chemical mediator generated by endothelial cells, macrophages, neurons, and involved in the regulation of various physiological processes. Excess concentration of NO is implicated in the cytotoxic effects observed in various disorders such as AIDS, cancer, Alzheimer's, and arthritis. Nitric oxide exhibits numerous physiological properties and it is also implicated in several pathological states. The interaction of nitric oxide with other radicals leads to the formation of more hazardous radical such as peroxy nitrile anion and hydroxyl radical. The absorption maximum of a stable NO radical in methanol was at 546 nm. The IC_{50} values of hexane and ethanol were found to be 46.34 µg/mL and 48.95µg/mL respectively by nitric oxide scavenging assay followed by ethyl acetate extract **(Figure 5).**

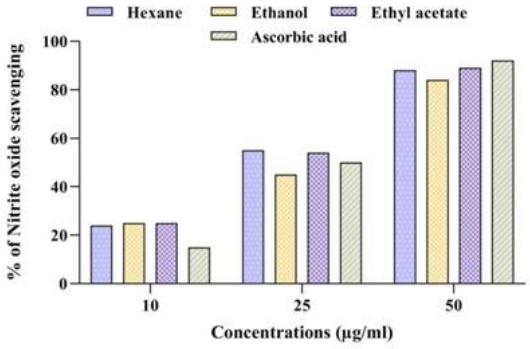

Figure5: Nitric Oxide scavenging activity using hexane, ethanol and ethyl acetate extracts of *C. halicacabum* leaves.

4. DISCUSSION

As reported in the literature, the ethyl acetate extract and the methanol extract of LJ flower buds had good ROS scavenging activities [25, 26]. Chang *et al.*, [27] demonstrated that the LJ extracts prepared by 70% methanol or 70% acetone extraction had good tyrosinase inhibition, xanthine oxidase inhibition, and nitrite scavenging activities. Choi *et al.*, [27] reported that the ingredients of ethyl acetate extract, such as luteolin, caffeic acid, protocatechuic acid, isorhamnetin-3-O-dglucopyranoside, quercetin 3-O-d-glucopyranoside, and luteolin 7-O-d-glucopyranoside, had high antioxidant activity. Most of these bioactive components are flavonoids. In UVB-irradiated fragments, diosmetin-3-O-β-D-glucuronide induced a significant decrease in hydrogen peroxide production and in the number of CDD-positive cells, reaching a maximal effect at the concentration of 2700 pg/mL (-48.6% and -52.0%), respectively [28]. DNA-sequence analysis of 14 strains from 26 non-aflatoxin producing strains were conducted and compared the three mycotoxin biosynthetic gene clusters (aflatoxin, cyclopiazonic acid, and aflatrem) and fermentation-related genes against those of reference strain Aspergillus oryzae RIB40 [29]. Furthermore, *C. halicacabum* showed a broad spectrum of pharmacological activities including anti-diabetic, antiulcer, anti-inflammatory, anxiolytic activity, apoptotic activity,nephroprotective, anti-arthritic, antibacterial, anti-diarrheal, antioxidant, antiviral, hepatoprotective properties [30].

Phenolic compounds and flavonoids are considered powerful antioxidants because they donate hydrogen or

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electrons to form stable radical intermediates [31-32]**(Figure6).** The antioxidant activities of many fruits, vegetables and edible plant extracts have a strong correlation with their total polyphenols content (TPC) and total flavonoids content (TFC) [33-36].*Cardiospermum halicacabum* leaf extract shows significant cytotoxic effect with 90% of inhibition when compared to standard drug [37]. The cyctoxicity level seems to increase with increase in concentration of the compound. While comparing test compound with the positive control the cyctotoxicity levels of the test compound were as less efficient as the cyclophosphamide but within the compound concentrations had good cytotoxicity capacity [38].

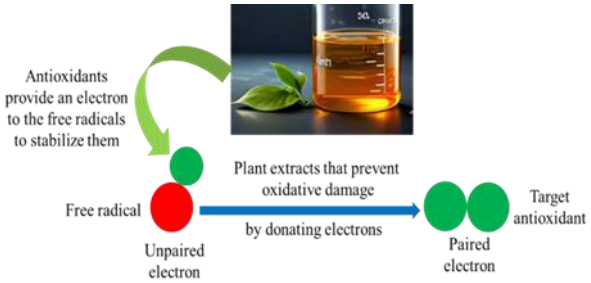

Figure6: The mode of action of antioxidants involves the donation of electrons by plant extracts.

The present investigation hexane extract of *C. Halicacabum* found to be good anticancer and antioxidant activity against HepG2 Human Liver Cancer Cell Lines significantly decrease the viability of cells at 50 µL recorded (36.77±1.12). The present finding coincides with the finding [39], evaluated the in vitro anticancer activity of the ethanol extract of root, stem and leaves of *Withaniasomnifer*against various Human cancer cell lines i.e. PC-3, DU-145 (prostrate), HCT-15 (colon), A-549 (lung) and IMR-32 (neuroblastoma). Result showed that root, stem and leaves extracts showed cytotoxicity activity ranging 0-98% depending on the cell lines but maximum activity was found in 50% ethanol extract of leaves of *Withaniasomnifera.* Ethanol extract leaves obtained from treatments T2, T3, T4 and T5 showed strong activity against PC-3 and HCT-15 with 80-98% growth inhibition, while the 50% ethanol extract of leaves from T1 treatment showed a minimum of 39% and T3 treatment showed maximum of 98% growth inhibition against HCT. Strawberry extract exhibits cytotoxic activity over the oral cancer cell lines. On administration of about 100 µg/ml of strawberry extract, about 50% of cell viability could be observed and assessed from the cell lines. Strawberries have a cytotoxic effect on oral cancer cell line due to the presence of anticancer constituents in the berries. These berries can be used as a natural medicine for cancer sufferers [40].

Our result is also agreement with the finding of Kumar *et al.*,[41], evaluated the anticancer properties of water, ethanol and acetone extract of *Andrographis paniculata* leaves against neuroblastima (IMR-32) and human colon (HT-29) cancer cell line. The results were found that ethanol extract showed nearly 50% i.e. inhibition concentration (IC_{50}) for IMR-32 and Ht-29 cell lines at 200 µg/ml, where other extracts display 50% inhibition at 250 µg/ml concentration for Ht-29 cell lines. Anticancer activity of water, ethanol and acetone extracts of *A. paniculata*leaves against Ht-29 cancer cell lines shows 50% inhibition at 200 µg/ml concentration. The mace extract shows the cytotoxic activity and induced the apoptosis through the modulation of its target genes Bcl-2 in the KB cell lines, suggesting the potential of mace as a candidate for oral cancer chemoprevention [42]. Furthermore, diosmetin-7-0-β-D-glucuronide (DMG) can inhibit the production of reactive oxygen species (ROS) and proinflammatory cytokines as well as upregulate the expression of anti-inflammatory cytokines, and DMG potent activity of IC50= 16.72±1.01µM[43].

The present results also corroborate the findings [44], reported that the anticancer activity of Quercetin isolated from an ethanol extract of *Carmona retusa* (Vahl.) masam and HepG2 cell lines by MTT assay, Hoechst 33342 staining and Caspase-3 colorimetric assay. Result showed significant and concentration dependent anticancer activity at 100 µg/ml and 80 µg/ml doses after 24 and 48 hours of treatment on HepG2 cell line in MTT assay, significant cell apoptosis have shown at 53 µg/ml concentration of extract yield of flavinoidquercetin from *Carmona retusa* (Vahl.) masamin Hoechst 33342 staining and a significant activation of caspase-3 observed at 100 µg/ml of extract yield of flavinoidquercetin from *Carmona retusa* (Vahl.) masamafter 24 hour and even 48 hour incubation. Likewise, the examined the anticancer activity of isolated fractions from *C. hallicacabum* methanol leaf extract on human hepatocellular carcinoma (HepG-2) cells [45]. Research done with the [46], evaluated the in vitro

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anticancer activity of capsaicinoids from capsicum Chinese against human hepatocellular carcinoma cells. The result showed that acetonitrile extract of capsicum Chinese on HepG2 cells showed reduction in the cell viability through MMT assay and it also significantly suppressed the release of LDH, LPO and NO production in a dose-dependent manner.

In recent years much attention has been devoted to natural antioxidants and their health benefits. Antioxidant-based drug for-mulations are used for the prevention and treatment of many complex diseases. Plants are a major source of natural antioxidants; they produce a wide range of secondary metabolites with antiox-idative activities that have therapeutic potential. Polyphenols are the most abundant antioxidant compounds of plant raw material. Their antioxidant activity is based on to their redox properties,which facilitate their activity as reducing agents, hydrogen donors,singlet oxygen quenchers, metal chelators and reductants of fer-rylhemoglobin. The reducing ability is generally associated with the presence of reductants which exert antioxidant action through breaking the free radical chain by donating a hydrogen atom orpreventing peroxide formation [47]. Medicinal plant tissues are commonly rich in phenolic compounds such as flavonoids, pheno-lic acids, stilbenes, tannins, coumarins, lignans and lignins. These compounds have multiple biological effects including antioxidant activity [48]. The cytotoxicty activity of fractions of the seed oil against MCF-7 breast carcinoma, with 50% growth inhibition value of <10 µg/ml, and GC-MS analysis of the most active fraction suggested the presence of active components that lead to cause of this effect [49].

Antioxidant activity of hexane, ethanol and ethyl acetate extracts of *C. halicacabum* leaves at different concentrations (10, 25 and 50µg/ml) were studied in various in vitro models. Results were compared with standard ascorbic acid. Antioxidant activity of extract was increased with the increasing concentration. The order of antioxidant potential according to models were found to be highest in nitric oxide scavenging activity followed by total antioxidant activity, reducing power assay and hydrogen peroxide scavenging activity. In conclusion, the results showed that there is significant influence of solvent type in preserving various phytocompounds of the *C. halicacabum* leave extract, and the highest antioxidant activity [50].

The results of DPPH inhibition by hexane, ethanol and ethyl acetate extracts significantly inhibited at the concentration of 50µL recorded (90.73±0.27), (91.17±0.32) and (93.93±0.36) when compared with standard drug Vitamin C (L-Ascorbic acid) Standard (93.93±0.36).The results of Nitric oxide radical inhibition assayby hexane, ethanol and ethyl acetate extract significantly inhibited at the concentration of 50µL recorded (88.16±0.46), (84.84±0.32) and (89.39±0.07) when compared with standard drug Vitamin C (L-Ascorbic acid) Standard (92.77±0.29). The present finding coincides with the findings, *C. halicacabum* whole plant ethanolic extracts were tested for anticancer activity using the MTT assay (5, 25, 50, 75 and 100µg), and exhibited mild to moderate cytotoxicity to L929 cell line, mild to severe cytotoxicity to PC-3 cells [51]. The eight plant species evaluated in the variability in antioxidant characteristics. Extracts of the Achilleaspecies studied, e.g., *A. grandifolia* and *A. crithmifolia*, demonstrated high antioxidant potential. This is the first report for antioxidant capacity of these species [52]. The extract of *A. crithmifolia* contained the highest level of polyphenols and flavonoids among all tested species and it demonstrated the highest antioxidant capacity in methanol extract. These results are in accord with high levels offlavonoid and phenol contents reported previously for *A. crithmifolia* as well as for other Achillea species [53]. In conclusion the active compound responsible for the anticancer and antioxidant activity was isolated and purified. Further work needs to be carried out to isolate identify andelucidate the active molecule from the active crude extract.

5. CONCLUSION

The present study on the different solvent extracts of *C. halicacabum* Linn. leaves revealed promising results in terms of antioxidant and anticancer activities. The ethanol extract exhibited the highest total phenolic and flavonoid content, indicating its potential as a rich source of bioactive compounds. The hexane and ethanol extracts demonstrated significant antioxidant effects, while the ethanol and ethyl acetate extracts showed cytotoxicity against HepG2 cells, suggesting their potential as anticancer agents. The findings suggest that the phytochemical constituents present in *C. halicacabum* play a crucial role in its antioxidant and anticancer properties. Overall, the study highlights the therapeutic potential of *C. halicacabum* as a natural source of

antioxidants and anticancer agents, emphasizing the importance of further research to explore its pharmacological benefits and potential applications in the field of medicine.

Conflict of interest

The authors declare no conflict of interest.

Abbreviations

HepG2: Human Hepatocellular Carcinoma Cell line; **DPPH:**2,2-diphenyl-1-picrylhydrazyl;**NO:** Nitric Oxide;**MTT:** (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide);**PBS:** phosphate buffer saline;**DMSO:** Dimethyl sulfoxide; **SNP:** Sodium nitroprusside;**NED:** Naphthylethylenediamine dichloride; **GAE/g:** gallic acid equivalents per gram;**QE:** Quercetin Equivalents; **NaNO2:** Sodium nitrite;**AlCl3:** aluminium trichloride;**NaOH:** Sodium Hydroxide.

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