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Phytochemical Profiling, Free Radical Scavenging Potential And Anticancer Activity Of Hydroethanolic Leaf Extract Of Hiptage Benghalensis

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ABSTRACT

Bioactive substances with substantial therapeutic potential can be found in medicinal plants. The current investigation sought to ascess the anticancer and antioxidant properties of silver nanoparticles made from *Hiptage benghalensis* hydroalcoholic leaf extract. The leaf extract was shown to contain a number of bioactive substances, including flavonoids, phenolic compounds, alkaloids, tannins, glycosides, and saponins, which are recognized for their pharmacological and antioxidant qualities. Fourier Transform Infrared and UV-visible spectroscopy were used to characterize the silver nanoparticles that were produced utilizing a green manufacturing technique. While FTIR analysis revealed the existence of functional groups involved in the reduction and stability of nanoparticles, UV–visible examination revealed a distinctive absorption peak at 340 nm, confirming the creation of silver nanoparticles DPPH, Ferric Reducing Antioxidant Power (FRAP), and Hydrogen Peroxide scavenging assays were used to assess the antioxidant activity of the produced nanoparticles. Significant free radical scavenging action was demonstrated by the data, with more inhibition seen at higher doses. The MTT assay was used to evaluate the anticancer activity against the HeLa cervical cancer cell line. With an IC_{50} value of 35.5 µg/mL and a maximal inhibition of 87.68% at 100 µg/mL, the results showed a dose-dependent cytotoxic impact. These results imply that silver nanoparticles mediated by *Hiptage benghalensis* have promising antioxidant and anticancer potential.

Keywords: *Hiptage benghalensis*, silver nanoparticles, Antioxidant activity, Anticancer activity, HeLa cell line, Phytochemicals.

INTRODUCTION

India ranks among the world's top producers and exporters of herbal raw materials, making it one of the world's greatest stores of medicinal plant diversity. In traditional medical systems, about 7,500 plant species have been used for therapeutic purposes. Among these, Ayurveda, Siddha, and Unani are examples of traditional, organized medical systems that have heavily depended on plant-based treatments for millennia. To restore physiological balance and enhance general well-being, these methods make use of various plant parts, including roots, leaves, bark, seeds, flowers, and fruits, to create decoctions, powders, oils, and extracts. Inflammation, microbial infections, metabolic disorders such as diabetes, cardiovascular diseases, liver disorders, skin abnormalities, and neurological disturbances are just a few of the many illnesses that medicinal plants have historically been used to treat. Scientific research on Indian medicinal plants has been greatly boosted by the increased demand for natural and plant-derived products around the world [1]. Through the discovery of bioactive phytochemicals such alkaloids, flavonoids, tannins, saponins, terpenoids, phenolic compounds, and glycosides, contemporary pharmacological research is progressively confirming the therapeutic claims of traditional medicine. Numerous biological functions, including as antioxidant, anti-inflammatory, antibacterial,

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antidiabetic, immunomodulatory, and anticancer qualities, are known to be shown by these secondary metabolites. For instance, curcumin, a polyphenolic substance found in Curcuma longa (turmeric), has been extensively researched for its ability to reduce inflammation and fight cancer. *Azadirachta indica* (neem) has potent antibacterial and anticancer properties, whereas *Withania somnifera* (ashwagandha) is prized for its immunomodulatory and adaptogenic properties [2]. In a similar vein, Catharanthus roseus has produced significant anticancer medications used in chemotherapy, including vincristine and vinblastine. Uncontrolled cell proliferation and the capacity of aberrant cells to infiltrate Neighbouring tissues and spread to distant organs are characteristics of the complex and interconnected disorders collectively referred to as cancer. Almost any kind of cell in the body can give rise to it. Most often, genetic alterations that interfere with regular cellular regulatory processes are linked to the development of cancer. Genes involved in cell cycle regulation, apoptosis (programmed cell death), DNA repair pathways, and immune surveillance may be impacted by these abnormalities. This leads to unchecked cell division, resistance to cell death, accumulation of further mutations, and the formation of malignant tumours. Cancer can develop and spread as a result of environmental causes such chemical carcinogens, radiation exposure, poor food, infections, and tobacco use. Medicinal plants' rich phytochemical composition makes them important for both cancer prevention and treatment. By triggering apoptosis, preventing angiogenesis (the development of new blood arteries supplying tumours), reducing metastasis, and altering signalling pathways implicated in tumour growth, a variety of chemicals produced from plants demonstrate cytotoxic actions against cancer cells. Curcumin, for example, has been demonstrated to control a number of molecular targets implicated in inflammation and the development of cancer [3]. Some plants contain resveratrol, which has antiproliferative and antioxidant properties. As scavengers of free radicals, flavonoids and phenolic compounds lessen oxidative stress, which is a factor in the development of cancer. Antioxidants derived from plants also strengthen the body's defences against harm and help shield DNA. The potential uses of medicinal plants in cancer treatment have been further increased in recent years by nanotechnology-based strategies, such as the creation of plant-mediated nanoparticles. Green synthesis techniques that use plant extracts are thought to be economical and environmentally beneficial, and early research has shown that the resulting nanoparticles have improved anticancer activity. Overall, the enormous therapeutic potential of Indian medicinal plants particularly in the prevention and treatment of cancer and other chronic diseases continues to be highlighted by the fusion of traditional knowledge with contemporary scientific study [4].

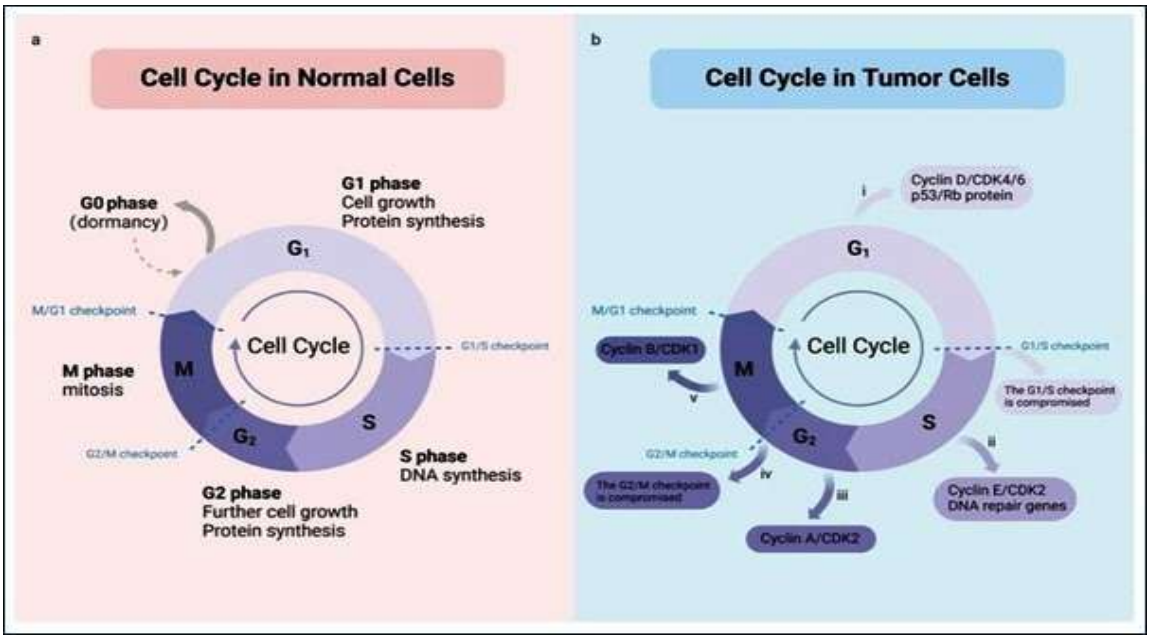


FIG 1: Stages Of Normal Cycle Vs Cancer Cell Cycle

Cervical cancer, for example, refers to the abnormal growth and unchecked multiplication of cells that start in the cervix. Cancer is usually termed for the specific body site where it originates. The narrow, lower part of the uterus that joins the uterus to the vagina is called the cervix. It is mostly made up of glandular epithelial cells lining the cervical canal (endocervix) and squamous epithelial cells on the exterior (ectocervix). Where these two cell types converge, the transformation zone is especially prone to malignant alterations. The cervix also has lymphatic veins and stromal connective tissue, which are involved in fluid drainage and immunological protection. One of the most prevalent malignancies afflicting women globally, especially in low- and middle-income nations, is cervical cancer. It is a huge public health hazard, with hundreds of thousands of new cases and deaths recorded each worldwide. A large percentage of cancers in women is cervical cancers. Treatment is made much more difficult by the possibility that it can spread to distant organs such the liver, lymph nodes, bones, and lungs. However, good outcomes and higher survival rates can result from early detection through routine screening and HPV immunization The cervix can develop a variety of lesions. Cervical intraepithelial neoplasia (CIN), an aberrant cell proliferation that could develop into invasive cancer if untreated, is one of the many benign or precancerous cervical abnormalities. A lower percentage of cervical malignancies develop from glandular cells of the endocervix (adenocarcinoma), whereas the majority originate from the squamous cells lining the ectocervix (squamous cell carcinoma). On rare occasions, malignancies may arise from different cervical tissues [5].

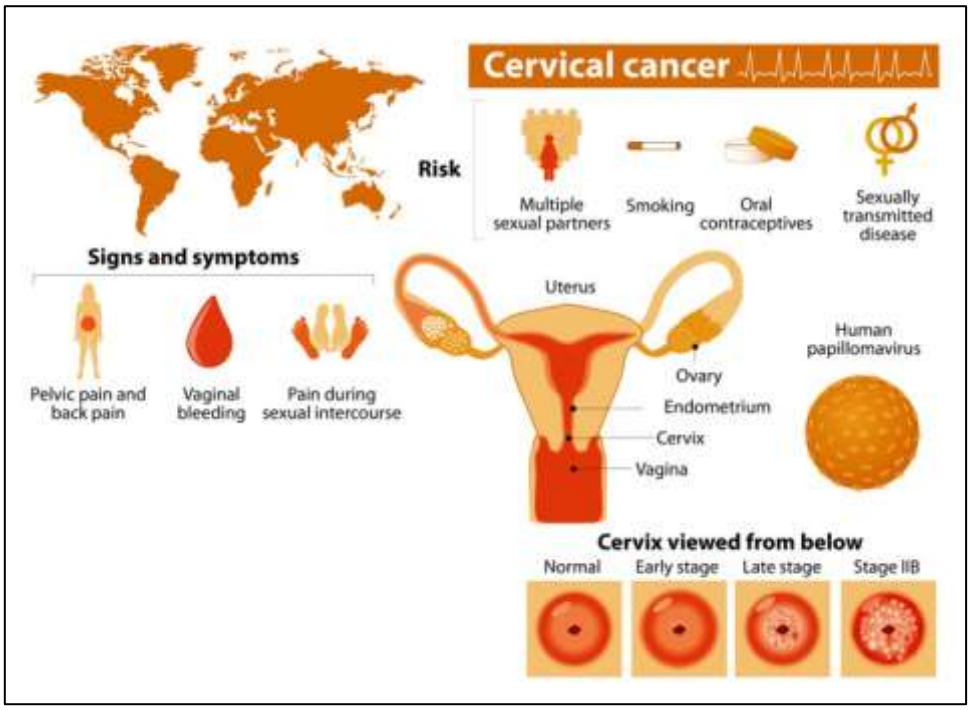


FIG 2: Etiology And Causes Of Cervical Cancer

CAUSES OF CERVICAL CANCER

Previous history

Women with a prior history of cervical precancer or cervical cancer have an increased risk of developing recurrent disease or cancer in the remaining cervical tissue.

Family history

Although cervical cancer is primarily associated with viral infection, a family history of cervical cancer may slightly increase risk, possibly due to shared genetic or environmental factors.

Genetic causes

Genetic susceptibility may influence how the immune system responds to HPV infection. Variations in genes related to immune regulation and cell cycle control can contribute to disease progression. Unlike breast cancer, strongly inherited mutations are not commonly linked to cervical cancer [6].

Hormonal factors

Long-term use of oral contraceptives, early age at first pregnancy, and multiple pregnancies are associated with an increased risk of cervical cancer, likely due to prolonged hormonal exposure and changes in cervical epithelial cells.

Lifestyle and dietary factors

Smoking is a major risk factor, as tobacco-related substances can damage cervical cells. Poor nutrition, obesity, early sexual activity, multiple sexual partners, and a weakened immune system also increase susceptibility, mainly by promoting persistent HPV infection.

Environmental factors

Long-term immunosuppression, including HIV infection or prolonged use of immunosuppressive drugs, increases the likelihood of persistent HPV infection and progression to cervical cancer [7-10].

Treatment of cervical cancer

Using natural plants to treat cervical cancer mostly focuses on complimentary and supportive care rather than taking the place of traditional treatments like radiation, chemotherapy, or surgery. Research investigations have demonstrated the anticancer potential of a number of medicinal plants. Curcumin, found in *curcuma longa* (turmeric), has anti-inflammatory and anti-cancer qualities. Green tea, or Camellia sinensis, is high in epigallocatechin gallate (EGCG), which has been shown to stop the growth of cancer cells. *Tinospora cordifolia* and *Withania somnifera* (ashwagandha) may lower oxidative stress and boost immunity. Garlic and aloe vera also have antiproliferative and antioxidant properties. However, in addition to conventional treatment, these natural therapies ought to be utilized under medical supervision [11].

NANOTECHNOLOGY:

Nanotechnology represents a domain dedicated to the development of contemporary systems, instruments, and materials by manipulating matter at the atomic and molecular scales, utilizing the unique properties that emerge at these levels. Its applications span various fields, including medical diagnostics, food production, pharmaceuticals, biotechnology, environmental science, energy, chemistry, and physics, thereby establishing it as an interdisciplinary and cross-sectoral technology [12-13]. The interdisciplinary essence of nanoscience and nanotechnology, which focuses on the precise manipulation of atoms and molecules, is poised to significantly influence the health and medical sectors Nanotechnology and nanoscience are burgeoning disciplines that enable the precise manipulation of materials, facilitating a deeper understanding and control over the fundamental components of physical entities [14-17]. Nanomaterials play a significant role in enhancing both human health and environmental conditions. The earliest documented application of nanomaterials in the realm of human health dates back over five millennia to the Indian Ayurvedic medicine system, where principles of nanoscience were utilized long before the term "nano" was established [18]. It was not until the 21st century that contemporary science began to explore nanoscience, leading to a swift expansion of research and advancements in this domain globally. A key result of this research has been the creation of innovative materials at the nano meter scale, particularly the synthesis of nanoparticles. Nanoparticles are defined as materials that possess at least one dimension measuring less than 100 nano meters (nm). This technology is rapidly advancing and holds significant promise for the development of novel materials with distinctive characteristics, as well as the enhancement of existing products across a wide range of applications [19-20].

SILVER NANOPARTICLES

The distinct physicochemical characteristics of silver nanoparticles (Ag NPs) and their potential uses in cancer treatment have drawn a lot of interest in biomedical research. Because of their remarkable optical, electrical, and catalytic properties, they are ideal for biological applications. A key characteristic

of Ag NPs is their high surface plasmon resonance (SPR), which improves their optical behaviour and makes it possible to use them for photothermal therapy, imaging, and sensing. The generation of reactive oxygen species (ROS) by silver nanoparticles is a major mechanism behind their anticancer action. Ag NPs have been demonstrated in experiments involving MCF-7 human breast cancer cells to cause oxidative stress by generating extracellular ROS. Excessive ROS causes membrane lipid peroxidation and upsets the cellular redox equilibrium, which compromises membrane integrity. Cells eventually experience apoptotic cell death, structural damage, and shrinking as a result. In order to eradicate cancer cells and stop tumour growth, this programmed cell death is necessary [21].

In addition to damaging membranes, Ag NPs also harm DNA and mitochondria. After being absorbed by cancer cells, silver nanoparticles alter the potential of the mitochondrial membrane and increase the generation of ROS Pro-apoptotic substances such cytochrome c are released as a result, triggering caspase enzymes and intrinsic apoptotic pathways that result in regulated cellular fragmentation. Proteotoxic stress and DNA damage brought on by ROS also accelerate the demise of cancer cells [22].

Additionally, silver nanoparticles have outstanding photothermal and photodynamic characteristics. Photothermal therapy is made possible by their effective conversion of light energy into heat due to their significant absorption in the near-infrared (NIR) region. With little effect on healthy cells, this focused heat generation can eliminate tumour tissues selectively. Additionally, Ag NPs generate a lot of reactive oxygen species (ROS) when exposed to light, which makes them useful tools for photodynamic therapy and increases the effectiveness of tumour cell death. Ag NPs have better stability, biocompatibility, and oxidation resistance than copper nanoparticles, despite being less conductive. Their high specific surface area makes it possible to load several anticancer medications efficiently, improving therapeutic efficacy and tailored drug delivery. Because malignant tissues exhibit altered silver ion interactions, clinical studies have also demonstrated the significance of metal ion homeostasis in the development of cancer.

ROS (Reactive oxygen species) overproduction, mitochondrial malfunction, DNA damage, and apoptotic pathway activation are some of the ways that silver nanoparticles exhibit substantial anticancer potential. They are promising candidates for sophisticated cancer diagnosis and therapy methods due to their multifunctional qualities [23]

PLANT PROFILE

Hiptage benghalensis (L.) Kurz is a climbing shrub or woody liana belonging to the family Malphigiaceae, a family predominantly distributed in tropical and subtropical regions. The family Malphigiaceae comprises about 75 genera and over 1,300 species, with members characterized by opposite leaves and distinctive winged fruits. *Hiptage benghalensis* is one of the most widely distributed species of the genus Hiptage. It is native to South and Southeast Asia, with a natural range extending across India, Sri Lanka, Nepal, Bhutan, Bangladesh, Myanmar, Thailand, Laos, Vietnam, southern China, Malaysia, and Indonesia. In India, the plant is commonly found in tropical and subtropical forests, forest margins, hedges, and scrublands, particularly in states such as Andhra Pradesh, Telangana, Karnataka, Kerala, Tamil Nadu, Maharashtra, and West Bengal the species is well known for its ornamental value, fragrant flowers, and extensive use in traditional medicine, where various plant parts are employed for treating inflammation, skin diseases, wounds, and gastrointestinal disorders [24].



FIG 3: Hiptage Benghalensis Plant

BOTANICAL DESCRIPTION

TABLE1: TAXONOMY AND CLASSIFICATION [25]

BOTANICAL NAME	***Hiptage benghalensis***
FAMILY	**Malphigiaceae**
SYNONYM	***Hiptage madablot*, *Banisteria benghalensis***
COMMON NAME	**Madhavi Lata, Madhumalati, Hiptage**
KINGDOM	**Plantae**
PHYLLUM	**Angiosperms**
CLASS	**Eudicots**
ORDER	**Malphigiales**
FAMILY	**Malphigiaceae**
GENUS	**Hiptage**
SPECIES	**Benghalensis**

VERNACULAR NAMES

TABLE 2: VERNACULAR NAMES OF HIPTAGE BENGHALENSIS [26]

HINDI	**Madhavi lata**
MALAYALAM	**Vasanthamalli**
MARATHI	**Hiptaj**
SANSKRIT	**Adimukta, Vasantaka**
TAMIL	**Kurukkathi, Madhumalati**
TELUGU	**Madhavi**

DISTRIBUTION

Hiptage benghalensis, commonly known as Hiptage or the helicopter flower, is widely distributed across tropical and subtropical regions of South and Southeast Asia. It is native to India, Sri Lanka, Nepal, Bangladesh, Myanmar, Thailand, Laos, Vietnam, Cambodia, and southern China. The species typically grows in warm, humid environments such as moist deciduous forests, forest edges, grasslands, riverbanks, and along roadsides. Its ability to tolerate a range of soil types and climatic conditions enables it to spread easily. Outside its native range, *Hiptage benghalensis* has been introduced as an ornamental plant to several tropical regions worldwide. It is now naturalized in areas such as Mauritius, Réunion, Madagascar, Malaysia, Indonesia, and parts of the Pacific islands. In many of these regions, it has become invasive, spreading rapidly in disturbed

habitats, plantation areas, and secondary forests. Its vigorous growth allows it to compete with and suppress native vegetation, contributing to ecological imbalance [27-30].

MORPHOLOGY

Habit

Hiptage benghalensis is a perennial woody liana that grows extensively over trees and shrubs. It exhibits vigorous climbing behaviour with long, flexible stems. The plant forms dense thickets, enabling rapid spread in forest edges and semi-tropical regions [31-32].

Stem

The stem is woody, flexible, and cylindrical with brownish to grey bark. Older stems develop a rough texture, while younger stems remain smooth. The plant produces branching tendrils that help in climbing and spreading across supporting vegetation [33-34].



FIG 4: Stem Of Hiptage Benghalensis

Leaves

Leaves are simple, opposite, and glamorous with an ovate to elliptic shape. They possess prominent veins and a glossy green surface. The leaf apex is acute, and the base is rounded, supported by short petioles. Leaves appear leathery and thick [35-36].



FIG 5: Leaves Of Hiptage Benghalensis

Flowers

Flowers are fragrant, showy, and arranged in elongated racemes. Each flower has five white to pinkish petals with a yellow throat. The corolla is slightly crinkled, enhancing attractiveness Flowers bloom in clusters, supporting pollination by insects [37-38].



FIG 6: Flower Of Hiptage Benghalensis

Calyx

The calyx consists of five sepals, with one enlarged sepal that often bears a nectar gland. Sepals are green, narrow, and persistent, protecting the inner floral structures. The calyx remains attached even during early fruit formation [39-40]

Corolla

The corolla has five petals that are broad, wrinkled, and slightly overlapping. Petals display white, pink, or pale rose colours with a yellowish central region. Their delicate texture and sweet fragrance attract a variety of pollinators, mainly bees [41].

Stamens

Flowers possess ten stamens arranged in two whorls. Stamens vary in length, with longer outer and shorter inner whorls. Anthers are dorsifixed, producing abundant pollen. This arrangement promotes effective cross-pollination and reproductive success [42].

Pistil

The pistil is single, slender, and elongated with a superior ovary. The style is filiform, ending in a small, receptive stigma. The ovary develops into a characteristic winged fruit. The pistil structure supports efficient fertilization after pollinator contact [43].

Fruit

The fruit is a distinctive three-winged samara, light brown and papery. Each wing is elongated, helping wind dispersal. The central seed chamber is small and ovoid. The fruit's aerodynamic design supports long-distance seed dispersal across habitats [44].



FIG 7: Fruit Of Hiptage Benghalensis

Seed

Seeds are small, brown, and enclosed within the central portion of the samara. They are lightweight and non-endospermic. Once dispersed, seeds germinate readily in moist soil, giving rise to vigorous seedlings that grow into woody climbers [45].



FIG 8: Seed Of Hiptage Benghalensis

REVIEW OF LITERATURE

1. **Maheshwari, *et al.*,** (2012) evaluated the hepatoprotective activity of methanolic leaf extract of *Hiptage benghalensis* against CCl_4-induced hepatotoxicity in rats. The extract (200 and 400 mg/kg) significantly reduced serum AST, ALT, ALP, and total bilirubin levels. Histopathological examination confirmed protection of hepatic tissue from CCl_4-induced damage The hepatoprotective

effect was comparable to silymarin and attributed to its antioxidant activity.

2. **Kumudhavalli, *et al*.,** (2010) carried out a detailed phytochemical investigation of *Hiptage benghalensis* leaves using various solvent extracts and identified the presence of flavonoids, phenolic compounds, tannins, and other secondary metabolites. The extracts were further subjected to pharmacological evaluation to validate their traditional medicinal use. Anti-inflammatory activity was assessed using experimental animal models, where the leaf extracts showed significant inhibition of inflammation compared to the control group. The study concluded that the bioactive phytoconstituents present in the leaves may be responsible for the observed therapeutic potential of the plant.

3.Hridi, *et al*., (2013) conducted a phytochemical screening and evaluated the analgesic and anti-inflammatory activities of the ethanol extract of *Hiptage benghalensis* leaves. The phytochemical analysis revealed the presence of steroids, flavonoids, alkaloids, tannins, phenols, terpenoids, carbohydrates, and mangiferin. The extract demonstrated significant anti-inflammatory activity in carrageenan-induced hind paw edema in rats. It also showed marked central and peripheral analgesic effects in hot plate and acetic acid-induced writhing models in mice. The study scientifically validated the traditional Ayurvedic use of *Hiptage benghalensis* for treating inflammation and pain.

4.**Bhukya and Yellu., (2018)** evaluated the anticancer potential of the methanolic extract of *Hiptage benghalensis* using in vitro cancer cell lines. The cytotoxic activity was assessed by MTT assay against HeLa (cervical), MCF-7 (breast), and IMR-32 (neuroblastoma) cells. The extract exhibited significant dose-dependent inhibition of cell viability in all tested cell lines. The IC_{50} values were reported as 50.73 µg/mL (HeLa), 47.90 µg/mL (MCF-7), and 53.76 µg/mL (IMR-32). The study concluded that *H. benghalensis* possesses promising anticancer activity and supports its potential for further anticancer research.

5.**Murugan M. and Mohan V.R., (2011)** evaluated the phytochemical composition and antibacterial activity of *Bauhinia purpurea* L. and *Hiptage benghalensis* L. Kurz. Different solvent extracts were prepared, among which the methanolic extract showed the highest antibacterial activity against pathogens such as *Staphylococcus aureus*, *E. coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. Phytochemical screening revealed the presence of alkaloids, flavonoids, phenols, tannins, coumarins, and terpenoids. The study concluded that these bioactive constituents are responsible for the significant antimicrobial potential of the plants.

6.**Yadav, S. and Kumar, P., (2012)** investigated the production, isolation, and identification of flavonoids from the aerial parts of *Hiptage benghalensis* (L.) Kurz. The study reported that flowers contained the highest free flavonoid content, while leaves showed the maximum bound and total flavonoid content. Stem exhibited comparatively lower flavonoid concentration than leaves and flowers. Their findings suggest that *Hiptage benghalensis* is a promising natural source of flavonoids with medicinal value.

7.**Hsu, C. L., *et al* (2013)** investigated the anti-inflammatory effects of triterpenes and a steroid derivative isolated from the stem bark of *Hiptage benghalensis*. Eight compounds were characterized, and several showed significant inhibitions of NO and PGE_2 production in LPS-stimulated RAW 264.7 macrophages. The isolates also suppressed I NOS and COX-2 expression and modulated NF-κB signaling. Their findings confirm the potent anti-inflammatory potential of bioactive compounds from *H. benghalensis*.

8.Meenaa *et al*., (2014) *Hiptage benghalensis* (L.) Kurz, belonging to the family Malphigiaceae, is a medicinal plant widely used in traditional systems of medicine. Various studies have reported that this plant possesses significant pharmacological activities such as antibacterial, antifungal, antidiabetic, anti-inflammatory, anticancer, antimutagenic and hepatoprotective effects. In Ayurveda, the plant is described as cooling, astringent, vulnerary and cardiotonic, and is traditionally used in the treatment of wounds, skin diseases, cough, asthma, rheumatism and cardiac disorders. The plant also shows potential in managing conditions such as obesity, ulcers and inflammatory diseases. These findings highlight the

ethnopharmacological importance and therapeutic potential

9.**Vitelli *et al.*, (2009)** studied the control of the invasive liana *Hiptage benghalensis* in Queensland, Australia. They evaluated seven herbicides using foliar, basal bark, and cut stump application methods. Cut stump treatment was found to be most effective, achieving over 95% plant mortality, particularly with fluroxypyr, triclopyr/picloram, and glyphosate. Foliar herbicides such as dicamba were effective mainly on seedlings. The study also reported a short-term seed bank and provided cost-effective management strategies for large infestations.

10.**Johnson Barnty., (2023)** investigated the ecological impacts and management of *Hiptage benghalensis* in tropical ecosystems of Southeast Asia. The study reported that the invasive vine significantly reduces native plant diversity, alters soil properties, and disrupts wildlife habitats. Different control strategies, including manual removal, chemical herbicides, and biological control methods, were assessed for their effectiveness and sustainability. The research highlights the importance of integrated management approaches with early detection, monitoring, and community involvement to protect biodiversity and restore ecosystem health.

11.**Tan, *et al*., (2019)** conducted the first molecular phylogenetic study of the genus *Hiptage* (Malphigiaceae) using ITS sequence data. Their analysis suggested that the genus likely originated in the South Indo-China Peninsula and diversified within this region. The study identified and described a new species, *Hiptage benghalensis*, from Southwest China based on both molecular and morphological evidence. The findings provide important insights into the evolutionary history, dispersal patterns, and biogeography of the genus.

12. **Samal *et al.* (2023)** investigated the hypolipidemic activity of aqueous and ethanolic extracts of *Hiptage benghalensis* leaves in high-fat-diet induced hyperlipidemic Wistar rats. The study reported that treatment with the plant extracts significantly reduced serum total cholesterol, triglycerides, LDL, and VLDL levels while increasing HDL levels compared with the hyperlipidemic control group. Phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, saponins, phenolic compounds, and steroids, which may contribute to the observed lipid-lowering activity.

13. **Li *et al.* (2023)** sequenced and analyzed the complete chloroplast genome of *Hiptage benghalensis* (L.) Kurz from the Malphigiaceae family. The chloroplast genome was 160,719 bp in length with a GC content of 36.7% and contained 132 genes, including protein-coding, tRNA, and rRNA genes. Phylogenetic analysis revealed that H. benghalensis is closely related to species of the genus Aspidopterys. This study provided the first chloroplast genome data for H. benghalensis, helping to understand its evolutionary relationship.

14. **Yadav and Kumar (2010)** studied the production, isolation, and identification of flavonoids from the aerial parts of *Hiptage benghalensis*. Flavonoids were extracted from dried stem, leaves, and flowers and separated into free and bound fractions. The study reported that free flavonoids were highest in flowers, while bound flavonoids were highest in leaves. Overall, the total flavonoid content was found to be maximum in the leaves compared to flowers and stem.

15.**Desai D.J *et al* (2023)** A comprehensive review on *Hiptage benghalensis* (L.) Kurz highlights its botanical characteristics, phytochemistry, and medicinal importance. The plant is traditionally used to treat various ailments such as rheumatism, asthma, skin diseases, and digestive disorders. Different parts of the plant including bark, leaves, roots, stem, and flowers possess several pharmacological activities like anti-inflammatory, antimicrobial, antioxidant, and hepatoprotective effects. These therapeutic properties are mainly attributed to phytoconstituents such as flavonoids, alkaloids, phenols, tannins, and terpenoids.

MATERIALS AND METHODS:

COLLECTION AND AUTHENTICATION:

The fresh Leaves of *Hiptage benghalensis* (Malphigiaceae) were gathered on 02 December 2025, in SMPG Office, Salem (Mettur Dam), Tamil Nadu, India. The plant's identification was verified by comparing it with reference specimens stored at the

Siddha Medicinal Plant Garden in Salem on 02 December 2025. The given specimen of the plant was identified by Dr. P. Radha Research Officer (Botany)And Head of Office the Siddha Medicinal Plant Garden in Salem

PLANT EXTRACT PREPARATION:

The Leaves of the plant was carefully chosen and thoroughly washed to remove any impurities, and then shade dried. The dried Leaves was processed into a fine powder using a mechanical grinder. The resulting fine powder was placed in an airtight container for later use. About 15 grams of the powdered material were extracted with 150ml of Hydro alcoholic (70%water+30%Etahanol) as a solvent through a Soxhlet apparatus, after extraction process 65 ml of leaf extract was obtained. A portion of the extract was preserved for preliminary phytochemical screening to identify various constituents of the plant and the other portion of the extract used for the further analysis.

QUALITATIVE PHYTOCHEMICAL ANALYSIS [46-49]

A qualitative phytochemical analysis was conducted on the extracts utilizing the standard tests listed below.

1. Test for Alkaloids (Dragendorff's Test):

A small amount of plant extract, around a few millilitres, was combined with 4-5 drops of Dragendorff's reagent. The appearance of a reddish-brown precipitate indicates the presence of alkaloids.

2. Test for Phenol (Ferric chloride Test):

Approximately 2ml of the extract was mixed with a 10% solution of ferric chloride and examined for the appearance of a deep brown or black colour.

3. Test for reducing sugars (Fehling's Test)

To 1 ml of the extract, a few drops of Fehling's (1&2) reagent were added, and the mixture was boiled in a water bath for 10 minutes, then monitored for the emergence of a blue colour

4. Test for Saponins (Foam test)

In a test tube, 1 ml of the plant extract was combined with 5 ml of water. The resulting mixture was shaken vigorously and monitored for the development of stable foam for several seconds. The formation of foam indicates the presence of saponins.

5. Test for Flavonoids

To approximately 2 ml of the plant extract, several drops of a Sodium hydroxide solution were introduced. The development of a yellow colour signifies the presence of flavonoids.

6. Test for Phytosterols (Salkowski's Test):

One millilitre of the plant extract was combined with two millilitres of chloroform, followed by the addition of a few drops of acetic anhydride. Subsequently, an equal volume of concentrated sulfuric acid was introduced to the mixture. The appearance of a Reddish-brown colour signifies the presence of phytosterols.

7. Test for Amino acids and Proteins (Ninhydrin test)

A small quantity of Ninhydrin reagent was added to a few millilitres of plant extract. The development of a purple or violet colour signifies the presence of amino acids and proteins.

8. Test for Steroids

Approximately 2 ml of chloroform and 0.2 ml of concentrated sulfuric acid were introduced to 1 ml of the Plant extract. The emergence of a red-coloured precipitate signifies the presence of steroids.

9. Test for Tannin

To approximately 1 millilitre of plant extract, a few drops of dilute ferric chloride solution were added. The presence of tannins is indicated by the development of a brown or brick black coloration.

10. Test for glycosides

A few millilitres of concentrated sulfuric acid were added to 1 millilitre of plant extract. The development of a red colour signifies the presence of glycoside.

SYNTHESIS OF NANOPARTICLE [50-51]

MATERIALS AND METHOD

MATERIALS

All of the analytical-grade compounds used in the experiment were used exactly as supplied, without any additional purification. The forerunner to silver was silver nitrate (Ag NO_3). The experiment was conducted using deionized water as the solvent. The reaction medium's pH was controlled by sodium hydroxide (NaOH), and the reducing agent was sodium borohydride (Na BH_4). As an antioxidant, ascorbic acid was added to improve the stability of the produced colloidal silver nanoparticles.

METHODS

A hydro alcoholic extract was prepared at a concentration of 0.02 M using deionized water. Separately, a 0.01 M silver nitrate (Ag NO_3) solution was prepared in deionized water and protected from light. The silver nitrate solution was then added dropwise to the plant extract under continuous magnetic stirring at room temperature. The pH of the reaction mixture was adjusted by the gradual addition of 1 M sodium hydroxide (NaOH) solution prepared in deionized water. After maintaining stirring for 30 minutes, a freshly prepared 0.1 M sodium borohydride ($NaBH_4$) solution in deionized water was introduced slowly into the reaction mixture under constant stirring. Stirring was continued for an additional 15 minutes under ambient atmospheric conditions to ensure complete reduction of Ag^+ ions. A distinct color change from colorless/pale yellow to yellowish-brown or dark brown was observed, indicating the formation of silver nanoparticles.



FIG 9: Silver Nanoparticle Synthesis

CHARACTERIZATION OF NANOPARTICLE

UV- SPECTROSCOPY

The Silver nanoparticles synthesized biologically from *Hiptage benghalensis* were characterized through UV-spectroscopy. The resulting sample was analyzed in the Ultraviolet-Visible spectrum within the wavelength range of 260-400 nm. A notable color change was observed in the reaction mixture, where the plant leaf extract combined with an aqueous solution of Ag NO3 transitioned from Brown or dark brown, indicating the formation of Nanoparticles.

FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY

For the analysis using Fourier transform infrared (FTIR) spectroscopy, a colloidal solution of silver nanoparticles (50 mL) was prepared under optimal conditions, including an EFE of 5% and a concentration of 1 millimolar of silver nitrite. This solution was then centrifuged at 20,000 revolutions per minute for a duration of 20 minutes. The resulting pellets were subsequently lyophilized for 16 hours to facilitate the identification of functional groups present in the synthesized silver nanoparticles. The absorption spectrum obtained provides valuable insights into the molecular structure.

ANTIOXIDANT ACTIVITY

DPPH RADICAL SCAVENGING ACTIVITY [52]

The free radical scavenging activity of samples was measured by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) The scavenging activity for DPPH free radicals was measured according to the procedure described by (Braca *et al.,* 2001). An aliquot of 3 ml of 0.004% DPPH solution in methanol and 10 to 100 µl of plant extract/ascorbic acid at various concentrations were mixed. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 30 min. Decolorization of DPPH was determined by measuring the absorbance at 517 nm. A control was prepared using 0.1 ml of respective vehicle in the place of plant extract/ascorbic acid. The percentage inhibition of DPPH radicals by the extract/compound was determined by comparing the absorbance values of the control and the experimental tubes.

$$\text{Scavenging activity \%} = \frac{\text{A518 (control) - A518 (sample)}}{\text{A518 (control)}} \times 100$$

FERRIC REDUCING ANTIOXIDANT POWER (FRAP) ASSAY [53-54]

The antioxidant capacity of Nanoparticle extract was estimated according to the procedure described by Benzie and Strain (1996) and as modified by Pulido *et al*. (2000). FRAP reagent (900µl), prepared freshly and incubated at 37 ºC, was mixed with 90µl of distilled water and 30µl of test sample, or acetone (for the reagent blank). The test samples and reagent blank were incubated at 37 ºC for 30 minutes in a water bath. The FRAP reagent contained 2.5 ml of 20 mmol/l TPTZ solution in 40 mmol/l HCl plus 2.5 ml of 20 mmol/l FeCl3.6H2O and 25 ml of 0.3 mol/l acetate buffer, pH 3.6 (Benzie and Strain, 1996). At the end of incubation period the absorbance readings were recorded immediately at 593 nm using a spectrophotometer. The known Fe (II) concentration ranging between 100 and 2000µ mol/l (FeSO4.7H2O) was used for the preparation of the calibration curve. The parameter Equivalent Concentration (EC1) was defined as the concentration of antioxidant has a ferric- TPTZ reducing ability equivalent to that of 1 mmol/l FeSO4.7H2O. EC1 was calculated as the concentration of antioxidant giving an absorbance increase in the FRAP assay equivalent to the theoretical absorbance value of a 1 mmol/l concentration of Fe (II) solution determined using the corresponding regression equation.

$$\text{Scavenging activity \%} = \frac{\text{A518 (control) - A518 (sample)}}{\text{A518 (control)}} \times 100$$

HYDROGEN PEROXIDE SCAVENGING ACTIVITY (H_2O_2) [55]

This activity of the Nanoparticle extract was evaluated by the method of Ruch *et al*. 1989. 850µL of the aqueous plant extract was added to 150µL of 4 mM hydrogen peroxide solution prepared in phosphate buffer (0.1 M, pH-7.4). This was incubated for 10 minutes, and absorbance was read at 230 nm. BHT was taken as a positive control and the reaction was carried out in triplicates. Percent inhibition of the assay was calculated.

$$\text{Scavenging activity \%} = \frac{\text{A518 (control) - A518 (sample)}}{\text{A518 (control)}} \times 100$$

ANTICANCER ACTIVITY

REQUIREMENT

- To determine the cytotoxicity study on HeLa cell line, 1 sample were received.

The samples details as below:

TABLE 3: DETAILS OF SAMPLE RECEIVED FOR THE STUDY

Sl. No.	Sample Name/Code	Concentrations	Cell lines
1	Cervical Cancer cell line	(6.25, 12.5, 25.0, 50 and 100 µg/ml)	HeLa

BACKGROUND OF THE STUDY

MTT assay is a colorimetric assay used for assessing cell proliferation and cytotoxicity. It is based on the reduction of the yellow, water-soluble tetrazolium dye MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) to insoluble purple formazan crystals by metabolically active cells. This reduction is primarily carried out by mitochondrial dehydrogenase enzymes, not lactate dehydrogenase. The formazan crystals are then solubilized using an appropriate solvent, and the resulting purple color can be quantified by measuring absorbance at 570 nm using a spectrophotometer. The intensity of the color is directly proportional to the number of viable cells. (Mosmann,1983)

MATERIALS

1. Cell lines: HeLa cell lines (NCCS, Pune)
2. Cell culture medium: Dulbecco's Modified Eagle Medium (DMEM) + Minimum Essential Medium (MEM)
3. Fetal Bovine Serum (#RM10432, Hi media)
4. MTT Reagent (5 mg/ml) (# 4060 Hi media)
5. D-PBS (#TL1006, Hi media)
6. 96-well plate for culturing the cells (Nest, India)
7. T25 flask (Nest, India)
8. 50 ml centrifuge tubes (# 546043 TARSON)
9. 1.5 ml centrifuge tubes (TARSON)
10. 10 to 1000µL tips (TARSON)

EQUIPMENTS

1. Centrifuge (Neuation).
2. Pipettes: 2-10µL, 10-100µL, and 100-1000µL
3. Inverted binocular biological microscope (ICX41 Inverted Biological Microscope)
4. 37°C incubator with humidified atmosphere of 5% CO_2 (Thermo Fisher, USA)
5. 96well plate reader (Epoch2, BioTek, CA, USA)

MAINTENANCE OF CELL LINES

1. The HeLa human Cervical Cancer cell line was obtained from the **National Centre for Cell Science (NCCS), Pune, India**. Cells were cultured in **Dulbecco's Modified Eagle Medium (DMEM) and Minimum Essential Medium (MEM)** supplemented with **10% fetal bovine serum (FBS)** and **1% antibiotic-antimycotic solution**. Cultures were maintained at **37 °C** in a humidified incubator with **5% CO_2 and 18-20% O_2**. Cells were sub-cultured every **three days** to ensure optimal growth and viability.

MTT Assay Protocol

1. **Cell Seeding**

Seed 200µL of cell suspension into each well of a 96-well plate at the required cell density (e.g., 20,000 cells per well), without the test agent. Allow the cells to adhere and grow for approximately 24 hours at 37 °C in a 5% CO_2 incubator.

2. **Treatment with Test Agents**

Add appropriate concentrations of the test agents (as specified in the Results/Excel sheet) to the wells.

3. **Incubation**

Incubate the plate for an additional 24 hours under standard culture conditions (37 °C, 5% CO_2).

4. **MTT Reagent Addition**

After incubation, carefully remove the spent media from each well. Add MTT reagent to each well to achieve a final concentration of 0.5 mg/mL in the total volume.

5. **Light Protection**

Cover the plate with aluminum foil to protect it from light.

6. MTT Incubation

Return the plate to the incubator and incubate for 3 hours.
Note: Incubation time may vary depending on the cell type. For consistency within an experiment, the same incubation time should be maintained across all conditions.

7. Solubilization of Formazan Crystals

After incubation, remove the MTT-containing medium and add 100µL of solubilization solution (e.g., DMSO) to each well.

8. Dissolution

Gently shake the plate on a gyratory shaker to facilitate dissolution. For dense cell cultures, pipetting up and down may be necessary to completely dissolve the formazan crystals.

9. Measurement

Measure the absorbance at 570 nm using a spectrophotometer or microplate (ELISA) reader. The absorbance values correlate with the number of metabolically active (viable) cells.

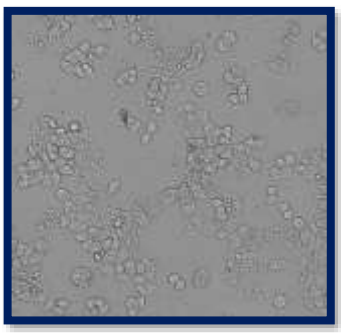


FIG 10:6.25PNG

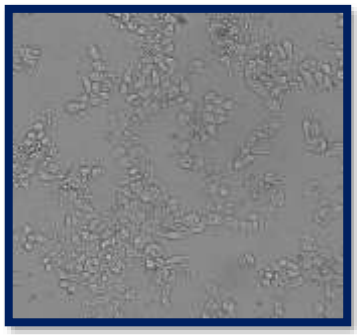


FIG 11:12.5 PNG

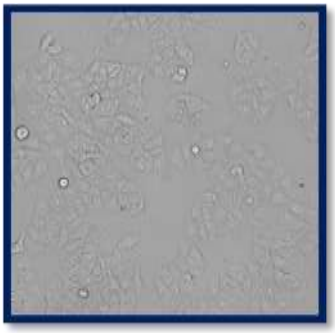


FIG 12:25 PNG

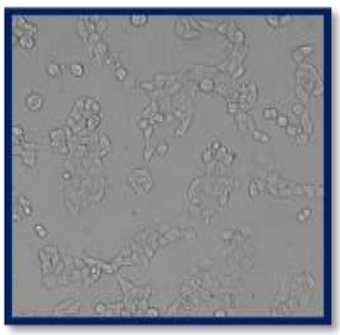


FIG 13: 50 PNG

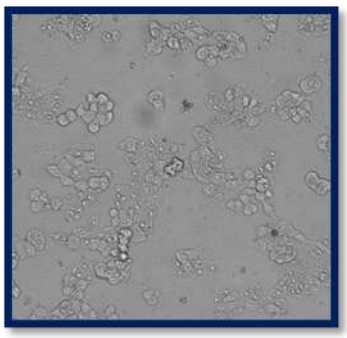


FIG 14:100 PNG

Cell viability is calculated using the formula,

Cell viability (%) = [Mean abs of treated cells/Mean abs of Untreated cells] x 100

RESULT AND DISCUSSION

1) **TABLE 4: PHYTOCHEMICAL ANALYSIS OF THE LEAVES**

S.NO	TEST	Hydroalcoholic extract of Hiptage benghalensis
1	Alkaloids	+ +
2	Flavonoids	+ +
3	Phenolic compounds	+ + +
4	Reducing sugar	+ + +
5	proteins	+ +
6	Phytosterol	+
7	Steroids	+
8	Glycosides	+
9	Tannins	+ +
10	Saponins	+

Note: Present (+) - Absent (-)

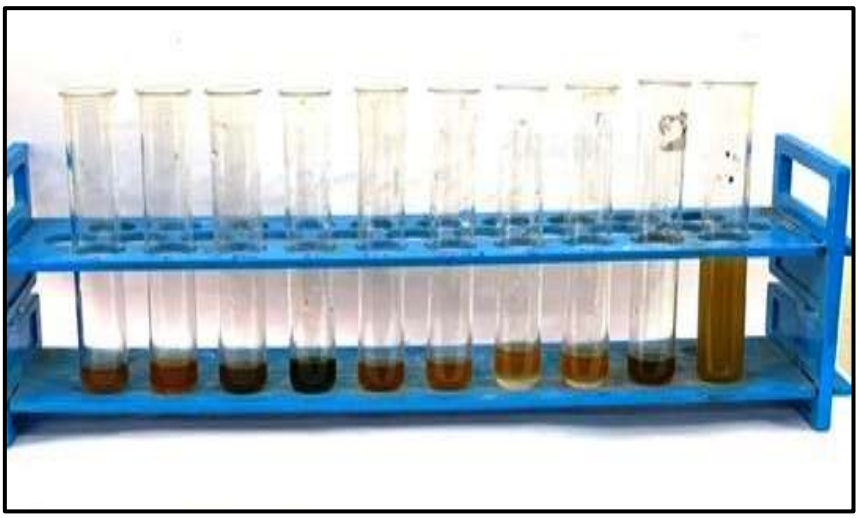


FIG 15: **Phyto Chemical Analysis Of Leaves**

CHARACTERIZATION OF NANOPARTICLE

1) UV-SPECTROSCOPY

At a wavelength of 340 nm, the ultraviolet-visible spectra of newly synthesized silver nanoparticles exhibit Plasmon resonance. Typically, the wavelength associated with silver nanoparticles range from 260-400 nm. In the analysis of silver nanoparticles, the

highest absorbance was noted at 340 nm. The conversion of silver nitrite to silver ions in the Leaf extract is validated by the peak value, which also facilitates the formation of silver nanoparticles.

TABLE 5: Uv Visible Spectroscopy

S.NO	Nanometer	Absorbance
1	260nm	1.938
2	280nm	2.795
3	300nm	2.795
4	320nm	2.95
5	340nm	3
6	360nm	1.564
7	380nm	1.104
8	400nm	1.367

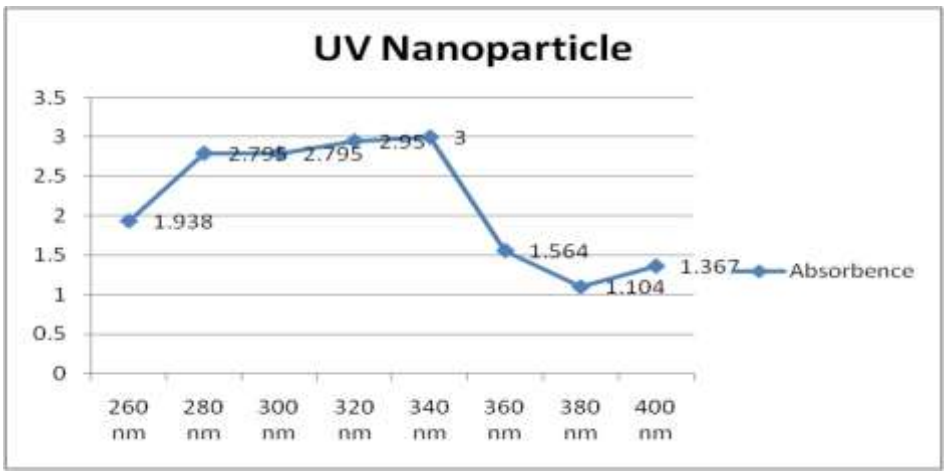


FIG 16: Graph Of Uv Spectroscopy

FTIR SPECTOSCOPY

TABLE 6: FTIR OBSERVATION

S. No	Peak (cm^{-1})	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	3819.06	12.5	0.08	3850.2	3785.4	6.12	0.05
2	3739.08	18.7	0.15	3780.6	3705.3	8.45	0.12
3	1634.35	82.4	45.1	1705.8	1550.4	52.8	30.25
4	1401.18	55.2	18.9	1455.6	1355.2	34.6	12.4
5	1044.5	68.9	22.75	1095.3	1010.2	41.85	15.6
6	686.6	40.3	9.8	735.4	650.3	26.15	6.9
7	601.78	32.1	7.25	645.9	570.4	21.3	5.15
8	495.42	25.6	4.1	525.8	465.2	18.75	3.4

The FTIR spectrum of the synthesized silver nanoparticles showed several characteristic absorption bands indicating the involvement of biomolecules in the reduction and stabilization process. Broad absorption bands observed at around 3819 and 3739 cm^{-1} correspond to O–H stretching vibrations of hydroxyl groups, suggesting the

presence of phenolic and alcoholic compounds that act as effective reducing agents. The prominent peak at approximately 1634 cm^{-1} is attributed to C=O stretching vibrations or N–H bending of amide I groups, indicating the participation of proteins or carbonyl-containing phytochemicals in nanoparticle stabilization. The absorption band near 1401 cm^{-1} is assigned to C–N stretching vibrations of amine groups, further supporting the role of biomolecules as capping agents. The peak observed at about 1044 cm^{-1} corresponds to C–O stretching vibrations of alcohols and ethers, confirming the presence of bioactive compounds on the surface of the nanoparticles. Additionally, absorption bands appearing in the lower wavenumber region between 686 and 495 cm^{-1} are characteristic of Ag–O vibrations, which confirm the successful formation of silver nanoparticles. Overall, the FTIR analysis reveals that hydroxyl, carbonyl, amide, and C–O functional groups derived from phytochemicals are responsible for the reduction of Ag^{+} ions and the stabilization of the synthesized silver nanoparticles, demonstrating the effectiveness of the green synthesis approach.

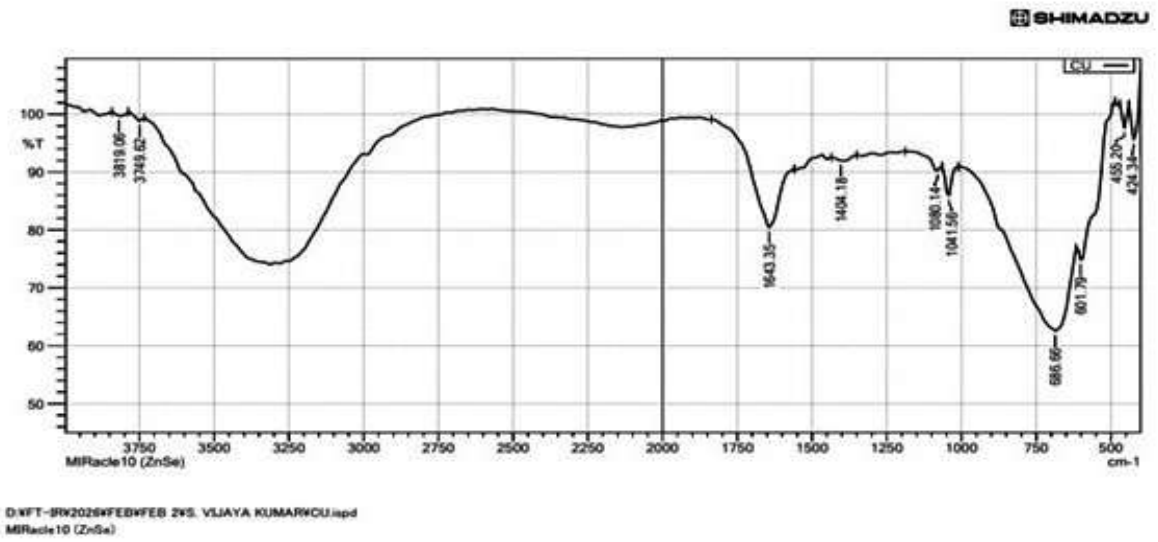


FIG 17: Graph Of Ftir Spectroscopyantioxidant Activity

A) DPPH RADICAL SCAVENGING ACTIVITY

FORMULA

% Inhibition = [(control-sample/control] × 100

Control = 0.980

STEP BY STEP CALCULATION:

- For 10µg = (0.980-0.823)/0.980 × 100 = 16.02%
- For 20µg = (0.980-0.564)/0.980 × 100 = 42.50%
- For 30µg = (0.980-0.368)/0.980 × 100 = 62.40%
- For40µg = (0.980-0.246)/0.980 × 100 = 74.8%
- For50µg = (0.980-0.101)/0.980 × 100 = 89.06%

TABLE 7: DPPH ASSAY

Concentration	Standard (Ascorbic acid)	Mean ± SD	Nanoparticle
10 µg	32	0.824±0.0025	16.02%***
20 µg	48	0.564±0.0025	42.50%***
30 µg	55	0.368±0.0021	62.40%***
40 µg	72	0.245±0.0031	74.8 %***
50 µg	87	0.104±0.0036	89.06 %***

Values are expressed as Mean ± SD, p<0.001* as compared to Control**

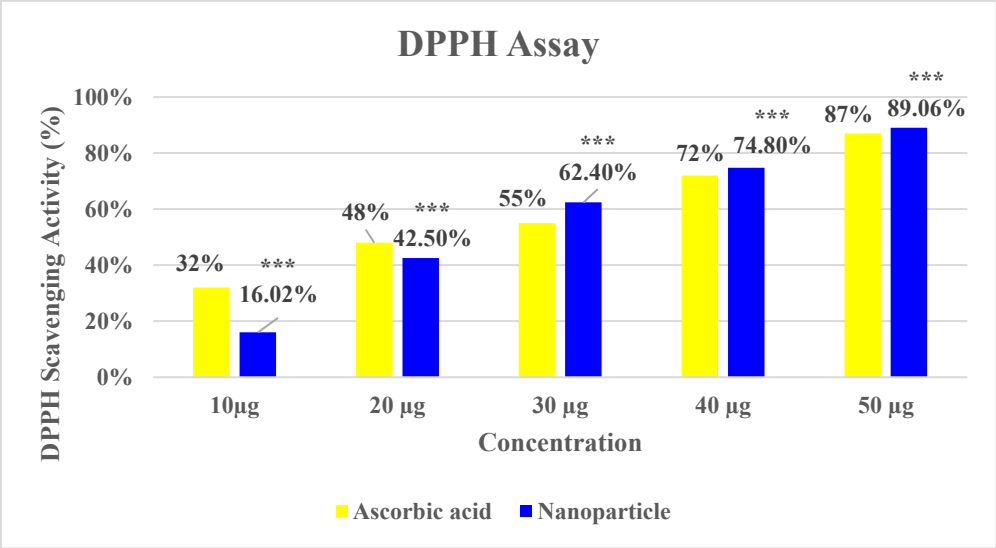


FIG 18: Graph Of Dpph Assay

DPPH free radicals Scavenging activity of silver nanoparticles of *Hiptage benghalensis* leaf extract and Standard Ascorbic acid were compared (Table:7 and Figure:18). The result was expressed as percentage inhibition. The highest percentage of inhibition Ascorbic acid shows 87% and synthesized nanoparticle leaf extract shows 89.06 % (p<0.001***) at the higher concentrations. The synthesized nanoparticle leaf extract shows almost similar antioxidant activity when compared with standard.

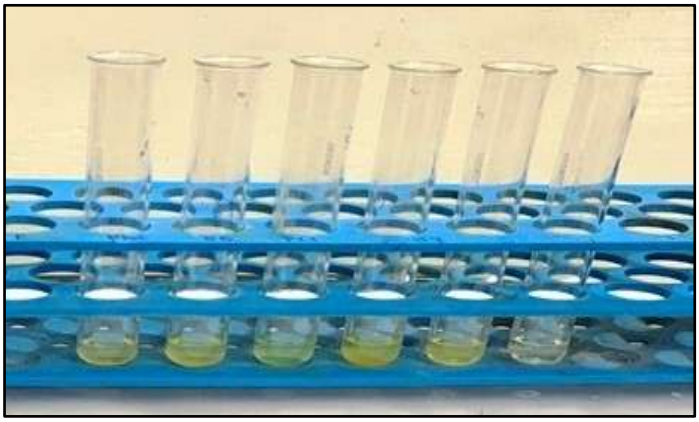


FIG 19: DPPH ASSAY

B) FERRIC REDUCING ANTIOXIDANT ASSAY (FRAP)

FORMULA

% inhibition = [(control-sample)/control] × 100

Control = 0.740

STEP BY STEP CALCULATION:

- For 10 µg = (0.740-0.687/0.740) × 100 = 7.16%
- For 20 µg = (0.740-0.628/0.740) × 100 = 15.13%
- For 30 µg = (0.740-0.443/0.740) × 100 = 40.00%
- For 40 µg = (0.740-0.302/0.740) × 100 = 59.18%
- For 50 µg = (0.740-0.229/0.740) × 100 = 69.05%

TABLE 8: FRAP ASSAY

Concentration	Standard (Ascorbic Acid)	Mean ± SD	Nanoparticles
10 µg	33 %	0.686±0.00252	7.16 %***
20 µg	44 %	0.624±0.00400	15.13 %***
30 µg	52 %	0.446±0.00252	40.00 %***
40 µg	71 %	0.305±0.00306	59.18 %***
50 µg	83 %	0.227±0.00153	70.00 %***

Values are expressed as Mean ± SD, p<0.001* as compared to Control**

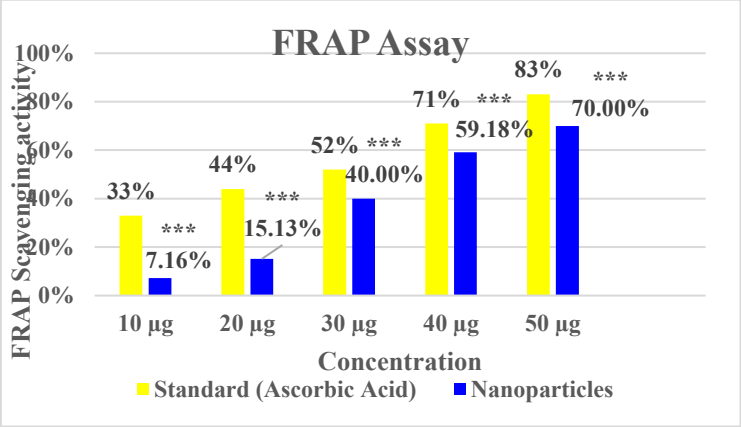


FIG 20: Graph Of Frap Assay

FRAP Assay of Silver nanoparticles of *Hiptage benghalensis* leaf extract and Standard Ascorbic acid were compared (Table:8 and Figure:20). The result was expressed as percentage inhibition. The highest percentage of inhibition Ascorbic acid shows 83% and synthesized nanoparticle leaf extract shows 70 % at the higher concentrations. The synthesized nanoparticle leaf extract shows the more than 70% ($p<0.001^{***}$) inhibitory concentration in TPTZ Solution

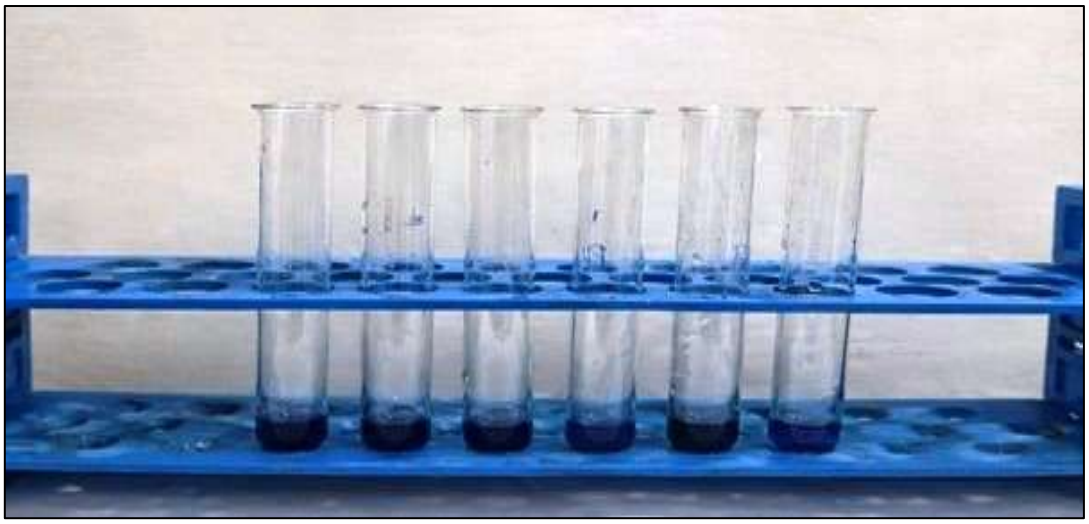


FIG 21: FRAP ASSAY

C) HYDROGEN PEROXIDE SCAVENGING ACTIVITY (H_2O_2)

FORMULA

% Inhibition = [(control-sample)/control × 100

Control = 0.700

STEP BY STEP CALCULATION:

- For 10 µg = (0.700-0.355/0.700) × 100 = 49.20%
- For 20 µg = (0.700-0.337/0.700) × 100 = 51.80%
- For 30 µg = (0.700-0.253/0.700) × 100 = 63.80%
- For 40 µg = (0.700-0.137/0.700) × 100 = 80.40%
- For 50 µg = (0.700-0.054/0.700) × 100 = 92.20%

TABLE 9: H_2O_2 ASSAY

Concentration	Standard (Ascorbic acid)	Mean ± SD	Nanoparticle
10 µg	28 %	0.354±0.0015	49.20 %***
20 µg	42 %	0.336±0.0036	51.80 %***
30 µg	53 %	0.252±0.0015	63.80 %***
40 µg	68 %	0.137±0.0015	80.40 %***
50 µg	80 %	0.054±0.0020	92.20 %***

Values are expressed as Mean ± SD, p<0.001* as compared to Control**

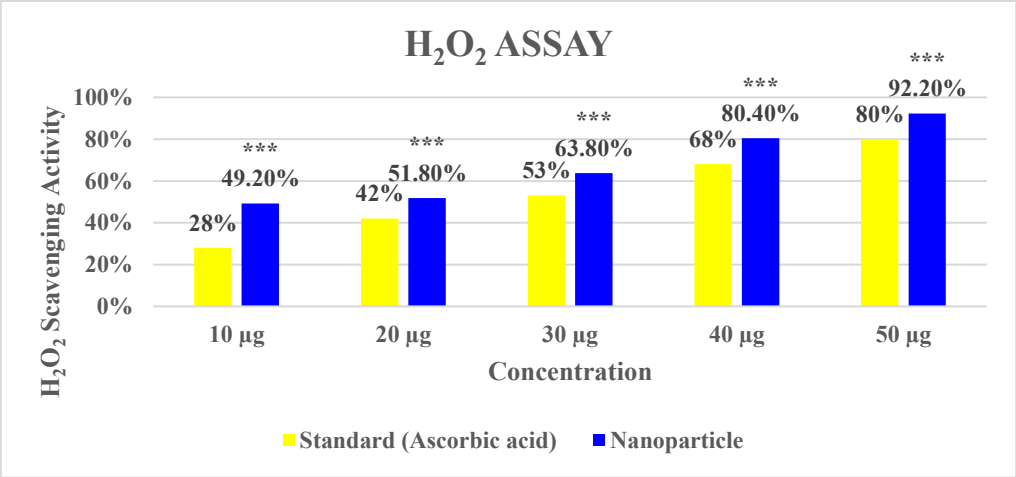


FIG 22: GRAPH OF HYDROGEN PEROXIDE ASSAY

H_2O_2 Free radical scavenging activity of silver nanoparticles of *Hiptage benghalensis* leaf extract and Standard Ascorbic acid were compared (Table:9 and Figure:22). The result was expressed as percentage inhibition. The highest percentage of inhibition Ascorbic acid shows 80% and synthesized nanoparticle leaf extract shows 92% ($p<0.001^{***}$) at the higher concentrations.

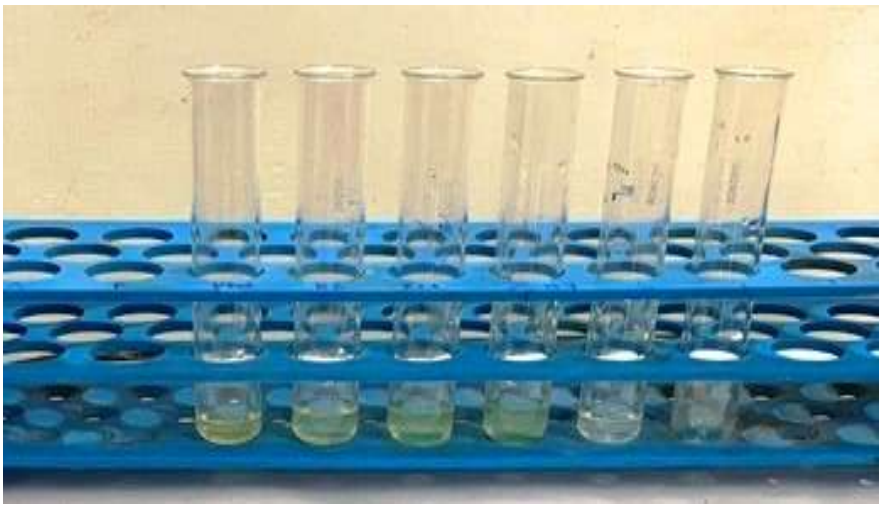


FIG 23: Hydrogen Peroxide Assay

OBSERVATIONS & CONCLUSIONS

TABLE 10: PERCENTAGE OF CELL VIABILITY IN HELA CELL LINE

SUMMARY - MTT ASSAY	
Concentration of sample (µg/ml)	% cell viability
6.25ug	90.51
12.5ug	77.57
25ug	59.01
50ug	41.02
75ug	20.82
100ug	12.32
IC50 value = 35.5 µg/ml	

PERCENTAGE OF CELL INHIBITION

% of cell inhibition = 100 - % of cell viability

TABLE 11: PERCENTAGE OF CELL INHIBITION IN HELA CELL LINE

Conc µg/ml	% of inhibition
6.25 µg	9.49
12.5 µg	22.43
25 µg	40.99
50 µg	58.98
75 µg	79.18
100 µg	87.68

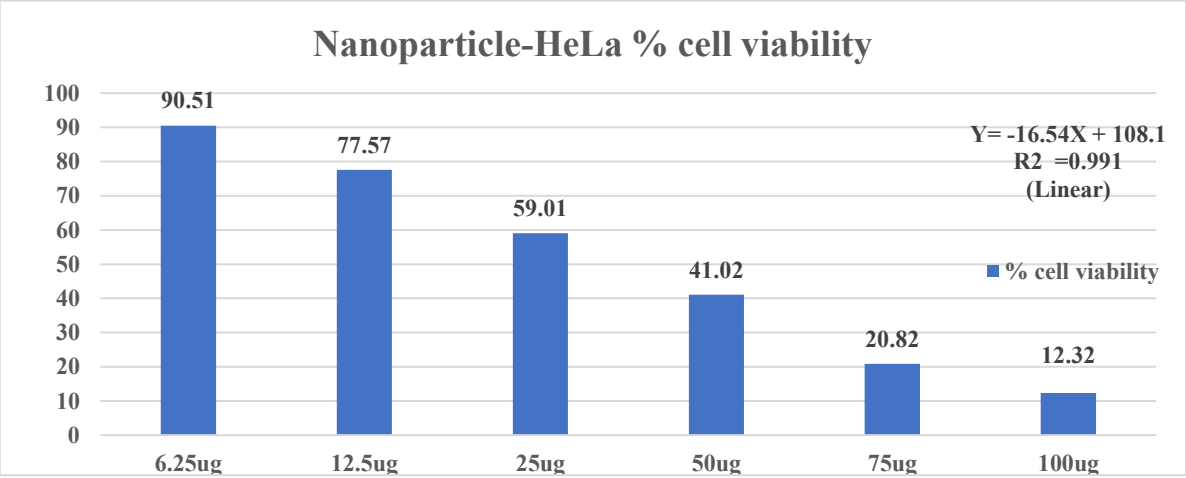


FIG 24: Graph Of Cell Viability

RESULT

- Nanoparticle was showed potent cytotoxic against *HeLa cells* after the treatment period of 24 h to 48 h.
- The hydroethanolic extract of *Hiptage benghalensis* exhibited 87.68% inhibition against the *HeLa cell* lines at a concentration of 100 µg/mL

DISCUSSION

The current study's results clearly highlight the promising therapeutic potential of silver nanoparticles (AgNPs) generated from *Hiptage benghalensis* leaves as a cutting-edge method of cancer treatment [56]. The leaf extract's phytochemical study showed that it contained a large number of bioactive substances, including glycosides, flavonoids, and phenolic compounds. The strong anti-inflammatory, anti-cancer, and antioxidant qualities of these phytoconstituents are widely recognized. Strong free radical scavengers, phenolic chemicals and flavonoids in particular shield cells from oxidative damage and help slow the growth of tumors. These naturally occurring bioactive compounds serve as stabilizing and reducing agents in the green synthesis of silver nanoparticles, producing physiologically active and biocompatible nanoparticles.

When combined with silver nanoparticles, these phytochemicals seem to have a synergistic impact that increases their total biological activity. The produced AgNPs' surface area-to-volume ratio is greatly increased by their nanoscale size, which enhances their reactivity and ability to interact with cellular constituents. They are more effective than traditional medicinal drugs at penetrating cancer cells because of their tiny size, which enables efficient cellular uptake by endocytosis. Their increased ability to internalize is essential for enhancing their cytotoxic effects on cancerous cells.

By causing oxidative stress in cancer cells, the silver nanoparticles work mechanistically to inhibit cancer growth. Reactive oxygen species (ROS) production upsets the redox balance, resulting in DNA damage, mitochondrial malfunction, and apoptotic pathway activation. AgNPs can also disrupt important signaling pathways in cancer cells that are involved in

metastasis, survival, and cell proliferation. The nanoparticles limit unchecked cellular growth and promote programmed cell death by modifying pathways like p53, caspase activation, and other apoptosis-related proteins.

The ability of plant-mediated silver nanoparticles to lessen the negative side effects frequently connected to traditional chemotherapy is another important benefit. Conventional chemotherapeutic drugs can cause serious systemic toxicity because they frequently lack selectivity and harm healthy cells. On the other hand, because green-synthesized AgNPs have natural phytochemical coatings, they may have more targeted activity, better biocompatibility, and lower toxicity. Better treatment results and lower effective doses may be possible because to this specific mechanism.

All things considered, the study offers compelling proof of the potential of silver nanoparticles made from the leaves of *Hiptage benghalensis* as safe and efficient anticancer treatments. These results create new opportunities for more thorough research, such as clinical trials, toxicity profiles, in vivo studies, and molecular mechanism analysis. Such plant-based nanotherapeutics may play a major role in the future development of safer, more effective, and sustainable cancer treatment approaches with more investigation and validation.

CONCLUSION

The results of the current study showed that the leaf extract had strong antioxidant capability, as evidenced by tests for hydrogen peroxide scavenging, ferric reducing activity, and DPPH radical scavenging. Reactive oxygen species (ROS) overproduction leads to oxidative stress, which is a major factor in the etiology of cancer and other chronic illnesses. Consequently, finding antioxidants in plants is crucial to creating potent medicinal substances.

The leaf extract has a strong ability to scavenge free radicals in the DPPH assay, its ability to contribute electrons or hydrogen atoms to neutralize free radicals. The extract's significant reducing power, which indicates its potential to donate electrons and transform ferric ions (Fe^{3+}) into ferrous ions (Fe^{2+}), was further substantiated by the ferric reducing activity. Furthermore, the extract successfully neutralizes hydrogen peroxide, avoiding the production of extremely reactive hydroxyl radicals that could harm cellular components, according to the hydrogen peroxide scavenging assay.

The presence of bioactive phytoconstituents including flavonoids and polyphenols in the leaf extract may be the cause of the notable antioxidant activity seen. These substances' strong oxidative chain-breaking, metal-chelating, and free radical scavenging capabilities are well known. Their combined effect strengthens the extract's total antioxidant potential.

A strong basis for future research is provided by the significant overall inhibition seen at 100 µg/ml, which indicates 88% antioxidant activity. The development of leaf extracts in nanoparticle form may improve their bioavailability, stability, and targeted distribution, increasing the likelihood that they may be used in anticancer treatments in the future.

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