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In vitro Anticancer Activity of *Curcuma caesia* on Human Skin Cancer Cell Line SK-MEL-28 (Skin Melanoma)

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ABSTRACT

Background: The hydroalcoholic extract derived from *Curcuma caesia* (CCHE) is recognized for its traditional use and well-established medicinal properties, often prescribed for the treatment of various ailments like skin cancer. Global trends favoring the utilization of non-toxic plant-based products, particularly those aligned with conventional medicinal practices and considering the reliance of developing countries on herbal remedies for primary healthcare.

Methods: This study aims to investigate the *in-vitro* cytotoxic effects of CCHE on the SK-MEL-28 human skin cancer cell line (specifically, skin melanoma) through MTT assays. The evaluation revealed that the hydroalcoholic extract of CCHE exhibited a notable inhibitory effect on the SK-MEL-28 cell line, with a percentage inhibition of 80.29% observed at lower concentrations (25 g/ml) and reaching 92.65% at higher concentrations (400 g/ml).

Result: These findings suggest that CCHE, herein referred to as CCHE (Potentiated Hydroalcoholic Extract), instigated a mechanism leading to cell arrest, thereby impeding the proliferation of cancer cells. In comparison to conventional medicines, the observed inhibitory effect of CCHE underscores its potential as a cytotoxic agent against human skin cancer cells.

Key words: *Curcuma caesia,* Cytotoxic activity, Human ckin cancer cell line SK-MEL-28 (Skin melanoma), IC_{50} value, MTT assay.

INTRODUCTION

Malignant melanoma represents a highly lethal manifestation of cutaneous malignancy, accounting for approximately 75% of fatalities associated with skin cancer globally. In recent years, there has been an observable escalation in the incidence of this pathology. The transition of melanoma towards the metastatic phase is an intricate process governed by a myriad of biochemical pathways, encompassing disruptions in the cell cycle, evasion of apoptosis, aberrations in cellular adhesion, degradation of the extracellular matrix, as well as orchestrated cellular migration and invasion. Treatment modalities for malignant melanoma remain constrained, with only a limited repertoire of chemotherapeutic agents available, all of which elicit significant adverse effects. Natural products represent a pivotal reservoir of prospective anticancer agents, constituting over 60% of clinically approved drugs employed in cancer therapeutics (Senderowicz *et al.,* 2003).

C. caesia, commonly denoted as black turmeric, possesses a rhizome characterized by a bluish-black hue and emits a bitter and pungent aroma. Existing literature attests to the multifaceted pharmacological attributes of this plant, encompassing anti-inflammatory, hepatoprotective, antioxidant, anti-asthmatic, antitumor, stomachic and carminative properties (Sahu and Saxena, 2018).

The prospective antitumor impact of CCHE is ascribed to its dual mechanism, involving direct cytotoxic effects and antioxidative properties. Through the alleviation of oxidative stress in diverse tissues of EAC-bearing mice, CCHE diminishes the viability of EAC cells (Karmakar *et al.,* 2013). Previous investigations have elucidated the anticancer potential of CCHE against a spectrum of cancer cell lines, inclusive of human breast cancer, human colon cancer and ovarian cancer (Shaikh *et al.,* 2016). The observed direct cytotoxic impact and antioxidant attributes of CCHE have been ascribed to its potential antitumor efficacy. Furthermore, CCHE displays promise bioactive constituents within CCHE may exert anticancer effects through the TNF α-mediated NF-κB signaling pathway (Hadem *et al.,* 2015).

The bioactive chemical constituents identified in the rhizomes of *C. caseia* (CCHE) encompass germacrone, zerumbone, furanodienone, curzerenone and curcuzederone.

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The identification of these biologically active compounds serves to substantiate the historical utilization of *C. caseia* rhizomes in the context of cancer, tumors and various human ailments. Furthermore, this investigation underscores *C. caseia* rhizomes as a prospective reservoir for the isolation of bioactive chemicals, warranting further exploration in scientific research endeavors (Mohammad Al-Amin *et al.,* 2021). Consequently, the present study focuses on the investigation of *in-vitro* cytotoxic properties of CCHE (Crude Chloroform Hibiscus Extract) against the SK-MEL-28 human skin cancer cell line, specifically targeting skin melanoma.

MATERIALS AND METHODS

We acquired the human skin cancer cell line SK-MEL-28 (skin melanoma) from the National Centre for Cell Science (NCCS) in Pune, India. The experimental cellular cultures were upheld at a temperature of 37°C within a controlled atmosphere of 5% carbon dioxide and were cultivated in Minimum Essential Medium (MEM, GIBCO)

Plant collection and preparation

Plant specimens were procured from the local market in the Bhopal region of Madhya Pradesh, India. The identification and authentication processes were meticulously conducted by Dr. Sandeep Kumar Singh, a Pharmacognosist at the Central Ayurvedic Research Institute in Jhansi, Uttar Pradesh. The verification of plant materials was carried out with precision and accession numbers CARI/H/13302021 were assigned for reference purposes. Additionally, the authentication was cross-verified and documented by the Botanical Survey of India, Central Regional Centre, Praygraj, U.P.

Extraction of plant material

The plant material from *C. caseia* (CCHE) was subjected to extraction through the cold maceration method. Plant samples were systematically collected, thoroughly washed, rinsed and appropriately dried. The powdered form of the plant sample was then subjected to extraction using a hydroalcoholic solvent (30:70 ratio), allowing it to stand for 4-5 days per iteration. Subsequently, the resulting extract underwent filtration using filter paper to eliminate all non-extractable components, including cellular materials and other constituents insoluble in the extraction solvent. The filtrate was then transferred to a beaker and subjected to evaporation, facilitating the removal of excess moisture. The resulting extract was collected in an airtight container. Qualitative analysis of extracts obtained through different solvents was conducted to ascertain the presence of various phytoconstituents, as outlined by (Kokate *et al.,* 2010).

Qualitative phytochemical tests of extracts

Phytochemical tests of extracts were performed using reported general methods for the detection of alkaloids, flavonoids, tannins, phenol, saponins and glycosides etc **(**Kokate *et al.,* 2010).

In-vitro anti cancer cell study

Cell plating and MTT assay

The evaluation of the *in-vitro* inhibitory effects of test chemicals on cell growth was conducted through slight adaptations to the Mosmann MTT assay protocol. The human skin cancer cell line SK-MEL-28 (skin melanoma), derived from T-25 flasks, was cultured in 96-well tissue culture plates at a seeding density of cell density was used to be 8,000 cells per well in a growth medium and maintained at 37°C with 5% CO_2. The growth media supernatant was aspirated from each well and replaced with 100 µl of dimethyl sulfoxide to dissolve the formazan material, yielding a colored solution. Subsequently, 20 µl of freshly prepared MTT solution (5 mg/ml in PBS) was added to each well, followed by a 24-hour incubation period at 37°C. The optical density (OD) was measured at 570 nm using an ELISA reader after a 30-minute incubation time (Sugathakumari *et al.,* 2022).

$$\% \text{ of cell viability} = \frac{(\text{OD of test})}{(\text{OD of control})} \times 100$$

Data interpretation

Reduced absorbance values compared to the control cells signify a deceleration in the rate of cellular proliferation. Conversely, an elevated absorption rate is indicative of augmented cell proliferation. Periodically, an upswing in proliferation may be counteracted by a decline in cellular mortality; manifestations of cell demise may encompass discernible morphological alterations.

RESULTS AND DISCUSSION

CCHE were taken and removed throughout the extraction process. It was discovered that the extract had a yield of 8.1%.

Phytochemical screening test

According to research, CCHE has the best potential for bioactivity of any extract when it comes to glycosides, alkaloids, glycosides, phenolic compounds, tannins, saponins, flavonoids and proteins. Hence, CCHE chose us for additional research. Results are shown in below Table 1.

The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-dipheny ltetrazolium bromide] assay methodology was employed

Table 1: Results of phytochemical screening test of hydroalcoha extract.

Name of tests	Results
Alkaloids	+
Steroids	-
Flavonoid	+
Tannins and phenolic compounds	+
Saponins	+
Glycosides	+

to ascertain the anticancer attributes of a poly-herbal formulation derived from selected botanical sources. In the context of *in-vitro* assessments directed at elucidating a cellular population's responsiveness to extrinsic stimuli, foundational metrics encompass measurements pertaining to cell viability and proliferation. The integration of radioactive thymidine into cellular deoxyribonucleic acid (DNA) was implemented in cell growth experiments. The MTT assay method induced a reduction in cell metabolic activity, concomitantly diminishing the activities of dehydrogenase enzymes, leading to the generation of dipping equivalents such as nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH). This method facilitated the assessment of cell proliferation capabilities, shedding light on growth rates and discerning reductions in cell viability associated with metabolic processes culminating in apoptosis (Hussein *et al.,* 2003). Through the establishment of the correlation between cell number and the signal generated specific to each cell type, alterations in the rate of cell proliferation could be precisely quantified.

Effect of CCHE on human skin cancer cell line SK-MEL-28 (skin melanoma)

Table 2 displays the results of the MTT cell growth inhibition assay performed for CCHE of medicinal herb composition at various concentration dosages of 25, 50, 100, 200 and 400 µg/ml. Inhibition of Caco-2 cancer cell growth caused by the composition of a herbal extract and a standard sample was compared in Fig 1. Using the MTT test method, the IC50 value for CCHE was discovered to be 85.08±0.01µg/ml. The consensus among experts supports the utilization of tetrazolium salts reduction as a consistent and reliable method for assessing cellular proliferation.

The impact of the test extract on cellular proliferation and viability was assessed employing the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Tetrazolium reduction catalyzed by dehydrogenase enzymes serves as an indicator of cellular metabolic activity, yielding NADH and NADPH in the process. The resulting formazan precipitates as purple crystals with limited water solubility (Fig 1). Dimethyl sulfoxide (DMSO) was employed to quantify the formazan content and assess the color intensity at 570 nm, aligning with the number of viable cells in the culture. Results were expressed as a percentage of viability (log) relative to untreated cells (basal), which served as a 100% viability control.

The percentage of inhibition of the human skin cancer cell line SK-MEL-28 (skin melanoma) by CCHE and conventional medicines was determined to be 80.29% and 92.65%respectively, when the concentration of CCHE increased from 25 to 400g/ml. This indicates that the CCHE activated a cell arrest mechanism and slowed the development of cancer cells (Fig 2).

Table 2: Dose response of test sample on human skin cancer cell line SK-MEL-28 (skin melanoma).

Concentration (ug/ml)	% Cell survival of STD	% Cell inhibition of STD	% Cell survival of CCHE	% Cel linhibition of CCHE
0	0	0	0	0
25	78.23	21.77	63.13	32.87
50	46.18	53.82	56.06	49.04
100	28.23	71.77	41.27	64.73
200	21.96	78.04	25.34	70.66
400	7.35	92.65	14.70	80.29

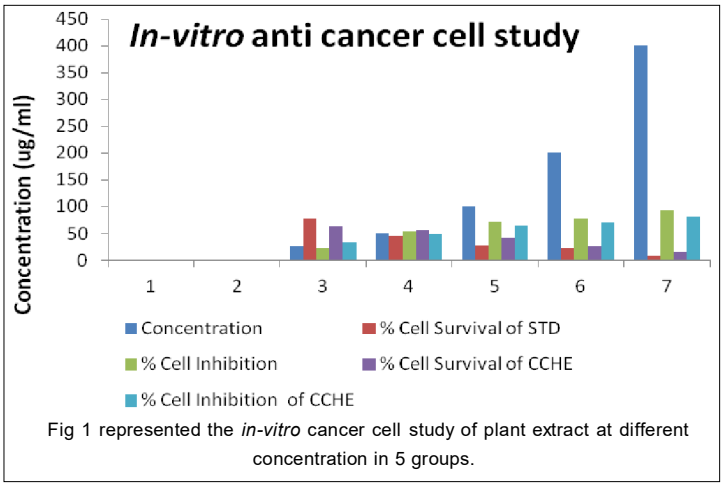
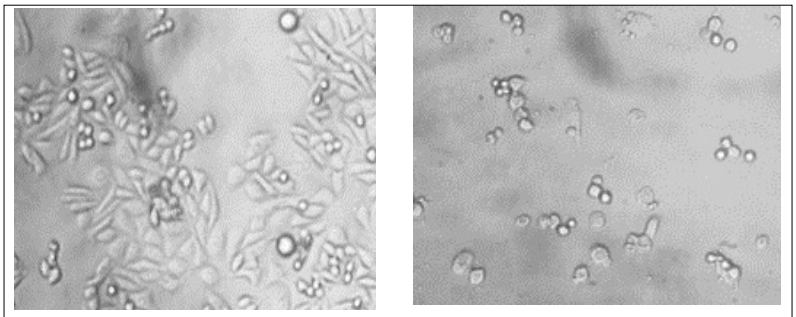


Fig 1 represented the *in-vitro* cancer cell study of plant extract at different concentration in 5 groups.

Fig 1: Effect of CCHE on human skin cancer cell line SK-MEL-28 (skin melanoma) growth inhibition.



a. Before treated cell lines.

b. After treated cell lines at 400 µg/ml.

Fig 2a represented the before large numbers cancerous cell and fig b represented the after treated cell line condition and numbers at 400 µg/ml.

Fig 2: Images of human skin cancer cell line SK-MEL-28 (skin melanoma).

Flora, botanicals and ethnomedicinals have been instrumental in human well-being since time immemorial, persisting as crucial agents in contemporary global healthcare systems for health promotion and disease mitigation. The escalating global demand for herbal remedies is attributed to their diminished toxicity. Natural compounds sourced from plants constitute the foundational elements of modern medicine, significantly influencing the development of pharmaceuticals. The assessment of anticancer potential in this investigation employed the *in-vitro* MTT screening method on cancer cell lines. Colorimetry, integral to the MTT test, facilitated the analysis of reagent-induced color changes, offering insights into cell viability. The cytotoxicity of viable cells is intricately linked to the activity of mitochondrial dehydrogenases.

The examination of anticancer activity in this study revealed notable outcomes. The MTT assay method demonstrated that both the conventional drug and CCHE (*C. caesia* hydroalcoholic extract) exhibited substantial growth inhibition of the human skin cancer cell line SK-MEL-28 (skin melanoma) by 82.6 per cent and 66.7 per cent, respectively. *In vitro* investigations of plant phenolic compounds have consistently demonstrated their ability to impede cell growth across various phases of the cell cycle (G1, S and G2). These compounds operate through direct and indirect mechanisms, downregulating cyclins and cdks and influencing the expression of genes such as p21, p27 and p53 by acting as prooxidants (Sugathakumari *et al.,* 2022; Daveri *et al.,* 2022). The research findings hold promise for pharmaceutical companies to develop environmentally friendly cancer treatments. *C. caesia*, with its diverse array of bioactive chemicals, appears to harbor potential substances capable of arresting the growth of specific cancer cells, particularly the human skin cancer cell line SK-MEL-28 (skin melanoma) (Hadem *et al.,* 2014, Hadem *et al.,* 2015). The observed apoptosis-inducing capacity of CCHE underscores its potential anticancer effects. This study suggests that CCHE contains polyphenolic chemicals, a subgroup of phytochemicals, warranting further research into these bioactive polyphenolic components (Noorjahan and Saranya, 2018, Kumar *et al.,* 2023, Lee *et al.,* 2018, Atchaya *et al.,* 2024).

Historically, secondary metabolites from herbal plants have found utility across diverse medical systems for treating various ailments, including diabetes, cancer and arthritis. The persistent reliance on phytoconstituents underscores their continued significance in anticancer drug formulations. The cytotoxic potential of the hydroalcoholic extract of *C. caesia* was evaluated in this study using MTT assays, revealing a substantial percentage of cell inhibition with increasing concentrations of the bioactive components in the extract.

CONCLUSION

Our investigation has led to the inference that the utilization of *in vitro* research diminishes the requisite for animal-based clinical trials. This facilitates the expeditious evaluation of a broader spectrum of substances with reduced resource consumption. The MTT assay conducted on the SK-MEL-28 (skin melanoma) human skin cancer cell line has provided evidence of the potentially potent and distinctive anticancer properties associated with the CCHE (extract from the indigenous tree). The cumulative results suggest the prospective utility of this native tree as an innovative chemotherapeutic agent for the treatment of skin cancer. This discovery holds the potential to catalyze advancements in state-of-the-art cancer research methodologies and plant-derived cancer therapies, obviating the need for toxic chemical dosages, conventional chemotherapeutic drugs, or associated side effects. The research outcomes underscore the imperative for further investigations aimed at identifying the specific bioactive constituent responsible for eliciting the observed anticancer effects.

Conflict of interest

Every author affirms that they have no competing interests.

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