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RESEARCH ARTICLE

Grewia asiatica: An In-depth Analysis of its Phytochemical Composition, Antioxidant Potency, and Implications in Cancer Therapeutics

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Abstract

Background: Cancer is a global health issue, and natural medicinal plants like *Grewia asiatica*, also known as 'Phalsa', offer a promising alternative to synthetic medications due to their antipyretic, antidiabetic, analgesic, antibiotic, and antimicrobial properties. *G. asiatica* leaves possess a wide range of therapeutic actions like antipyretic, antidiabetic, antibiotic, and anticancer properties. **Aim:** This study aimed to explore the pharmacognostic characteristics, phytochemical composition, *in vitro* antioxidant activity, and anticancer potential of *G. asiatica* leaves. **Methods:** *G. asiatica* leaves, pharmacognostical, phytochemical and proximal analysis were carried out by standard qualitative and quantitative methods. The antioxidant activity was determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay and Ferric Reducing Antioxidant Power (FRAP) assay whereas anticancer activity was determined using five different human cancer cell line (DU-145, MDA-MB-231, HT-29, SK-OV-3, A-549) by SRB assay. **Results:** The micromorphological evaluation of *G. asiatica* leaves revealed unique characteristics, aiding in species identification. Physicochemical parameters, including total ash and extractive values, assessed quality control. Preliminary phytochemical screening identified alkaloids, flavonoids, and glycosides. Quantitative analysis demonstrated a total phenolic content of 20.19 ± 1.34 mg gallic acid equivalent/g in EEGA, emphasizing its potential antioxidant properties. The total flavonoid content was 7.59 ± 0.71 mg rutin equivalent/g, further supporting the antioxidant potential. *In vitro* antioxidant assays, including DPPH and FRAP, confirmed EEGA's strong scavenging activity and reducing power. *In vitro* cytotoxicity studies using five human cancer cell lines revealed minimal cytotoxic effects, with GI_{50} values exceeding 80 µg/mL. **Conclusion:** The study provides comprehensive insights into the micromorphological, phytochemical profile and antioxidant properties of *G. asiatica* leaves. While exhibiting promising antioxidant activity, further research is warranted to explore its anticancer potential at higher concentrations. The findings underscore the importance of *G. asiatica* leaves as a potential source of natural antioxidants and warrant further investigation for its role in cancer prevention and treatment.

Keywords: Anticancer, Antioxidant, DPPH, FRAP, *Grewia asiatica*

Abbreviations: EEGA: Ethanolic extract of *G. asiatica;* ADR: Adrimycine; TPC: Total Phenolic content TFC: Total Flavanoid content; DPPH: 1,1-diphenyl-2-picrylhydrazyl; FRAP: Ferric Reducing Antioxidant Power Assay

1. Introduction

Cancer, a complex of diseases resulting from disrupted cell cycle control, entails uncontrolled cell growth. Combating cancer remains a paramount global health challenge[1]. As per the estimations from the Global Cancer Observatory (GLOBOCAN), the global count of incident cancer cases reached 19.3 million in 2020. These projections indicate a prospective surge in cancer cases in India, foreseeing a 57.5% increase to reach 2.08 million by the year 2040 compared to the figures recorded in 2020[2]. Numerous synthetic

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pharmaceuticals elicit adverse reactions, deemed tolerable only in dire cases like terminal illnesses, for instance, cancer. In contrast, metabolites identified in medicinal plants offer a potential avenue to circumvent the undesired side effects associated with synthetic drug interventions[3].

Herbs and natural products, historically revered for their medicinal attributes, serve as vital repositories of healing compounds. The exhaustive exploration of bioactive elements, predominantly sourced from botanical entities such as spices, reveals their distinctive characteristics and therapeutic prowess. Phytochemicals, intrinsic to medicinal plants, though non-essential for routine bodily functions, actively contribute to health benefits and disease mitigation. Despite the extensive knowledge of numerous phytochemicals, a considerable array remains undiscovered, awaiting meticulous identification in the realm of medicinal science[4].

Although the genus *Grewia* (*Tiliacae*) includes over 150 species of shrubs originally from the Himalayas, only *G. asiatica* produces edible fruits. These bushes are often cultivated in tropical and subtropical climates around the globe[5,6]. *G. asiatica,* commonly known as Phalsa, because of its exceptional nutritional and therapeutic properties[7]. *G. asiatica*, a plant species, has been traditionally utilized in *Ayurveda*, an ancient Indian medical book, for the treatment of several conditions such as ulcerative colitis, inflammation, fever wound healing, hypermenorrhea, and diabetes[8,9]. *G. asiatica* comprises a diverse range of primary and secondary metabolites in its phytochemistry, including alkaloids, saponins, glycosides, steroids, amino acids, mucilage, myricetin, hydroxybenzoic acid, cyanidin, quercetin, kaempferol, and fixed seed oil[9,10]. Examining the phytochemical profile of *G. asiatica* is crucial to determine the health-promoting properties of its leaves and their potential contribution to disease prevention[11]. Therefore, our current study aims to investigate the *in vitro* antioxidant and anti-cancer potential of *G. asiatica* leaves.

2. Materials and Methods

2.1 Plant Material

Grewia asiatica leaves that had been grown naturally in various districts of Surat and Vadodara were harvested fresh from various sources. The plants were collected during March-June 2019 which was authenticated by Dr. M. N. Reddy. Herbarium specimens of plants were deposited with voucher No. BVBRC1403 at Bioscience Department, Veer Narmad South Gujarat University, Surat.

2.2 Characterization of Plant Material

2.2.1 Micromorphological Evaluation

A comprehensive morphological analysis was conducted on the leaves of *G. asiatica*, encompassing dimensions, shape, colour texture, surface, venation, apex, margin, base, and lamina. This investigation adhered to consistently reported protocols[12,13]. A transverse section of *G. asiatica* leaf was cut by the free hand section method. The sections were cleared and stained[14]. The thin stained sections were affixed with glycerine and examined using a microscope (Olympus Magnus Magcam DC) at magnifications of 10X and 45X[15]. The leaves of *G. asiatica* were dried in the shade and finely powdered. Powders were passed through a sieve #60[15]. The powder characteristics were studied under a microscope and photomicrography was carried out using cameras[15-17].

2.2.2 Physicochemical Parameters

A physicochemical evaluation was conducted on the powdered leaves of *G. asiatica* using the established standard method[18]. The assessed criteria were loss on drying, extractive value, and total ash.

2.2.3 Preparation of Extract and Preliminary Phytochemical Screening

In the cold-maceration technique, 500g of powdered leaves of *G. asiatica* were immersed in 5L of Ethanol (99.9% v/v) for 24 hours at room temperature and filtered. The rotary evaporator (Rotavapor R-300) was used to dry the resulting Ethanolic Extract of *G. asiatica* leaves (EEGA). Afterwards, according to the standard procedure, the dried extract was qualitatively tested according to the standard procedure[17]. The dried extract was collected and stored in an amber colour glass vial in desiccators at room temperature.

2.2.4 Total Phenolic Content (TPC) Estimation

EEGA was tested for TPC as per the Singleton *et al.* method. In this study, a precise volume of 20 µL of

extract from each sample and subsequently combined with 1.580 µL distilled water. After that, a hundred µL of FC reagent was mixed and waited for 2 minutes to add. 300 µL Na_2CO_3 (7.5%) into each sample. The mixture was undisturbed for 2 hours at 20°C temp after thoroughly mixing. The results were expressed in milligrams equivalent to Gallic acid[18,19].

2.2.5 Total Flavonoid Content (TFC) Estimation

Similarly, EEGA was also tested for TFC employing the Chang *et al* method. The extracted sample underwent dilution with methanol to prepare 100 mg/mL concentration. Then 2.0 mL of extract, hundred microliters aluminum chloride solution (10% w/v) along hundred microliter potassium acetate solution (0.1 mM) were mixed and kept at room temperature for 30 minutes. The UV-VIS spectrophotometer was used to measure the absorbance at 415 nm. TFC was quantified and reported in mg of rutin equivalent per g of extract[20].

2.2.6 Antioxidant Activity of EEGA

2.2.6.1 DPPH Assay

EEGA was evaluated for antioxidant activity using a well-established method for inhibiting 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay. Prepared test samples (EEGA) were produced with concentrations ranging from 25 to 500 µg/mL in methanol. Ascorbic Acid (AA) served as the benchmark for antioxidants. A 100 µL test sample was combined with 3 mL of DPPH. Following a 30-minute incubation period, the sample was examined via spectrophotometer at a wavelength of 517 nm, compared to a blank solution of methanol. The control was made by combining DPPH solution (3 mL) and methanol (100 µL). The formula used to quantify the fraction of free radical scavenging or inhibition was as follows:

$$Scavenging\ Activity = \left(\frac{A_{517}ofcontrol - A_{517}ofsample}{A_{517}ofcontrol} \right) \times 100$$

IC_{50} value was calculated to report the extract's ability to inhibit DPPH radicals by 50%[21].

2.2.6.2 FRAP Assay

The measurement of ferric reducing antioxidant power of extract ability was tested using Huang *et al.,* method[22]. EEGA (100 µL) and ascorbic acid (100 µL) of different concentrations (25 - 250 µg/mL) and FRAP and FRAPL) were mixed and set aside for 30 min at a 37°C temperature in the absence of light. The absorbance of the prepared samples was measured at a wavelength of 593 nm and was used to measure the absorbance (n=3) of each solution. Calibration was done using freshly prepared $FeSO_4$ solutions. The antioxidant potential of the sample was expressed by its ability to eliminate ferric ions and the results were noted as µmol Fe^{+2} equivalents per mg of sample[23].

2.2.7 In Vitro Anticancer Activity (SRB Assay)

2.2.7.1 Cell Line

In this study, the cytotoxic evaluation primarily uses DU-145 for (prostate cancer), MDA-MB-231 (breast adenocarcinoma), HT-29 (colorectal adenocarcinoma), SK-OV-3 (ovarian cancer), and A-549 (lung cancer) cell line. It was kept in RPMI 1640 medium with 10% fetal bovine serum and 2 mML-glutamine. The study was performed at the Advanced Center for Treatment, Research, and Education in Cancer (ACTREC) lab of Tata Memorial Center (TMC) Hospital in Mumbai, India.

2.2.7.2 In Vitro Cytotoxicity Study

The Sulforhodamine B (SRB) assay was employed to analyse the cytotoxicity of EEGA, while adriamycin was utilized as a positive control. The cell lines were grown in their culture media.

For screening, a total of 5000 cells were placed in 96-well microliter plates, with each well containing a hundred microliter. Following a 24-hour incubation at controlled conditions (37°C, 5% CO_2, 100% relative humidity), the experimental drugs were diluted to 1mg/mL, stored in a frozen state, and subsequently thawed. The dilution of samples was to prepare different concentrations of 20, 40, 80, and 160 (µg/mL). After the introduction, the plates were subjected to a 48-hour incubation period, which was concluded by the application of cold trichloroacetic acid. A sulforhodamine B solution (0.4% w/v in 1% acetic acid) was added, followed by the mixture was incubated, washed, and allowed to air dry. The bound stain was released using Prisma base, and subsequent absorbance measurements were recorded at a wavelength of 540 nm (reference 690 nm). Percentage growth was determined in different drug concentration levels using

six absorbance measurements (Tz, C, and Ti at four concentration levels).

2.2.8 Statistical Evaluation

The results were expressed as the value of mean ± standard deviation and performed in triplicate.

3. Results

3.1 Micromorphological Evaluation

The morphology of *G. asiatica* shrub and leaves were well depicted in Figure 1, the leaves exhibited a variety of shapes, including ovate, sub-orbicular, acute, sub-acuminate, or cuspidate. Their leaves became sharp and coarse double serrates, a sub-glabrous appearance above, a fuzzy and whitish appearance underneath, and a rounded or slightly heart-shaped base.

Grewia asiatica leaves observed as dorsiventral leaves observed in Figure 2. Both the upper and lower epidermis have collenchyma cells in the midrib portion.

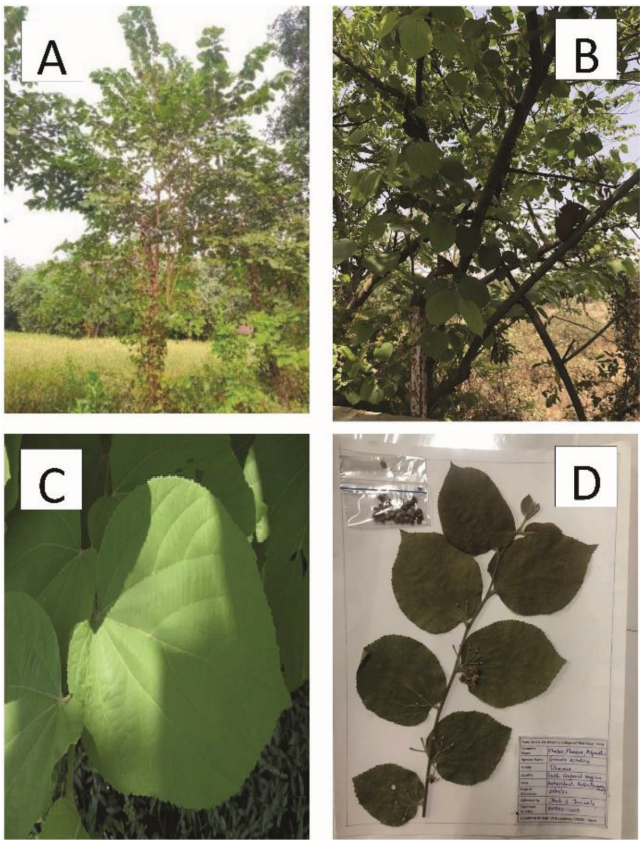


Figure 1. Morphological figures: **(A).** Shrub of *G. asiatica*, **(B).** Branches along with fruits, **(C).** *G. asiatica* leaves, **(D).** Herbarium sheet of *G. asiatica.*

Beneath the upper epidermis are two to three layers of Palisade cells. Leaves have collateral vascular bundles surrounded by pericyclic fibres. Leaves having unique lignified stellate trichomes. Powder study of plant observed xylem vessel, stellate trichomes, normocytic stomata, epidermal cell, starch and aleurone grains.

3.2 Physicochemical Parameters

The proximal evaluation of *G. asiatica* leaves was conducted as per standard method and the results obtained were mentioned in Table 1.

3.2.1 Preliminary Phytochemical Screening

The chemical analysis of EEGA leaves distinctive qualitative variations in their phytochemical

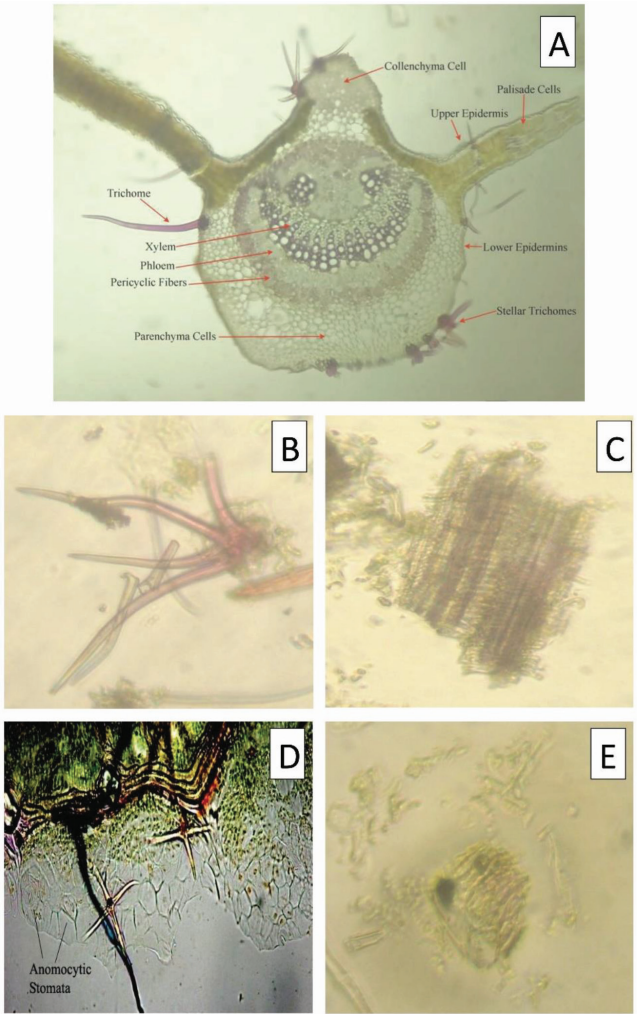


Figure 2. Microscopical and power characteristic study of *G. asiatica* leaf : **(A).** TS of *G. asiatica* leaf, **(B).** Stellectrichomes, **(C).** Xylem vessels, **(D).** Anomocytic stomata, **(E).** Epidermal cells.

profiles (Table 2). Alkaloids, tannins, carbohydrates, flavonoids, and glycosides were found in EEGA suggesting the existence of a variety of bioactive constituents. The total yield of EEGA was 16.86 % w/w.

3.2.2 Total Phenolic Content

The present investigation aimed to ascertain the phenolic content of *G. asiatica* leaves through the utilization of a standardized methodology. The study revealed the phenolic content of EEGA was 20.19 ± 1.34 mg GAE/g of dried extract. Phenolic compounds, renowned for their remarkable chain-breaking antioxidant properties, have been extensively studied due to their pivotal role in the inhibition of free radicals that are implicated in chain formation.

3.2.3 Total Flavonoid Content

EEGA contains 7.59 ± 0.71 mg rutin equivalent/g of ethanolic dried extract as a total flavonoid. The observed high antioxidant potential, linked to the phenolic hydroxyl groups in flavonoids, highlights the research significance for further exploration.

Table 1. Proximal Analysis of *G. asiatica* leaves

Physicochemical parameters		Value (%w/w)
Ash value	Total ash	2.70
	Acid Insoluble ash	0.33
	Water Soluble ash	2.37
Extractive value	Aqueous	13.28
	Alcohol	17.85
Moisture content	Loss on Drying	10.83

Table 2. Preliminary phytochemical screening of Ethanolic extract of *G. asiatica* leaves

Chemical Test	EEGA
Alkaloids	+
Carbohydrates	++
Protein	-
Phytosterol	-
Saponin	+
Flavonoids	++
Glycosides	++
Tannins	+
–, absent; +, moderately present; ++, high presence.	

3.3 Antioxidant Activity

3.3.1 DPPH Assay

DPPH compound is widely recognized for its strong and stable nature, making it a valuable tool for evaluating the scavenging activity of free radicals. Calculated IC_{50} values for EEGA (171.22 µg/mL) and ascorbic acid (90.44 µg/mL) suggest that extract has a concentration-dependent effect on suppressing DPPH radicals (Figure 3).

3.3.2 FRAP Assay

The reductive ability of EEGA exhibited a concentration-dependent increase, as depicted in Figure 4. This observation suggests that EEGA possesses superior reducing power compared to standard ascorbic acid.

3.4 *In vitro* Cytotoxicity Study

The screening of anticancer experiments using EEGA was repeated thrice with 20, 40, 60, 80, and 160 µg/mL extract concentration, to evaluate cytotoxic effects

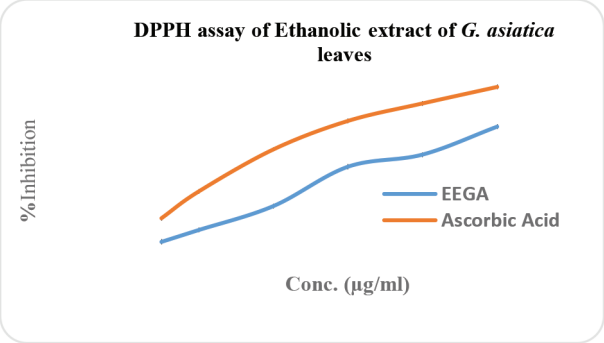


Figure 3. The DPPH radical scavenging activity of EEGA and Ascorbic acid.

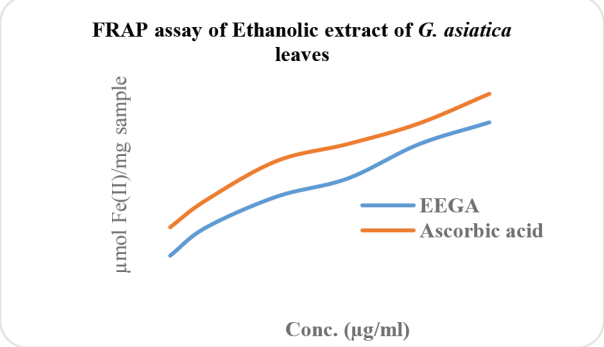


Figure 4. Ferric reducing antioxidant power of EEGA and Ascorbic acid.

and average values were calculated. Figures 5, 6 and Table 3 show that the GI_{50} value of EEGA was >80 µg/mL in DU-145, MBA-MB-231, HT-29, SK-0V-3 and A-549 cell line while >160 µg/mL in the HT-29 cell line whereas, the ADR treated cell lines showed cell death at <20 µg/mL. As per ACTREC, extracts with GI_{50} value less than 20 µg/mL ($GI_{50} \leq 20$) are considered to have anticancer activity. Based on our experimental results, it is evident that the EEGA evaluated has shown a significantly low cytotoxic effect against the human cancer cell lines used. However, further work

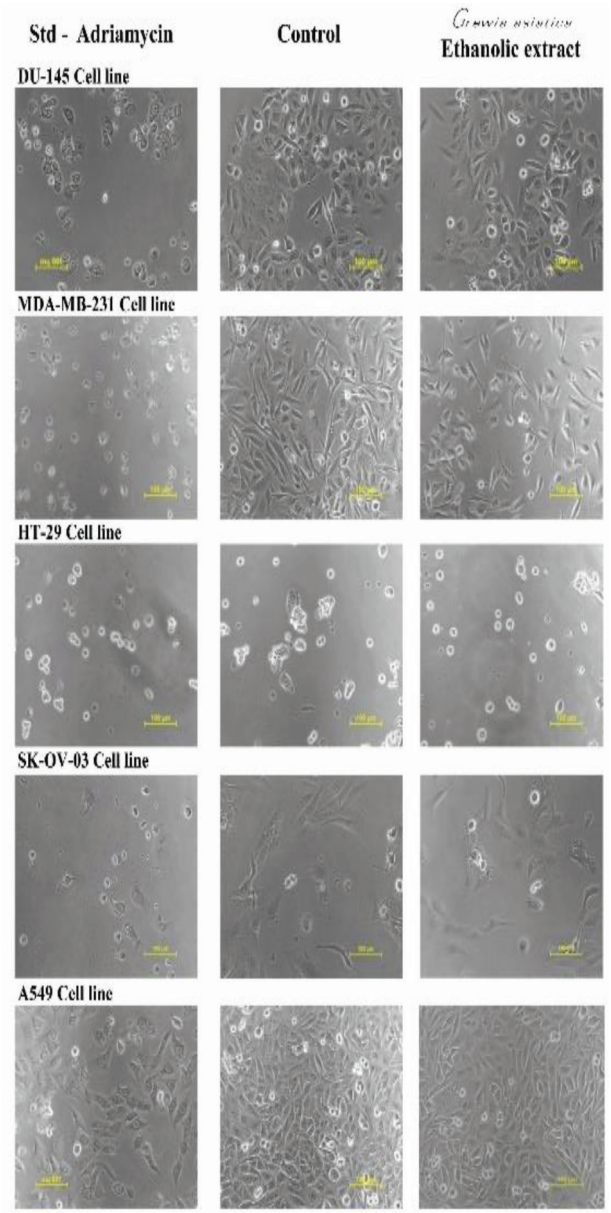


Figure 5. Cytotoxicity of Adriamycin, control and EEGA on different cell lines.

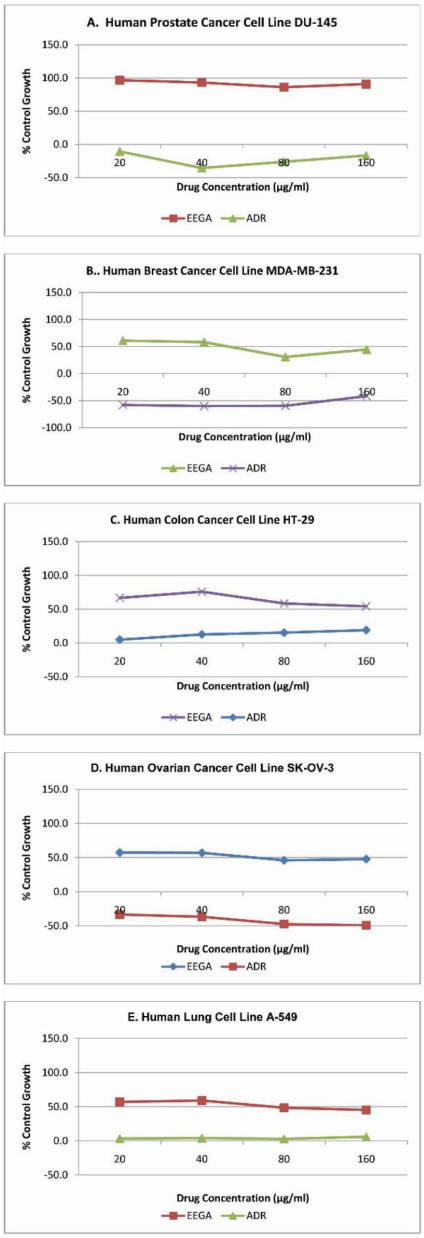


Figure 6. Percent growth inhibition of Ethanolic extract of *G. asiatica* leaves and standard drug Adriamycin.

Table 3. GI_{50} values of EEGA and Std-Adrimycine on five human cancer cell lines using the SRB assay



is needed by using different higher concentrations of test samples.

4. Discussion

Standardization is crucial for quality control, purity evaluation, and sample identification. The current study involved conducting a pharmacognostic investigation, and physiochemical analysis, along with *in vitro* antioxidant and *in vitro* cytotoxicity studies using EEGA. Pharmacognostical investigations and the assessment of various physiochemical characteristics are crucial for the standardization of drugs and the establishment of their pharmacological effectiveness. The micromorphological characteristics of *G. asiatica* leaves used in these investigations help in identifying species and plant materials. Epidermal characteristics are commonly employed in taxonomic identification at the genus and species levels[9,24]. The evaluation of physicochemical parameters is of utmost importance in the identification of adulterants and the detection of drug mishandling[25]. The ash values identified in this study hold significant promise in their ability to serve as standards for assessing adulteration, quality and purity. The lower total ash value indicates a reduced occurrence of inorganic salts[26]. These results were also confirmed by acid insoluble ash value. This feature might be used as a diagnostic tool in the future[25,26].

This study provides information on the chemical components found in the plant and helps determine the precise elements that can dissolve in the chosen solvent for extraction, as well as identifying any materials that have been depleted[25]. The alcohol-soluble extractives exhibited greater levels compared to the water-soluble extractives. Therefore, these solvents are an optimal alternative for extracting leaves of *G. asiatica*. Furthermore, a preliminary phytochemical screening was performed to detect the presence of secondary metabolite derivatives in the leaves. In the present study, ethanolic extract of *G. asiatica* leaves has been identified as potentially valuable for conducting biological research due to the presence of various phytoconstituents. The estimation of antioxidant and anticancer activity of the EEGA was conducted by analyzing its phytoconstituent composition.

Fruits, vegetables, and herbs are frequently discovered to have phenolic chemicals that have a strong ability to prevent oxidation. The hydroxyl groups of phenolic compounds in plants are crucial since they provide scavenging activity[27,28]. Moreover, the Folin Ciocalteu technique for quantifying phenolic compounds has a resemblance to the assessment of antioxidant activity. Consequently, the obtained values should, to some extent, reflect the antioxidant activity[29]. Results of the study showed 20.19 ± 1.34 mg GAE/g of dried extract of the phenolic compound in EEGA. However, the total phenolic content of *G. asiatica* leaves is lower than fruit (144.11 mg of GAE/g)[30].

The overall flavonoid content of the EEGA was quantified using rutin equivalents. Plants contain several types of phenolic chemicals, with the most important ones being the flavonoids that exhibit strong antioxidant properties[31]. Phenolic and flavonoid components are natural substances that have a strong ability to prevent oxidation. Our study provides evidence for a significant correlation between the IC_{50} value for radical scavenging activity and the quantity of phenolic or flavonoid content[32].

An experiment was performed to find antioxidant potential using the DPPH radical scavenging activity technique. DPPH is a persistent derivative that maintains its stability. During antioxidant interactions with DPPH, the unpaired electron becomes paired, resulting in the decolourization of the DPPH solution[33,34]. The scavenging activity of the antioxidant is directly influenced by the quantity of electrons absorbed[35]. The findings from the present investigation demonstrated that EEGA exhibited a notable antioxidant capacity in comparison to ascorbic acid. The EEGA exhibited a high scavenging capacity on DPPH, which can be attributed to the hydrogen donation capability of the polyphenolic chemicals included in the extracts.

The potential antioxidant activity of the sample may anchor as a strong indicator of its reduction ability. Antioxidant compounds can transfer electrons to reactive ions, hence rendering them non-reactive species[36,37]. The capacity of EEGA to reduce was assessed by FRAP experiments, which yielded encouraging findings. The FRAP assay findings expressed a positive correlation between the quantities of EEGA and its capacity to decrease ferric ions.

Plants have long been a valuable source of strong traditional medicines used to treat various types of cancer. Although the specific components extracted from plants may not directly function as pharmaceuticals,

they offer valuable clues for the creation of new and promising therapeutic agents. With the emergence of new technology, several drugs that previously proved unsuccessful in clinical tests are now generating renewed attention. The SRB test provides more consistency, accuracy, and reduced variability compared to the MTT assay for adherent cell culture[38]. Previous investigations have involved the examination of *G. asiatica* leaves' *in vitro* cytotoxicity on various cell lines showed significant cytotoxicity in the MTT assay[39-41]. However, no study reported the use of SRB assay for *in vitro* cytotoxicity study of *G. asiatica* leaves. The study was conducted according to the guidelines and requirements of ACTRECT. According to these recommendations, in an *in vitro* experiment, an extract is regarded to indicate activity if its GI_{50} value is less than 20 µg/mL. The investigation determined that the GI_{50} of EEGA at its highest concentration is greater than 20µg/mL for the DU-145, MBA-MB-231, HT-29, SK-0V-3, and A-549 cell lines. This indicates that the EEGA has extremely minimal or no cytotoxic effects. Therefore, this study offers a promising opportunity for further exploration into the cytotoxic effects of various solvent extracts of *G. asiatica*, employing elevated concentrations of samples.

5. Conclusion

Micromorphological profiling of *G. asiatica* leaves, coupled with physiochemical analysis provides a distinct identification of the drug and contributes to establishing purity. Moreover, high yield and phytoconstituents like alkaloids, flavonoids, glycosides, phenolic compounds, tannins, and carbohydrates, were found in the ethanolic extract. Our investigation was aimed at conducting a scientific validation of the antioxidant and anticancer properties of *G. asiatica* leaves. In this study, the findings presented compelling evidence supporting the robust antioxidant properties of ethanol extracts derived from *G. asiatica*, as demonstrated through the DPPH and FRAP assays. The observed correlations between antioxidant activities and Total Phenolic Content (TPC) show that the antioxidant properties of the extracts are due to the presence of phenolic compounds. However, EEGA showed minimal to no cytotoxic activity on five human cancer cells. Therefore, further comprehensive investigation is needed to support the anticancer potential of *G. asiatica* leaves.

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