Journal of Applied Biology & Biotechnology Vol. X(XX), pp. 1-8, 2024
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2024.194161

Assessment of *in vitro* antioxidant properties and anticancer potential of *Cucumis pubescens* Willd. a medicinal fruit, utilizing human lung cancer cell line (A549)

T. Sundari[1], R. Kavitha[1]*, B. Mythili Gnanamangai[2], S. Saranya[2]

[1]PG & Research Department of Chemistry, Sri Sarada College for Women (Autonomous), Salem - 636 016, Tamil Nadu, India.
[2]Department of Biotechnology, K.S.Rangasamy College of Technology (Autonomous), Tiruchengode - 637215, Tamil Nadu, India.

ARTICLE INFO

Article history:
Received on: March 13, 2024
Accepted on: June 02, 2024
Available online: ***

Key words:
Cucumis pubescens,
Wild Cucumber,
Anticancer potential,
Antioxidant properties.

ABSTRACT

This study explores the antioxidant properties and anticancer potential of *Cucumis pubescens* Willd. (Cucurbitaceae) fruits, focusing on enzymatic, nonenzymatic, and MTT [3-(4,5-dimethylthiozol-2-yl)-3,5-diphenyl tetrazolium bromide] assays. The investigation reveals the presence of key antioxidant enzymes, known for their antioxidant properties. Furthermore, MTT assay within *C. pubescens* exhibits significant anticancer effects by inducing cell growth arrest and inhibition. The aim of this study was to assess the *in vitro* antioxidant potential and anticancer properties of a human lung cancer cell line (A549). Antioxidant property evaluation methods are carried out through SOD (superoxide dismutase) assay, catalase activity assay, GPx (glutathione peroxidase) assay, and DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. GST enzyme estimation established a detoxifying effect, and the MTT assay established the cytotoxic potential of *C. pubescens* fruits. The fruit extract of *C. pubescens* showed promising antioxidant properties and anticancer effects. The IC_{50} (50% inhibition concentration) value was estimated to be 7.5 ± 1.5, which is significantly higher compared to its positive control (doxorubicin). This comprehensive exploration not only enhances our understanding of the health-promoting properties of *C. pubescens* but also emphasizes its potential as a nature-derived source for medical applications, particularly in the field of oncology.

1. INTRODUCTION

India, endowed with rich biodiversity, boasts an array of wild fruits that have been an integral part of traditional diets and folk medicine for centuries. These fruits, sourced from diverse ecosystems across the subcontinent, not only contribute to culinary diversity but also harbor a rich source of bioactive compounds with potential health benefits. Incorporating Indian fruit varieties can have a variety of health benefits, including improved digestive health, heart health (lowering blood pressure and cholesterol), enhanced immunity (vitamin C-rich fruits), skin health, and blood sugar control. Fruits are also a rich source of antioxidants [1]. The exploration of antioxidant-rich fruits aligns with a global paradigm shift toward preventive healthcare and the recognition of dietary interventions as pivotal components of well-being.

Common Indian wild fruits, such as *Phyllanthus emblica*, *Syzygium cumini*, *Ziziphus mauritiana*, *Punica granatum*, *Aegle marmelos,* and *Annona squamosa*, have drawn significant scientific interest due to their rich phytochemical compositions. These fruits are replete with polyphenols, flavonoids, and other bioactive compounds, contributing to their antioxidant potential [2]. Traditional medicinal practices in India have long harnessed the therapeutic properties of these fruits, recognizing their ability to bolster the body's defense against oxidative stress and associated ailments.

As India generally bears the burden of communicable and noncommunicable diseases, perceiving the antioxidant properties of common Indian wild fruits becomes paramount. This exploration not only sheds light on traditional wisdom but also provides a scientific basis for incorporating these fruits into modern dietary patterns and wellness strategies. In this context, this study aims to delve into the antioxidant properties and anticancer potential of wild cucumber fruit (*C. pubescens*), unraveling their phytochemical intricacies and potential health implications.

C. pubescens (fruit), colloquially known as the prickly cucumber or gooseberry cucumber, has been regarded as a subject in medicinal plant research. Although usually not cultivated, this plant commonly thrives as a weed amidst other crops. It tends to flourish in fields

*Corresponding Author:
R. Kavitha,
PG & Research Department of Chemistry,
Sri Sarada College for Women,
Salem, Tamil Nadu, India.
E-mail: kavitha.rsk@gmail.com

where sorghum, maize, and groundnut were cultivated, particularly in arid and infertile soils. Its growth is widespread across the drier regions of India. Characterized by a hairy stem, the plant produces yellow flowers, and its fruit skin displays various colors, including yellow, striped green, and brown. It is capturing attention owing to its promising health-promoting properties. Belonging to the Cucurbitaceae family, this fruit has a history of traditional medicinal uses across various cultures [3]. As the demand for natural remedies and functional foods continues to grow, exploring the therapeutic properties of *C. pubescens* has become essential. The interest in *C. pubescens* is driven by its composition of bioactive compounds, including polyphenols and flavonoids, known for their potential antioxidants. A variety of antioxidants contribute to reducing oxidative stress and are also associated with different diseases, especially cancer [4]. Oxidative stress happens when there is a disproportion between reactive oxygen species (ROS) production and the body's capability to reduce their effect, followed by causing cellular damage and contributing to initiation of cancer-related diseases.

The relationship between antioxidant potential and anticancer properties has been discussed in biomedical research using various medicinal plants. Multiple scientific articles explore the interplay between cellular redox balance and carcinogenesis. Antioxidants, by nature, are capable of neutralizing ROS and moderate oxidative stress, play a pivotal role in maintaining cellular homeostasis, and prevent DNA damage [5]. Elevated levels of oxidative stress are characterized by an inequity between the production of ROS and the defense system of antioxidants, and they have been implicated in the initiation and progression of various cancers. Antioxidants, sourced from both endogenous cellular mechanisms and exogenous dietary components, act as frontline defenders against ROS-induced cellular damage. Importantly, the capacity of antioxidants to scavenge free radicals and oxidative stress is reduced. The exploration of their potential is used in cancer prevention and treatment. Preclinical studies have demonstrated that compounds with robust antioxidant potential, such as polyphenols and flavonoids found in various fruits and vegetables, exhibit anticancer activities by interfering with cancer cell proliferation, inducing apoptosis, and suppressing angiogenesis [6]. However, the intricate balance between pro-oxidant and antioxidant effects poses challenges in extrapolating these findings to clinical settings, highlighting the need for a detailed understanding of the role of antioxidants in cancer biology. While antioxidant-rich diets have shown promise in reducing cancer risk, the complex nature of cancer demands a robust approach that considers individual variability, the specific type of cancer, and the stage of its development [7]. Thus, elucidating the dynamic relationship between antioxidant potential and anticancer effects remains a crucial area of research, with implications for both preventive strategies and therapeutic interventions in the study of oncology.

While preliminary investigations hint at the antioxidant potential of *C. pubescens*, a comprehensive elucidation of its bioactive constituents and their specific modes of action are paramount. This research endeavors to bridge existing knowledge gaps, delving into the molecular intricacies of how *C. pubescens* may mitigate oxidative stress and, consequently, contribute to the prevention or treatment of diseases, with a particular focus on cancer. By scrutinizing the fruit's pharmacological attributes, this study seeks to understand the scientific need for the utilization of *C. pubescens* as a potential therapeutic agent, thus aligning with the global pursuit of effective and sustainable health interventions.

2. MATERIALS AND METHODS

2.1. Plant Collection and Identification

The *C. pubescens* (fruit) used in this study was collected from in and around Namakkal in the state of Tamil Nadu. The collected plant sample was identified with the support of plant taxonomists at the Department of Botany, Arignar Anna Government Arts College, Namakkal, Tamil Nadu, following the guidelines of botanical flora [8]. The botanical nomenclature of the plant was as per the flora of Tamil Nadu [9]. The sample was stored in a shaded area for subsequent analysis. A voucher specimen number BOT-AAGAC-08/2018) was assigned to the sample. The freshly collected fruits were washed thoroughly and dirt and debris were removed. The fruit was uniformly cut into slices to ensure even drying. The thinner slices generally dry faster. The slices were placed in trays and dried in a sunny and well-ventilated area. The trays were covered with cheesecloth or mesh to protect the fruit from insects and debris. After consistently drying the fruit slices under the sun, the shade-drying process was followed. After shade drying of the fruit slices for 20 days, they were ground, powdered, sieved with No. 40 mesh, and stored in a cool place. To ensure the quality and integrity of the fruits during the shade-drying process, several measures were implemented. The fruits were regularly monitored throughout the 20-day period to detect any signs of microbial contamination. The drying area was maintained in a clean and well-ventilated condition to minimize the risk of fungal and bacterial growth. The powder was later stored in airtight containers.

2.2. Evaluation of Antioxidant Properties

2.2.1. Fruit extract preparation

The fruit was rinsed in running water, and with a vegetable peeler, the outer skin was removed. The seeds and pulp were discarded, retaining the mesocarp for enzyme extractions. A 10 g portion of the mesocarp was homogenized with the help of a mortar and pestle in 30 mL of ice-cold 100 mM potassium phosphate buffer solution (pH 7.5) and stored for subsequent enzymatic activity measurements [10].

2.2.2. Estimation of superoxide dismutase

The activity of superoxide dismutase (SOD) was estimated using a standard assay method, using ascorbic acid as a control to generate a comparative curve. In a conical flask, 25 mL of 216 mM potassium phosphate buffer (pH 7.8), 1 mL of 10.7 mM EDTA, 1 mL of 1.1 mM cytochrome C solution, 50 mL of 5 units/mL xanthine oxidase solution, and 23 mL of purified water were added. The components were mixed, and the pH was adjusted to 7.8. The absorbance at 550 nm was monitored until it equilibrated. Varying quantities of 10× diluted fruit extract were added to this cocktail mix, and the increase in absorbance at 550 nm was measured [11]. The percentage of inhibition was recorded accordingly.

$$\%\, inhibition = \frac{\Delta A_{uninhibited} - \Delta A_{inhibited}}{\Delta A_{uninhibited} - \Delta A_{blank}} \times 100$$

where

$\Delta A_{uninhibited}$—Absorbance of uninhibited extract – blank at 550 nm,

$\Delta A_{inhibited}$—Absorbance of inhibited extract – blank at 550 nm,

ΔA_{blank}—Absorbance of blank at 550 nm.

2.2.3. Estimation of catalase

Catalase stands as a vital antioxidant enzyme, playing a key role in catalyzing the decomposition of peroxides into nontoxic substances. The assessment of catalase activity often involves measuring its ability to scavenge hydrogen peroxide, which is indirectly proportional to the quantity of catalase present in the fruit extract. To gauge this, the absorbance of a hydrogen peroxide solution, prepared in mM phosphate buffer, is measured as a blank at 230 nm. Subsequently, 1 mL of the fruit sample extract was taken out at various concentrations, introduced to 2 mL of this solution, and incubated at room temperature for 10 min. The reaction is halted using 100 mM H_2SO_4, and absorbance was taken at 230 nm against the blank [12]. The activity of catalase was estimated using a standard assay method, with curcumin used to generate a standard curve for comparison. The scavenging action is graphed against concentration, facilitating the evaluation of catalase activity using a predefined formula:

$$\% \ scavenging \ activity = \frac{A_{230} \ control - A_{230} \ sample}{A_{230} \ control} \times 100$$

where

A_{230}control—Absorbance of control at 230 nm,

A_{230}sample—Absorbance of fruit sample at 230 nm.

2.2.4. Estimation of glutathione peroxidase

An essential antioxidant enzyme that is vital to cellular defense against oxidative stress is glutathione peroxidase (GPx). Using reduced glutathione (GSH) as a substrate, the main role of GPx is to catalyze the reduction of hydrogen peroxide (H_2O_2) and organic hydroperoxides. The guaiacol oxidation method is used to measure GPx activity [12], using 1 mL of fruit sample extract, 8 mL of guaiacol, and 10 mL of potassium phosphate buffer (pH 7.0). The addition of 2.75 mM hydrogen peroxide starts the process. Tetraguaiacol production is shown by a 30-min spike in absorption at 470 nm. The calculation of % inhibition is performed using the provided formula.

$$\% \ inhibition = \frac{A_{470} \ control - A_{470} \ sample}{A_{470} \ control} \times 100$$

where

A_{230}control—Absorbance of control at 470 nm,

A_{230}sample—Absorbance of sample at 470 nm.

2.2.5. Estimation of DPPH

DPPH is a dark crystalline solid with a strong ability to scavenge radicals and generate a deep violet solution. The DPPH assay, utilizing 2,2-diphenyl-1-picrylhydrazyl (DPPH), is a widely employed colorimetric method for determining antioxidant potential in plant varieties. DPPH, a dark crystalline solid yielding a deep violet solution, exhibits robust radical scavenging capacity, causing a pale yellow or colorless transition upon reacting with free radicals. The higher antioxidant content in samples correlates with increased discoloration of the purple pigment, emphasizing the electron-donating role of the antioxidant source. Trolox and ascorbic acid serve as standard antioxidants in DPPH assays [13]. Quantifying antioxidant potential is expressed through various methods such as µmol trolox equivalent antioxidant capacity (TEAC)/100 g dried material and EC_{50}.

Antioxidant potential can be measured using multiple techniques, including EC_{50} and µmol TEAC/100 g of dry material.

$$\% \ inhibition = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where

Acontrol—Absorbance of control at 515 nm,

Asample—Absorbance of sample at 515 nm.

2.3. Assessment of Anticancer Potential

2.3.1. Estimation of glutathione S-transferase

By catalyzing the conjugation of the tripeptide glutathione (GSH) to a range of hydrophobic and electrophilic chemicals, the enzyme glutathione S-transferase (GST) is essential to cellular detoxification. In the enzymatic assessment of GST activity, conducted at 25°C, the substrate employed was 1-chloro-2,4-dinitrobenzene (CDNB). The reconstitution medium for enzyme activity consisted of phosphate buffer (pH 6.5), 20 mM GSH, and 25 mM CDNB. Enzyme units, a standard measure in enzyme research, represent the quantity of enzyme catalyzing the conversion of 1 µmol of a substrate to a product within 1 min. Spectrophotometric measurements of absorbance changes at 340 nm were recorded over a 1-min interval using a spectrophotometer. The GST enzyme activity, utilizing reduced GSH and CDNB as substrates, was quantified spectrophotometrically at 25°C. This methodological approach allows for a comprehensive evaluation of GST catalytic efficiency under the given experimental conditions [14].

2.3.2. Morphological study

The National Centre for Cell Sciences (NCCS), Pune, India, provided the A549 human lung cancer cells. A balanced salt solution (BSS) containing 1.5 g/L Na_2CO_3, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate, 2 mM L-glutamine, 1.5 g/L glucose, 10 mM (4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid) (HEPES), and 10% fetal bovine serum (GIBCO, USA) was added to the Dulbecco's modified Eagle's medium (DMEM) for the cancer cells that were chosen. Furthermore, 100 IU/100 µg of penicillin and streptomycin were added at a 1 mL/L dosage. The cells were grown in a humidified environment with 5% CO2 at 37°C.

To study the morphology, the selected cells, cultivated on coverslips at a density of 1×10^5 cells per coverslip, underwent incubation with a complex at various concentrations. Following that, a 3:1 (v/v) ethanol:acetic acid solution was used to fix the cells. To conduct morphometric analysis, the coverslips were carefully attached to glass slides. Using micrography, three monolayers from each experimental group were recorded. Nikon (Japan) bright-field inverted light microscopy was used to examine the morphological changes in the cells at 10× magnification [15].

2.3.3. Evaluation of cytotoxicity

To find out the inhibitory concentration (IC_{50}) value, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide test was used. In a 96-well plate, cells were grown at 1×10^4 cells/ well for 48 h, until 80% confluence was reached. Later, the cells were cultured for an additional 48 h, until 80% confluence was reached. Then, the cells were cultured for an additional 48 h in a new medium that contained a serially diluted sample. After discarding the culture medium, 100 µL of MTT [3-(4,5-dimethylthiozol-2-yl)-3,5-diphenyl tetrazolium bromide] solution (Hi-Media) was added to each well and incubated at 37°C for 4 h. Following the deletion of the supernatant, 50 µL of DMSO was added to solubilize the formazan crystals, with an additional 10-min incubation. The OD was measured at 620 nm using an ELISA multi-well plate reader (Thermo Multiskan EX, USA).

The OD value was used for calculating the percentage of viability, employing the following formula [16].

$$\%\ of\ viability = \frac{OD\ value\ of\ experimental\ sample}{OD\ value\ of\ experimental\ control} \times 100$$

2.3.4. Statistical analysis

Each *in vitro* experiment was conducted in triplicate, and the entire set of experiments had three replicates. The statistical differences among the fruit extracts were evaluated using a one-way ANOVA. Every value is significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

The antioxidant properties of *C. pubescens* (Fruit) were determined through enzymatic activity assays, including SOD, catalase, and glutathione peroxidase, along with the estimation of redox potential through the DPPH assay. The comparison was done to identify the antioxidant potential between standard and fruit extract. Notably, the *C. pubescens* fruit extract demonstrated exceptional performance, surpassing the standard in terms of % inhibition in all assays, except for the catalase assay, where it performed well. This observation was consistent across varying concentrations for each respective assay.

3.1. Superoxide Dismutase

The SOD assay of the *C. pubescens* fruit extract showed various concentrations from 20 to 100 µg/mL, inferring that the activity increased when the concentration of the fruit extract increased. The results showed a gradual increase in the dosage of fruit extract concentration. The maximum SOD activity was observed in 100 µg/mL and the 44.30 ± 0.24 percentage of inhibition was recorded against the standard ascorbic acid [Figure 1].

3.2. Catalase

The catalase activity was reduced when the concentration of fruit extract increased. The maximum activity of catalase recorded in standard curcumin is as follows: 13.56 ± 0.64, 24.37 ± 0.23, 36.02 ± 0.46, 45.15 ± 0.71, and 59.24 ± 0.15. But in *C. pubescens* the catalase activity was reduced, and the percentage of inhibition was 11.03 ± 0.74, 2.62 ± 0.52, 31.89 ± 0.11, 39.22 ± 0.31, and 52.84 ± 0.42, respectively, from the concentration of 20–100 µg/mL of fruit extract [Figure 2].

3.3. Glutothione Peroxidase

The *C. pebescens* fruit extract was subjected to glutothione peroxide analysis at the concentrations of 20, 40, 60, 80, and 100 µg/mL. When compared with the gallic acid used as a standard, the percentage of inhibition was 11.33 ± 0.43, 13.90 ± 0.67, 17.46 ± 0.7, 26.45 ± 0.32, and 40.66 ± 0.26. The fruit extract recorded 18.47 ± 0.43, 20.81 ± 0.68, 22.58 ± 0.22, 28.41 ± 0.85, and 43.18 ± 0.53 as percentages of inhibition. When increasing the concentration of fruit extract, the glutathione peroxidase activities also increased [Figure 3].

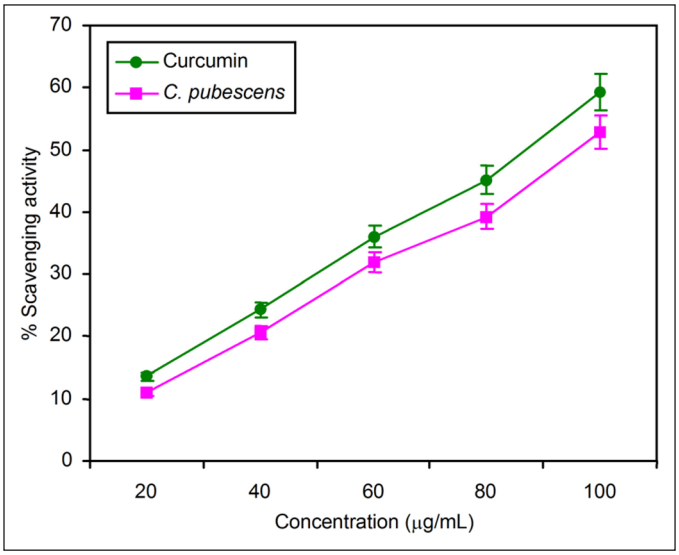

Figure 2: Comparison of the catalase activity of standard curcumin and *C. pubescens* fruit extract at different concentrations. All the values are expressed as the mean ± standard deviation with three replicates.

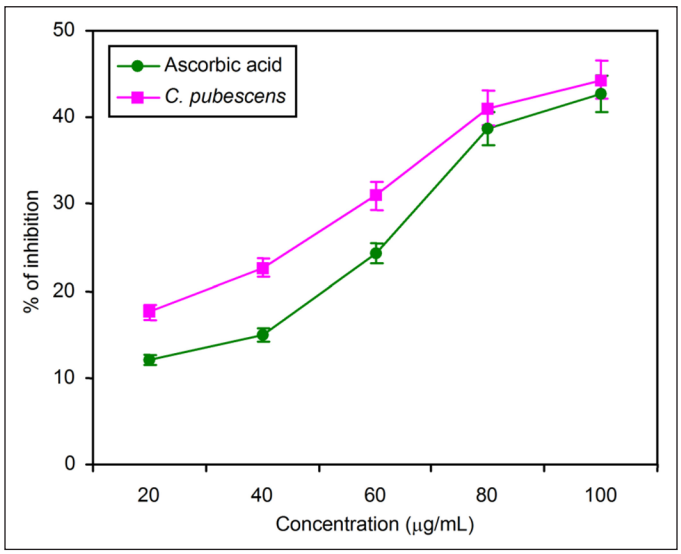

Figure 1: Comparison of the superoxide dismutase activity of standard ascorbic acid and *C. pubescens* fruit extract at different concentrations. All the values are expressed as the mean ± standard deviation with three replicates.

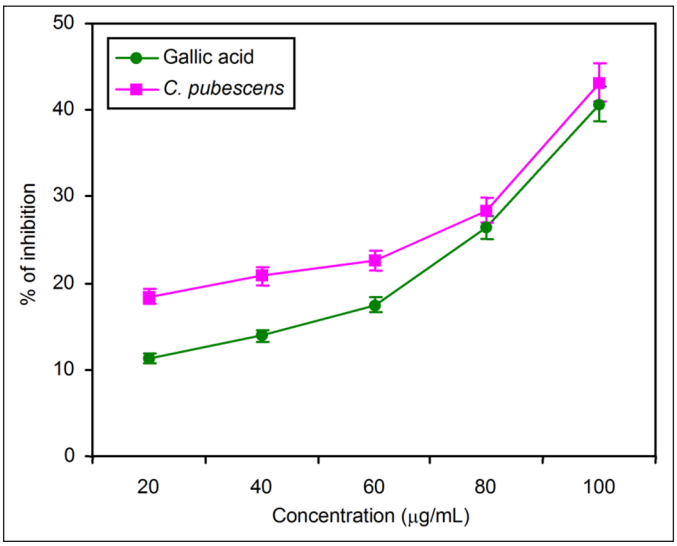

Figure 3: Comparison of glutathione peroxidase activity of standard gallic acid and *C. pubescens* fruit extract at different concentrations. All the values are expressed as the mean ± standard deviation with three replicates.

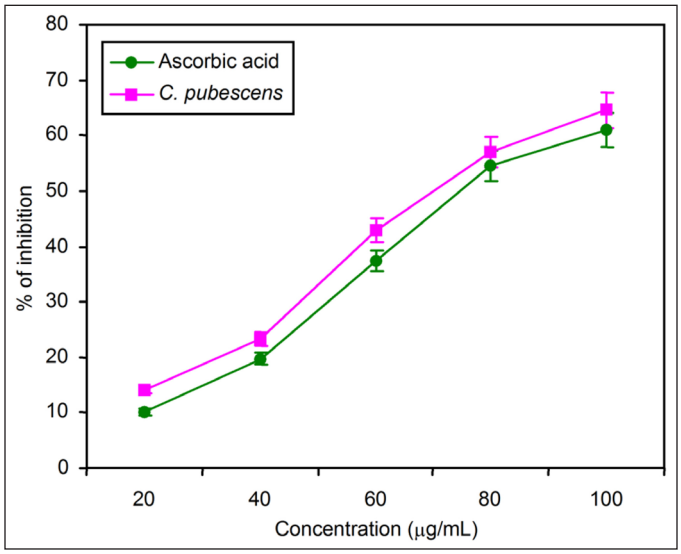

Figure 4: Comparison of redox potential of standard ascorbic acid and *C. pubescens* fruit extract by DPPH assay at different concentrations. All the values are expressed as the mean ± standard deviation with three replicates.

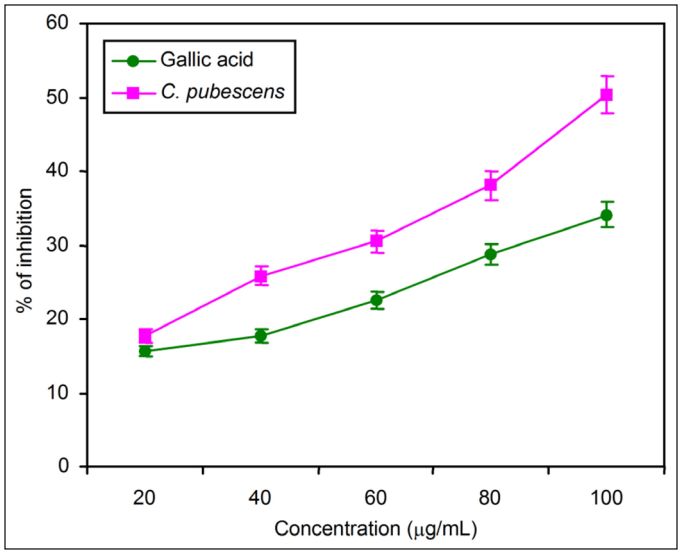

Figure 5: Comparison of glutathione S-transferase activity of standard gallic acid and *C. pubescens* fruit extract at different concentrations. All the values are expressed as the mean ± standard deviation with three replicates.

3.4. DPPH

The results of the DPPH (α,α-diphenyl-B-picrylhydrazyl) assay also aligned with the trends observed in other methods for estimating antioxidant properties. The percentage of scavenging activity was shown in 100 µg/mL at its maximum when increasing the concentrations of fruit extract as 20, 40, 60, 80, and 100 µg/mL. When increasing the concentration of standard ascorbic acid and *C. pubescens* fruit extract, it increased the DPPH free radical scavenging activity [Figure 4].

3.5. Glutothione S-Transferase

GST activity was measured by the biochemical method, and MTT assay was carried out by *in vitro* method. The *C. pubescens* fruit extract showed better inhibition potential when compared to its standard in GST assay as given in Figure 5. The effect of fruit extract in various concentrations ranging from 20 to 100 µg/mL. The maximum GST activity was recorded at 100 µg/mL, and the lowest percentage of inhibition was recorded at 20 µg/mL of fruit concentration against the standard gallic acid [Figure 5].

3.6. Cytotoxicity

For anticancer potential assessment, both biochemical and *in vitro* methods were employed. In the *in vitro* analysis, a comprehensive assessment of cell morphology and cytotoxicity was conducted, with the detailed outcomes presented in Figures 6 and 7, and Table 1. Figure 6 clearly illustrates a dose-dependent response of the cells to the incremental addition of the fruit extract. Notably, the introduction of 50 µg/mL of the fruit extract resulted in a reduction of cells within the defined area compared to the control sample. Moreover, this concentration exhibited enhanced morphological features, showcasing well-defined cells with fairly regular shapes.

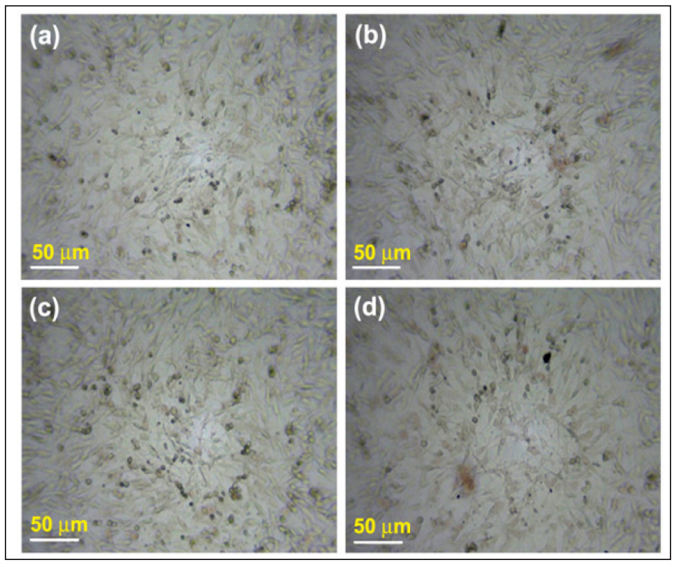

Figure 6: 10× image of bright-field inverted light microscopy of A549 lung cancer cells with varying concentrations of *C. pubescens* fruit extract. **(a)** Control, **(b)** 10 µg/mL, **(c)** 25 µg/mL, and **(d)** 50 µg/mL.

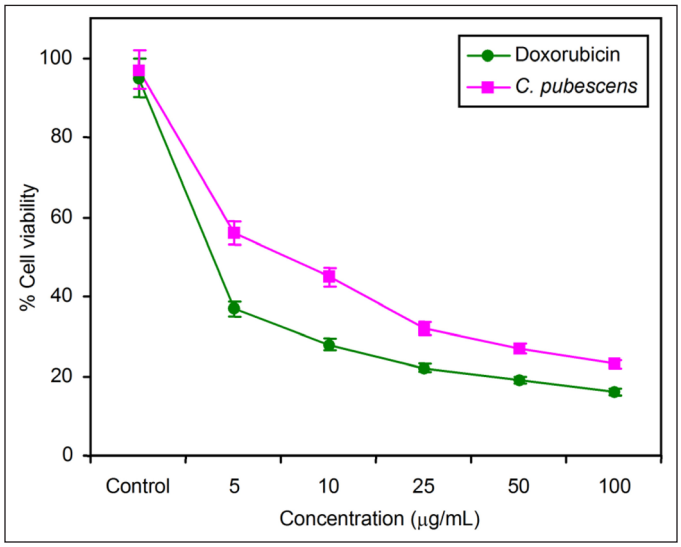

Figure 7: Comparison of cell viability between *C. pubescens* fruit extract and doxorubicin (positive control) by MTT cell cytotoxicity assay.

3.7. Cell Viability

The concentration-dependent impact on cell viability is evident in Figure 7, where an increase in the fruit extract concentration from 0 to 100 µg/mL is associated with a gradual decrease in cell viability. These findings are further elucidated and organized in Table 1, providing a comprehensive overview of the observed effects on cell morphology and viability in response to varying concentrations of the fruit extract. Table 2 shows that the IC_{50} value of the fruit extract was estimated to be 7.5 ± 1.5, in comparison to doxorubicin, employed as a positive control, which demonstrated an IC_{50} of 6 ± 0.5 over a 24-h time period.

C. pubescens, an Indian wild plant variety, has been relatively underexplored in prior research, resulting in limited available resources on its characteristics, uses, and potential medicinal benefits. While the existing literature is sparse, some sources suggest a synonymous relationship between *C. pubescens* and *C. melo*, the widely cultivated honeydew melon in India [17]. This relationship allows for meaningful comparisons to be drawn not only between these two distinct species but also with other members of the *Cucumis* genera. Exploring such associations provides a valuable foundation for understanding the unique features and potential applications of *C. pubescens* within the broader context of its plant family.

An extensive array of resources exist for comprehending the therapeutic potential of *C. melo* globally. Researchers in the study of food and pharmaceuticals have examined plant extracts from diverse parts of the *C. melo* plant, seeking therapeutic molecules and elucidating its potential mechanisms of action. Notably, investigations have highlighted the antioxidant, anti-inflammatory, and analgesic properties inherent in the seeds of *C. melo* [18]. Traditional uses have attributed to its diuretic properties. The plant has garnered significant attention for its antioxidant potential, as evidenced by numerous studies [19]. In contrast, *C. pubescens* remains relatively unexplored, presenting a compelling avenue for exploration. It holds the potential to yield health benefits equal to or even surpassing those found in cultivated honeydew melons. Hence, a comprehensive investigation into *C. pubescens* is of paramount importance to understand its untapped therapeutic potential and contribute to our understanding of its health-promoting properties.

This investigation assessed both the antioxidant properties and anticancer potential of *C. pubescens* through a series of gauging tests. Given the straightforward nature of studying SOD as an enzyme for comprehending antioxidant potential, it is noteworthy that prior research has already established *C. melo* as a notable source of SOD enzyme [20]. Several common enzymes utilized to assess antioxidant potential include SOD, catalase, GPx, ascorbate peroxidase, ascorbate oxidase, guaiacol peroxidase, and glutathione reductase. Additionally, nonenzymatic methods, such as measurement of DPPH reduction, total phenolic content, flavonoids, saponins, ascorbic acids, anthocyanins, FRAP, and ABTS, constitute a diverse array of techniques available for comprehending antioxidant potential. It is not mandatory to employ all methods to establish the antioxidant properties of a sample. Simultaneously, not all methods measure similar substrates to elucidate antioxidant properties. Therefore, the choice of methods depends on the researcher's discretion, laboratory capabilities, and the feasibility of conducting a specific assay to determine the antioxidant properties of a test substance. Being a wild variety rather than a cultivated one, *C. pubescens* exhibits a higher tolerance to both drought and salinity. Previous studies have identified the abundance of antioxidant enzymes, including SOD, catalase, and ascorbate peroxidase, in salinity-tolerant varieties of *C. melo* [21]. We possess substantial evidence indicating a correlation between antioxidant properties and the potential for anticancer effects. While cancer employs various mechanisms to invade normal cells, these mechanisms ultimately lead to the generation of free radicals. A robust antioxidant has the capacity to mitigate the damage caused by free radicals to a considerable extent, potentially counteracting the harmful effects of cancer cells [22]. The inherent qualities observed in *C. pubescens* make it a compelling candidate for further exploration in evaluating its potential anticancer properties. Similar results were reported for *Limonia elephantum,* which is commonly known as wood apple in Indian folk medicine [23,24].

The assessment of anticancer potential encompassed both biochemical and *in vitro* methods, with the evaluation of GST activity constituting the biochemical approach, while the MTT assay served as an *in vitro* method. Within the *Cucumis* genus, one noteworthy compound of interest is cucurbitacin, a secondary metabolite prevalent in the Cucurbitaceae family. Extensively studied for its anti-inflammatory properties, antitumor effects, and antidiabetic activity, cucurbitacin holds promise for various therapeutic applications [25]. It is important to note that the current study does not focus on isolating specific molecules responsible for anticancer properties. Instead, the objective is to utilize the crude extract of the whole fruit to explore potential therapeutic benefits.

The presence of GST in cucumber varieties has been documented, particularly in response to cold stress in plants [26]. This enzyme is involved in one of the most prevalent pathways crucial for the plant's detoxification response, potentially playing a significant role in mitigating the impact of carcinogenic cells. Another mechanism under consideration involves the efficient elimination of xenobiotic substances post their interaction with GST. These mechanisms collectively highlight the multifaceted role of GST in contributing to the detoxification processes within cucumber varieties. The fruit extract also possesses neuroprotective activity *in vivo* using rat models [27].

Cell cytotoxicity assays conducted on cancer cell lines serve as a crucial method for assessing the potential anticancer activity of the molecules or extracts under investigation. Numerous studies have explored the anticancer effects of various Indian fruits, employing cancer cell lines such as A549, HepG2, MDA-MB-231, among others [28,29]. Remarkably, some of the most effective anticancer drugs have been derived from common Indian fruits and vegetables. This underscores the significance of investigating natural sources for potential therapeutic agents in the fight against cancer. Cucurbitacins

Table 1: Experimental results of cell viability analysis by MTT assay.

Concentration (µg/mL)	*Cucumis pubescens* Fruit Extract % of Cell Viability	Doxorubicin Positive Control % of Cell Viability
0	97	95
20	56	37
40	45	28
60	32	22
80	27	19
100	23	16

Table 2: IC_{50} estimation by MTT assay.

Sample Name	IC_{50} (µg/mL)
C. pubescens fruit extract	7.5 ± 1.5
Doxorubicin	6 ± 0.5

IC_{50}—Values of respective sample (at 24 h).

elicit arrest of cell growth and induce apoptosis in a diversity of cancer cells. This action occurs through the suppression of Akt phosphorylation, subsequently leading to the modulation of p21/cyclin signal, activation of mitochondria-dominated caspase pathways, and interference with signaling pathways associated with the migration of cancer cells and invasion [30,31]. *Moringa concanensis* is active against anticancer properties by using HepG2 cell lines. The plant is reported to be a novel and natural phytomedicine against various diseases [32]. Nevertheless, a comprehensive study is essential for the isolation and characterization of the specific molecules responsible for the anticancer effects observed in *C. pubescens* fruits. This necessitates the utilization of robust and systematic methods, including *in vivo* studies, to firmly establish the research findings. Such an investigation holds the potential to create a market for this fruit, paving the way for the utilization of naturally derived substances for medical purposes.

4. CONCLUSION

The present study delves into the antioxidant properties and anticancer potential of *C. pubescens* fruits. While the exploration of antioxidant enzymes and nonenzymatic methods provided valuable insights, the study suggests a promising avenue for further research on this wild variety. The documented presence of GST and the potential implications of its anticancer properties in inducing cell growth arrest and apoptosis underscore the potential use of *C. pubescens*. However, it is imperative to conduct a more detailed investigation, involving the isolation and characterization of specific anticancer molecules. Robust methodologies and *in vivo* studies are warranted to firmly establish these findings and potentially unlock the market for this easily cultivable fruit, offering nature-derived substances for medical applications. The study opens a gateway for harnessing the therapeutic potential of *C. pubescens* in the area of oncology and underscores the importance of furthering our understanding of its health-promoting properties.

5. ABBREVIATIONS

MTT: 3-(4,5-dimethylthiozol-2-yl)-3,5-diphenyl tetrazolium bromide; SOD: Superoxide dismutase; GST: Glutothione S Transferase; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; IC_{50}: Inhibitory concentration; ROS: Reactive oxygen species; BOT-AAGAC: Botany-Arignar Anna Government Arts College; pH: Potential of Hydrogen; g: gram; mM: Milli molar; EDTA: Ethylene diamine tetra acetic acid; nm: Nano meter; %: percent; H_2SO_4: Sulphuric acid; (GPx): glutathione peroxidase; H_2O_2: Hydrogen peroxide; µmol: micromole; EC_{50}: half maximal effective concentration; °C: Degree celsius; CDNB: 1-chloro-2,4-dinitrobenzene; CO_2: Carbon di oxide; v/v: Volume by volume; µL: microlitre; OD: Optical density; FRAP: Ferric ion reducing antioxidant power; ABTS: 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid.

6. ACKNOWLEDGMENTS

We extend our acknowledgments and sincere thanks to DST-FIST-sponsored Laboratory, Sri Sarada College for Women (*Autonomous*), Salem-16 for providing instrumentation facilities. We are thankful to the Head, Department of Biotechnology, K.S. Rangasamy College of Technology (Autonomous), Tiruchengode, for providing valuable suggestions.

7. AUTHOR CONTRIBUTIONS

TS performed the experiments, analyzed the data, and wrote the manuscript; BM interpreted the data and meticulously planned the work; SS analyzed the data and helped in writing the manuscript; RK raised the problem, supervised, made final correction of the manuscript, and finalized the work. All authors have thoroughly read the manuscript and approved their final version.

8. FINANCIAL SUPPORT AND SPONSORSHIP

This research work did not receive any grants from funding agencies.

9. CONFLICT OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

10. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

11. DATA AVAILABILITY

All the data are available with the authors and shall be provided upon request.

12. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

13. PUBLISHER'S NOTE

All claims expressed in this article are solely those of the authors and do not necessarily represent those of the publisher, the editors and the reviewers. This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

REFERENCES

1. Sharifi-Rad M, Anil Kumar NV, Zucca P, Varoni EM, Dini L, Panzarini E, et al. Lifestyle, oxidative stress, and antioxidants: back and forth in the pathophysiology of chronic diseases. Front Physiol. 2020;11:694. https://doi.org/10.3389/fphys.2020.00694
2. Sidhu J, Zafar T. Fruits of the Indian subcontinent and their health benefits. In: Sen S, Chakraborty R, editors. Her Med India. Singapore: Springer; 2020. p. 451–78.
3. Mukherjee PK, Nema NK, Maity N, Sarkar BK. Phytochemical and therapeutic potential of cucumber. Fitoterapia. 2013;84:227–36. https://doi.org/10.1016/j.fitote.2012.10.003
4. Insanu M, Rizaldy D, Silviani V, Fidrianny I. Chemical compounds and pharmacological activities of *Cucumis* genus. Biointerface Res App Chem. 2022;12(1):1324–34.
5. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, et al. Oxidative Stress: Harms and Benefits for Human Health. Oxid Med Cell Longev. 2017;2017:8416763. https://doi.org/10.1155/2017/8416763

6. Ullah A, Munir S, Badshah SL, Khan N, Ghani L, Poulson BG, et al. Important flavonoids and their role as a therapeutic agent. Molecules. 2020;25(22):5243. https://doi.org/10.3390/molecules25225243
7. Aghajanpour M, Nazer MR, Obeidavi Z, Akbari M, Ezati P, Kor NM. Functional foods and their role in cancer prevention and health promotion: a comprehensive review. Am J Cancer Res. 2017;7(4):740–69. PMID: 28469951
8. Gamble JS. Flora of the presidency of madras. Calcutta: Botanical Survey of India; 1935.
9. Henry AN, Kumari GR. Chitra V. Flora of Tamil Nadu India. Series 1: Analysis. Vol. 2. Coimbatore: Botanical Survey of India; 1987.
10. Lester GE, Hodges DM, Meyer RD, Munro KD. Pre-extraction preparation (fresh, frozen, freeze-dried, or acetone powdered) and long-term storage of fruit and vegetable tissues: effects on antioxidant enzyme activity. J Agric Food Chem. 2004;52(8):2167–73. https://doi.org/10.1021/jf030713b
11. Lester GE, Jifon JL, Crosby KM. Superoxide dismutase activity in mesocarp tissue from divergent *Cucumis melo* L. genotypes. Plant Foods Hum Nutr. 2009;64(3):205–11. https://doi.org/10.1007/s11130-009-0124-1
12. Haida Z, Hakiman M. A comprehensive review on the determination of enzymatic assay and nonenzymatic antioxidant activities. Food Sci Nutr. 2019;7(5):1555–63. https://doi.org/10.1002/fsn3.1012
13. Gnanamangai BM, Saranya S, Ponmurugan P, Kavitha S, Pitchaimuthu S, Divya P. Analysis of antioxidants and nutritional assessment of date palm fruits. In: Naushad M, Lichtfouse E, editors. Sustainable Agriculture Reviews. Vol. 34. Cham: Springer. 2019; p. 19–40.
14. Gokçe B. Some anticancer agents as effective glutathione S-transferase (GST) inhibitors. Open Chem. 2023;21(1):20230159.
15. Lee SH, Jaganath IB, Wang SM, Sekaran SD. Antimetastatic effects of *Phyllanthus* on human lung (A549) and breast (MCF-7) cancer cell lines. PLoS One. 2011;6(6):e20994. https://doi.org/10.1371/journal.pone.0020994
16. Farshori NN, Al-Sheddi ES, Al-Oqail MM, Musarrat J, Al-Khedhairy AA, Siddiqui MA. Cytotoxicity assessments of *Portulaca oleracea* and *Petroselinum sativum* seed extracts on human hepatocellular carcinoma cells (HepG2). Asian Pac J Cancer Prev. 2014;15(16):6633–8. https://doi.org/10.7314/apjcp.2014.15.16.6633
17. Renner SS, Pandey AK. The Cucurbitaceae of India: accepted names, synonyms, geographic distribution, and information on images and DNA sequences. PhytoKeys. 2013;(20):53–118. https://doi.org/10.3897/phytokeys.20.3948
18. Gill NS, Bajwa J, Dhiman K, Sharma P, Sood S, Sharma PD, et al. Evaluation of therapeutic potential of traditionally consumed *Cucumis melo* seeds. Asian J Plant Sci. 2011;10:86–91.
19. Asif HM, Rehman SU, Akram M, Akhtar N, Sultana S, Rehman JU. Medicinal properties of *Cucumis melo* Linn. J Pharm Pharma Sci. 2014;2(1):58–62.
20. Vouldoukis I, Lacan D, Kamate C, Coste P, Calenda A, Mazier D, et al. Antioxidant and anti-inflammatory properties of a *Cucumis melo* LC. extract rich in superoxide dismutase activity. J Ethnopharmacol. 2004;94(1):67–75. https://doi.org/10.1016/j.jep.2004.04.023
21. Furtana GB, Tipirdamas R. Physiological and antioxidant response of three cultivars of cucumber (*Cucumis sativus* L.) to salinity. Tur J Biol. 2010;34(3):8.
22. Liaudanskas M, Žvikas V, Petrikaitė V. The potential of dietary antioxidants from a series of plant extracts as anticancer agents against melanoma, glioblastoma, and breast cancer. Antioxidants (Basel). 2021;10(7):1115. https://doi.org/10.3390/antiox10071115
23. Kamalakkannan K, Balakrishnan V. Antioxidant and hepatoprotective activity of ethanolic and aqueous extracts of *Limonia elephantum* corr. Leaves. Ban J Pharma. 2014;9:383–8.
24. Kamalakkannan K, Balakrishnan V. Hypoglycemic activity of aqueous leaf extract of *Limonia elephantum* Corr.in normal and alloxan-induced diabetic rats. Ban J Pharma. 2014;9:443–6.
25. Kaushik U, Aeri V, Mir SR. Cucurbitacins - an insight into medicinal leads from nature. Pharmacogn Rev. 2015;9(17):12–8. https://doi.org/10.4103/0973-7847.156314
26. Duan X, Yu X, Wang Y, Fu W, Cao R, Yang L, et al. Genome-wide identification and expression analysis of glutathione S-transferase gene family to reveal their role in cold stress response in cucumber. Front Genet. 2022;13:1009883. https://doi.org/10.3389/fgene.2022.1009883
27. Kumar H, Sharma S, Vasudeva N. Evaluation of nephroprotective and antioxidant potential of *Cucumis pubescens* in rats. J Med Pharma Alli Sci. 2021;10(5):3517–20.
28. Hanif A, Ibrahim AH, Ismail S, Al-Rawi SS, Ahmad JN, Hameed M, et al. Cytotoxicity against A549 human lung cancer cell line via the mitochondrial membrane potential and nuclear condensation effects of *Nepeta paulsenii* Briq., a perennial herb. Molecules. 2023;28(6):2812. https://doi.org/10.3390/molecules28062812
29. Garg M, Lata K, Satija S. Cytotoxic potential of few Indian fruit peels through 3-(4,5-dimethylthiazol-yl)-2,5-diphenyltetrazolium bromide assay on HepG2 cells. Indian J Pharmacol. 2016;48(1):64–8. https://doi.org/10.4103/0253-7613.174552
30. Kalebar VU, Hoskeri JH, Hiremath SV. *In-vitro* cytotoxic effects of *Solanum macranthum* fruit. Dunal extract with antioxidant potential. Clin Phytosci. 2020;6:24.
31. Wu D, Wang Z, Lin M, Shang Y, Wang F, Zhou J, et al. *In Vitro* and *In Vivo* antitumor activity of Cucurbitacin C, a novel natural product from cucumber. Front Pharmacol. 2019;10:1287. https://doi.org/10.3389/fphar.2019.01287
32. Balamurugan V, Balakrishnan V, Philip Robinson J, Ramakrishnan M. Anticancer and apoptosis-inducing effects of *Moringa concanensis* using HepG-2 cell lines. Ban J Pharm 2014; 9 (4) 604-609.

How to cite this article:
Sundari T, Kavitha R, Mythili Gnanamangai B, Saranya S. Assessment of *in vitro* antioxidant properties and anticancer potential of *Cucumis pubescens* Willd. a medicinal fruit, utilizing human lung cancer cell line (A549). J App Biol Biotech. 2024. http://doi.org/10.7324/JABB.2024.194161