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Comprehensive Investigation Of *Couroupita Guianensis* Leaves Pharmacognostical, Phytochemical And Pharmacological Approaches For Colorectal Cancer

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Abstract

Couroupita guianensis Aubl., commonly known as the cannonball tree, has long-standing ethnomedicinal usage for treating infections, inflammation, and gastrointestinal disorders. This study provides a multidisciplinary evaluation—pharmacognostical, phytochemical, and pharmacological—of the plant's leaves, emphasizing its potential in colorectal cancer (CRC) therapeutics. Macroscopic and microscopic analyses established key diagnostic features, while physicochemical evaluations confirmed the quality and purity of the crude drug. Phytochemical screening revealed the presence of alkaloids, flavonoids, terpenoids, phenolics, and tannins, corroborated by quantitative estimations indicating high total phenolic (92.08 mg GAE/g) and flavonoid content (61.5 mg QE/g). TLC and HPTLC confirmed the presence of quercetin and other bioactive markers. The hydroalcoholic extract exhibited notable antioxidant activity (IC_{50} = 48.43 µg/mL) and significant in-vitro anticancer effects against HCT-116 colorectal carcinoma cells (IC_{50} = 109.5 µg/mL), along with dose-dependent anti-mutagenic activity in Salmonella typhimurium TA100 assays. These findings scientifically validate the traditional uses of C. guianensis leaves and highlight their promise as a source of natural agents for managing oxidative stress and colorectal cancer. This integrated approach supports their future application in standardized herbal formulations.

Keywords: *Couroupita guianensis*, Pharmacognosy, Phytochemicals, Colorectal cancer, Antioxidant activity.

Introduction

Global Trends and Market: Herbal medicine is enjoying rapid global growth and acceptance. An estimated 80% of the world's population uses herbal remedies in some form. Industry reports note the global herbal products market reached roughly $70–87 billion in 2023, with projected growth at ~20–21% CAGR into the 2030s. This surge is driven by rising consumer health awareness, preference for "natural" treatments, and integration of traditional systems (e.g. Ayurveda, Traditional Chinese Medicine) into mainstream wellness. Popular botanicals include *Curcuma longa* (turmeric) and *Withania somnifera* (ashwagandha), whose anti-inflammatory and adaptogenic properties have been validated in recent trials. Alongside dietary supplements, herbal products now span tablets, tinctures, teas and topical formulations. Analysts emphasize that leaves and roots of many plants are the fastest-growing segments. There is a growing body of rigorous research on herbal efficacy and mechanisms. Modern studies confirm many traditional claims: for example, curcumin from turmeric shows anti-inflammatory and anticancer activity in preclinical models, while ginsenosides from ginseng modulate immunity. Advances in phytochemistry have identified active compounds (alkaloids, flavonoids, terpenes etc.) and elucidated their targets. For instance, clinical and animal studies have shown certain Ayurvedic herbs can improve glycemic control, reduce oxidative stress, or alleviate chronic pain. Quality-control research (e.g. using DNA barcoding or HPLC) is enhancing standardization to ensure consistent potency. A 2023 review highlights that rigorous quality testing is now viewed as essential to ensure safety and consumer trust in herbal products. Integration of traditional knowledge with modern methods (e.g. network pharmacology, genomics) is leading to new discoveries, such as novel plant-derived compounds for neuroprotective and immunomodulatory use.

India, with its rich herbal tradition (Ayurveda, Siddha, Unani, etc.), is both a major consumer and producer of botanicals. The Indian herbal market alone was valued at about $4.6 billion (USD) in 2023 and is forecast to grow at an ~28% CAGR to exceed $26 billion by 2030. Ayurveda remains the dominant segment, reflecting strong domestic demand. Exports of Indian AYUSH and herbal products have risen steadily (e.g. reaching ~$651 million in 2023–24, up 3.6% from prior year). Multinational interest is evident (for example, India now supplies ~25% of U.S. demand for Ayurvedic/herbal supplements. Notably, demand for adaptogenic and immunity-boosting herbs (like ashwagandha, tulsi/holy basil, giloy) surged during the COVID-19 pandemic, reflecting consumers' interest in preventive health. In Tamil Nadu and other southern states, Siddha medicine (a traditional Tamil system) continues to flourish in clinics and research. Tamil Nadu hosts major institutions (e.g. National Institute of Siddha) and contributed a large share of India's Siddha research output. State initiatives have promoted herbal cultivation and clinical studies (for example, Siddha formulations were piloted in COVID care centers) to integrate these remedies into public health. Herbal medicine continues to draw on millennia of traditional knowledge even as it embraces modern science. Many plants long used in folk remedies are being re-examined in rigorous clinical studies. For example, formulations like "Triphala" (a three-herb blend) are under study for gastrointestinal health. In practice, clinicians sometimes integrate herbs with conventional care (e.g. using turmeric or ginger to complement anti-inflammatory therapy). Wellness tourism and personalized care trends also support tailored herbal use.

Research on multi-ingredient formulations and herbal synergy is expanding, reflecting the holistic ethos of systems like Ayurveda. While skepticism remains in some medical circles, the convergence of strong research data and high patient demand is fostering greater acceptance of integrative approaches.

The cannonball tree (*Couroupita guianensis*), native to South America but widely cultivated in India (where it is considered sacred), has a long ethnobotanical record. Traditionally, the leaves are used as a poultice or juice to treat skin infections, toothache (odontalgia), stomach aches and dysentery. South American shamans also used leaf extracts against protozoal infections. ecent pharmacological studies of leaf extracts support these uses. For example, methanolic and aqueous extracts of *C. guianensis* leaves significantly reduced diarrhea in rodent models, corroborating its traditional antidiarrheal use. Alcoholic leaf extracts have demonstrated strong analgesic and anti-inflammatory effects in experimental assays. Other reported activities of leaf compounds include antioxidant, antimicrobial and antinociceptive effects, likely due to bioactive constituents such as β-sitosterol, tannins, and indirubin derivatives. Modern research is also exploring anticancer and neuroprotective potential of *C. guianensis* phytochemicals. Overall, *C. guianensis* leaves are being validated as a rich source of medicinal compounds. Current ethnopharmacological reviews emphasize that more controlled studies are needed to translate these findings into clinical applications.

Colorectal cancer (CRC) is a major global health challenge. It is the third most common cancer worldwide (~10% of all cases) and the second leading cause of cancer deaths. In 2020 there were roughly 1.9–2.0 million new CRC cases and 0.93–1.0 million deaths globally. Incidence and mortality vary widely: rates are highest in developed regions (e.g. North America, Europe) due to diet and lifestyle factors, but numbers are rising fast in developing countries as populations adopt Western diets. By 2040 the burden is projected to grow by over 60% (to ~3.2M cases and 1.6M deaths per year) if no major interventions occur. In India, CRC is among the top cancers (around 64,800 new cases in 2022, fourth most common in both sexes). Urban areas show higher incidence than rural, reflecting lifestyle and dietary risk differences. Notably, CRC often strikes at older ages (predominantly >50 years), but younger-onset cases are increasing alarmingly in many countries.

The primary objective of this study is to scientifically investigate the pharmacognostical, phytochemical, and biological properties of the leaves of Couroupita guianensis, a traditionally valued medicinal plant known for its diverse therapeutic applications. To ensure the correct identification and standardization of the plant material, a comprehensive pharmacognostical evaluation will be conducted, beginning with a detailed morphological analysis of fresh leaves. This includes assessment of macroscopic features such as colour, odour, taste, shape, phyllotaxy (arrangement), apex, base, margin, length, and width. Further, microscopical studies following Wallis' (1965) standard methods will be performed on transverse sections of the leaf to characterize its internal tissue structures. Quantitative microscopy will include the analysis of stomatal number and index, vein-islet number, vein termination number, palisade ratio, and powder microscopy to support crude drug authentication and quality control.

The second objective focuses on phytochemical investigations, involving the preparation of hydroalcoholic leaf extract for the quantitative estimation of phytoconstituents, particularly total flavonoid content, which are known to contribute significantly to the plant's antioxidant and therapeutic potential. Additionally, chromatographic profiling using Thin Layer Chromatography (TLC) will be carried out to identify and compare the phytochemical fingerprints, aiding in compound profiling and potential bioactive molecule identification.

The third objective is to explore the biological activities of *Couroupita guianensis* leaf extract, especially its potential antioxidant and anticancer effects. The in-vitro antioxidant activity will be assessed using standardized free radical scavenging assays to determine its capacity to neutralize oxidative stress. To evaluate the plant's anticancer efficacy, in-vitro cytotoxicity studies will be performed against HCT-116 human colorectal carcinoma cell lines, a widely used model for colorectal cancer research. Furthermore, the anti-mutagenic activity of the extract will be investigated to assess its potential in preventing genetic mutations, thereby supporting its use as a chemopreventive agent.

Based on traditional usage and existing preliminary pharmacological reports, it is hypothesized that Couroupita guianensis leaf extract will exhibit significant pharmacognostic markers, high flavonoid content, and promising antioxidant, anticancer, and anti-mutagenic activities. These outcomes would scientifically validate the ethnomedicinal claims associated with this plant and may pave the way for the development of novel herbal therapeutics for oxidative stress-related disorders and colorectal cancer.

Materials and Methods

Drying of Leaf and pulverization

Following collection, the leaves underwent a drying process under shade. Once dried, they were finely powdered using a mixer, and the resulting powder was sieved through a No. 60 sieve. Subsequently, the sieved powder was stored in a tightly sealed container in a dry environment.

Pharmacognostical Studies

Pharmacognostic studies have always placed great emphasis on the morphological and micromorphological study of medicinal plants. An analysis of a plant's medicinal properties requires an understanding of its botanical identity. A researcher may discover a new compound or discover a plant's pharmacological properties. When a plant's botanical identity is uncertain or erratic, the entire study becomes invalid. Pharmacological investigations begin with identifying the crude drug's botanical identity. The researchers should have access to all diagnostic parameters of the plant they intend to study.

Morphological studies:

Fresh leaves are evaluated for their morphological characteristics such as colour, odour, taste, shape, arrangement, apex, base, margin, length, and width.

Microscopy Studies (Wallis TE, 1965) Specimen collection

To ensure that the plants were healthy and the organs were normal, care was taken in selecting them. Collected leaf and petiole specimens from a healthy plant by cutting the petioles. Immediately after cutting, the materials were placed in fixative fluid FAA (Formalin + Acetic acid + 70% ethanol - 90ml).

Transverse section

After preservation in fixative FAA for over 48 hours, the specimens were carefully sectioned into thin slices using a sharp blade. These sections were then stained with safranin and observed under a Nikon ECLIPSE E200 trinocular microscope equipped with a Zeiss AxioCam Erc5s digital camera, utilizing bright field illumination. Magnifications were determined and indicated using a scale bar for accurate measurement.

Microscopy quantitative

Based on standard procedures, the following quantitative microscopical studies were conducted on fresh leaves.

Vein termination number and vein islet number determination

Using chloral hydrate, a fresh leaf was cut between the midrib and margin, cleaned, and mounted on slides. On the paper, a 1mm square was drawn using a stage microscope, a camera lucida, and a stage micrometer. Following that, the sample slides were replaced with the stage micrometer, allowing the veins to be mapped on the square.

Stomatal index

Stomatal index represents the proportion of stomata to the total number of epidermal cells, with each stoma counted as one cell.

Stomatal index = S/S+E x 100

Where,

S = Stomata per Unit Area.

E = Number of epidermal cells per unit area.

Stomatal index determination

The stomatal number of the plant was measured at a high power (45X). We counted the epidermal cells and stomata. The formula above was used to calculate the stomatal index based on these values.

Palisade ratio determination

The chloral hydrate solution was used to boil an ice cube under a microscope. Tracing four epidermis cells was done with the camera lucida, drawing board, and 4mm objective. This meant that enough epidermal cells covered the epidermal trace for the palisade layer to be focused. An outline is formed by the intersection of

epidermal walls and palisade cells. The epidermal walls are surrounded by palisade cells. The palisade cell structure was observed under four epidermal cells.

Leaf powder preparation

After thoroughly washing the leaves with cold water, the water was drained completely, the leaves are dried under shade until they become moisture free, then they are powdered and passed through Sieve no:40. In order to conduct further studies, the sieved powder was collected and stored correctly.

Microscopy of Powder

Powdered sample was mixed with equal volumes of phloroglicinol and con.HCl, then mounted on a microscopic slide with glycerol. The characters were observed under bright field light with a Zeiss ERc5s digital camera and a Nikon ES- E200 trinocular microscope. A photomicrograph of diagnostic characters was taken and documented.

Physicochemical Parameters

The powder is tested for ash value, organic matter, and loss on drying (WHO guidelines 1996, 1998, 2001).

Detection of foreign organic material

The air-dried coarse drug was spread thinly. As much foreign organic matter as possible was manually separated and weighed from the sample drug, either by unaided eye or with a 6x lens. Based on drug weight, foreign organic matter was calculated.

Determination of ash values

As per the official method, air dried leaf powder was used to determine ash content.

Total ash: Leaf powder was accurately weighed. A fine layer of powder was spread in the bottom of the crucible and incinerated until it was carbon-free. To ensure a constant weight, it had to be weighed after cooling. Based on the air dried powder, an ash percentage was determined.

Water soluble ash: The insoluble matter from total ash is collected on ashless filter paper after it has been boiled in 25ml of water for 5 minutes. Following ignition, it was maintained at 450°C for 15 minutes. Total ash weight was reduced by subtracting insoluble matter. Ash that is water soluble is represented by the difference in weight. Based on air dried powder, we calculated water soluble ash content.

Acid insoluble ash: 25ml dilute hydrochloric acid was boiled with five grams of total ash. A tarred sintered glass crucible was used to collect the insoluble matter. Afterward, hot water was used to wash, dry, and weigh the residue. In air dried drugs, acid insoluble ash is calculated as a percentage.

Loss on Drying

A method described by Wallis was used to determine loss on drying. In a tarred petri dish that had previously been dried according to IP'96, powdered leaf was weighed accurately. Using gentle sideways shaking, the

powder was distributed as evenly as possible. For 1 hour, 100 - 105°C was used to dry the dish. After cooling in a desiccator, it was weighed again. Based on the amount of dried powder taken, a drying loss was calculated.

Extractive value determination

For drugs whose constituents cannot be readily determined by other methods, the extractive value of crude drug can be used as a measure. Further, these values provide an indication of the relative amounts and types of chemical constituents present in crude drugs. The chemical nature and properties of drugs make it necessary to use various solvents for determining extractives. Extraction liquids are capable of dissolving large quantities of the desired substance.

Petroleum ether soluble extractive value

In a closed flask, five grams of coarsely powdered drug were macerated for 24 hours in 100ml of petroleum ether. The mixture should be shaken frequently for the first six hours and allowed to stand for 18 hours. Following that, the petroleum ether was rapidly filtered to prevent loss. A tarred dish was used to evaporate the filtrate to dryness, dried, and weighed. Based on the air-dried powder, we calculated extractives soluble in petroleum ether. Additionally, extractive values were determined for ethanol, water, acetone, ethyl acetate soluble, chloroform, hydroalcohol, and diethyl ether soluble.

Phytochemical Studies

Plants serve as natural factories, synthesizing a myriad of organic compounds such as carbohydrates, proteins, lipids, flavonoids, glycosides, alkaloids, volatile oils, and tannins, which have been utilized for their physiological effects by humans since ancient times. The medicinal efficacy of a plant hinges on its chemical composition, often referred to as its active principle. In our current investigation, we focused on powdered leaves of Couroupit aguianensis, Abul., aiming to extract and identify its active constituents. Utilizing chromatographic techniques and various solvent systems, we isolated compounds and confirmed their presence through spectral studies. (Chaudhri, RD, 1999, Kokate, CK, 2005).

Extraction

Previously dried, powdered, sieved and stored crude drug of the leaves of *Couroupita guianensis* was taken. It was extracted with hydroalcohol (70% ethanol+ 30% water) by cold maceration. Prior to that the powdered leaf material was defatted by using the petroleum ether. For about 72 hours, the defatted content was macerated in hydroalcohol (70% ethanol + 30% water). A filter sheet No. 42 (125 mm) was used to remove all non- extractable matter, such as cellular materials and other constituents that cannot be extracted. With a reduced pressure buchi rotary evaporator, the entire extracts were concentrated to dryness. Sterile bottles were labeled with sterile samples and stored at appropriate temperatures.

Screening of phytochemicals at the preliminary stage

Many compounds found in plants are phytochemicals, which can be defined as chemicals derived from plants. An important tool for analyzing bioactive compounds is the simple, quick, and inexpensive phytochemical screening assay. This hydroalcoholic extract of Couroupita guianensis leaves was tested for polyphenols, glycosides, carbohydrates, carotenoids, proteins, tannins, amino acids, and steroids by using the standard procedures. (Junaid R Shaikh et al., 2020)

Alkaloids test

Mayer's test: A small quantity of the extract is treated with Mayer's reagent. Cream colour precipitate indicates the presence of alkaloids.

Wagner's test: A small quantity of extract is treated with Wagner's reagent. Reddish brown precipitate indicates the presence of alkaloids.

Hager's test: A small quantity of extract is treated with Hager's reagent. Yellow precipitate indicates the presence of alkaloids.

Test for carbohydrates

Molisch's test: The extract of the powdered drug is treated with 2-3 drops of 1% alcoholic alpha naphthol and 2 mL of concentrated sulphuric acid was added along the sides of the test tube. Formation of purple colour indicates the presence of carbohydrates.

Fehling's test: The extract of the powdered leaf is treated with Fehling's solution I and II and heated on a boiling water bath for half an hour. Formation of reddish brown precipitate indicates the presence of free reducing sugars.

Benedict's test: The extract of the powdered leaf is treated with equal volume of Benedict's reagent. A red precipitate formation indicates the presence of reducing sugar.

Anthraquinone glycoside test

1. Borntrager's test: The powdered leaf was boiled with dilute sulphuric acid, filtered and to the filtrate benzene was added and shaken well. The organic layer was separated and ammonia solution was added slowly. Pink colour observed in ammoniacal layer shows the presence of anthraquinone glycosides.

2. Modified borntrager's test: About 0.1gram of the powdered leaf was boiled for two minutes with dilute hydrochloric acid and few drops of ferric chloride solution was added, filtered while hot and cooled. The filtrate was then extracted with benzene and the benzene layer was separated. Equal volume of dilute ammonia solution was added to the benzene extract and shaken well. Pink colour was observed in ammoniacal layer indicates the presence of glycosides.

Cardioglycoside test

Keller killiani's test: About 1gram of the powdered leaf was boiled with 10ml of 70% alcohol for two minutes, cooled and filtered. To the filtrate 10ml of water and 5 drops of solution of lead sub acetate were added and filtered. The filtrate was then extracted with chloroform and the chloroform layer was separated and evaporated to dryness. The residue was dissolved in 3ml of glacial acetic acid containing a trace of

ferric chloride. To this 3ml of concentrated sulphuric acid was added along the sides of the test tube carefully. No reddish brown layer was observed indicating the absence of deoxysugars.

Legal's test: To the extract of the powdered drug, pyridine and alkaline sodium nitro prusside solution gives no pink to red colour.

Detection of cyanogenic glycosides

Small quantity of the powder is placed in a stoppered conical flask with just sufficient water, to cover it. A sodium picrate paper strip was inserted through the stopper so that it was suspended in the flask and it was set aside for 2 hours in a warm place. Brick red colour was produced on the paper indicating the presence of cyanogenetic glycosides.

Phytosterol test

The powdered leaf was first extracted with petroleum ether and evaporated. The residue obtained was dissolved in chloroform and tested for sterols.

Salkowski test: Few drops of concentrated sulphuric acid was added to the above solution, shaken well and set aside. The lower chloroform layer of the solution turned red in colour indicating the presence of sterols.

Libermann – burchard's test: To the chloroform solution few drops of acetic anhydride was added and mixed well. 1ml of concentrated sulphuric acid was added through the sides of the test tube and set aside for a while. At the junction of two layers a brown ring was formed. The upper layer turned green indicating the presence of sterols.

Test for saponins

Foam test: About 0.5gram of the powdered leaf was boiled gently for 2 minute with 20 ml of water and filtered while hot and allowed to cool. 5ml of the filtrate was then diluted with water and shaken vigorously. Frothing if produced indicates the presence of saponins.

Test for tannins

Ferric chloride test: A small quantity of the powdered drug was extracted with water. To the aqueous extract, few drops of ferric chloride solution was added. A bluish black colour if produced will indicate the presence of tannins.

Proteins and free amino acids should be tested

Millon's test: The acidulous extract of the powdered leaf was heated with Millon's reagent. A white precipitate turned red on heating will indicate the presence of proteins.

Biuret test: To the extract of the powdered leaf 1ml of dilute sodium hydroxide was added. Followed by this one drop of dilute copper sulphate solution was added. A violet colour will be obtained which indicates the presence of proteins.

Ninhydrin test: 1ml of the extract was treated with few drops of ninhydrin reagent. Appearance of purple colour shows the presence of amino acid.

Nitric acid test: To 2ml of extract, 3drops of nitric acid were added by the sides of thetest tube. Appearance of yellow colouration indicates the presence of proteins and free amino acids.

Mucilage test

Powdered leaf extract was mixed with ruthenium red solution. In the presence of mucilage, the colour red will appear.

Flavonoids test

Shinoda test: A little amount of the powdered leaf was heated with alcohol and filtered. To the alcoholic solution a few magnesium turnings and few drops of concentrated hydrochloric acid were added, and boiled for 5 minutes. Red colour formation indicating the presence of flavonoids.

Alkaline reagent test: To the alcoholic extract of the powdered leaf, few drop of sodium hydroxide solution was added. Yellow orange colour formation indicating the presence of flavonoids.

Lead acetate test: To the test solution add a mixture of 10 % lead acetate. It gives white precipitate.

Acid test: To the small quantity of test solution, few drops of concentrated sulphuric acid is added. Yellow orange colour is obtained indicates the presence of flavonoids.

Zinc hydrochloride test: To the alcoholic extract, mixture of zinc dust and concentrated Hydrochloric acid was added.

Terpenoids

After shaking the powdered leaf with petroleum ether, it was filtered. A small amount of chloroform was used to dissolve the residue after the filtrate was evaporated. An addition of tin and thionyl chloride was made to chloroform solution. The solution contained terpenoids, which gave it a pink hue.

Test for triterpenoids

1 ml of chloroform was combined with 1 ml of acetic anhydride and 2 ml of condensation before electrolysis. It is composed of sodium sulfate. Triterpenoids produce a reddish violet colour.

Test for fixed oil and fats

A small amount of the powdered leaf was pressed in between in the filter paper and the paper was heated in an oven at 1050C for 10 minutes. The formation of soap or partial neutralization of alkali indicates the presence of fixed oils.

Test for coumarins

3 ml of 10% NaOH was added to 2 ml of aqueous extract, formation of yellow colour indicates the presence of coumarins.

Test for gums

The small quantity of extract is added with few drops of alcohol to form white precipitate which indicates the presence of gum.

Quantitative Estimation of Phytoconstituents Preparation of hydroalcoholic extract

Couroupita guianensis leaf powder was macerated with 60 - 80 percent petroleum ether for 72 hours. Extract was filtered and evaporated into a residue, the defatted marc is extracted with hydroalcohol by maceration for 72 hours than this alcohol extract was filtered and evaporated and the yield was calculated.

Estimation of total flavonoid content

In all plant extracts, flavonoids were quantified using aluminum chloride, one of the most common methods for estimating flavonoids. Aluminumsulfonates are prepared using flavonoids and C3-C5 hydroxyl groups. It was estimated that the color intensity at wavelength 510nm is proportional to the amount of flavonoids (TahiraAkther et al., 2017).

Separate test tubes were used for extracts of 0.5ml and 1ml. We added 0.5ml of FCR and 1ml of sodium carbonate to this solution, then added distilled water to make up a final volume of 10ml. As soon as the mixture had stood for one hour, it was intermittently shaken. At 765nm, the absorbance was measured. With gallic acid as a standard, calibration curves were generated for a variety of concentrations (2, 4, 6, 8, 10µg/ml). As a blank, we used the reaction mixture without sample.An extract's phenolic content is expressed in milligrams of gallic acid equivalent (GAE).

Total tannin content

These experiments were conducted in calibration flasks and calibrated volumetric flasks with HAECGL solutions prepared as above (10µg/ml-50µg/ml) and standard solutions (tannic acid). Approximately 100 ml of 10% sodium carbonate solution should be added to each flask, along with 1 ml Folin-Denis reagent. In a 10 mL container, prepare distilled water solution. Using 775nm as a wavelength, we report the mean value and color of the complex after 30 minutes of tannic acid-free reaction. We graphed the linear correlation between concentration (X axis) and absorbance (Y axis) and calculated the slope (b), intercept (a), and coefficient correlation (r) from linear equation (Y=mx+c) by regression analysis. By using the calibration curve, the corresponding tannin concentration against the respective absorbance was determined

as tannic acid. In milligrams per gram, leaf extracts were expressed as tannin content.

Chromatographic studies

Thin layer chromatography (TLC)

Analysis using TLC, a method based on adsorption chromatography, was demonstrated by Stahl in 1958. A number of natural products may be analyzed qualitatively and quantitatively using it as a tool to separate, identify, and estimate different components.Adsorption is the basis for separation. Chromatographic plates are coated with thin layers of adsorbent to spot one or more compounds. Solvent flows through capillary action (against gravity) in the mobile phase. When a component has a higher affinity for a stationary phase, it travels slower than when it has a lower affinity. Components with a lower affinity for the stationary phase travel

faster. Thus, the components are separated. A finished chromatography provides information about how the separated substances migrate. The Rf value is shown below. Rf must be between 0.01 and 1.When investigating and cultivating medicinal plants, TLC can be exploited. There is the possibility of running many samples of extracts based on Rf = Solute travel distance / Solvent travel distance. Individuals of high performance can be identified, selected, and bred simultaneously. Chemical races can also be discovered using this technique. In separating, identifying and estimating different components, thin layer chromatography plays an important role. Compounds that are readily soluble but not strongly adsorbable move up with a mixture of components on a TLC plate, while compounds that are less soluble but highly adsorbable move up less readily (Raja et al., 2012).

Preparation of the Standard solution

1 mg of standard quercertin was dissolved with 1ml of ethanol. Then the volume is made upto 10 ml with ethanol (100 µg/ml – 0.1mg/ml).

Preparation of the Sample solution

A 100 mg extract was diluted with 1 ml of ethanol. Then the volume is made upto 10 ml with ethanol then 1ml is again diluted with ethanol to make upto 10ml (1000 µg/ml – 1mg/ml).

Steps involved in TLC

1. Preparation of adsorbent-coated plates
2. Activation of the adsorbent
3. The sample is applied to the chromatographic plate as spots or bands
4. Selection of the solvent system
5. Ultraviolet light for detecting agents

Procedure

- The required quantity of Silica gel G was weighed. With sufficient distilled water, a homogenous slurry was made.
- A uniform layer of silica gel was applied to TLC glass plates using spreading technique and layered to a thickness of 0.25 mm.
- Before TLC, coated plates were dried in the air and activated for an hour in a hot air oven at 100-105°C.
- Extracts were made in different test tubes with the respective solvents, such as ethanol and distilled water.
- With the help of capillary tubes, extracts were spotted on TLC plates, which were developed in TLC chambers previously saturated with different solvents.
- In a trial-and-error method, hydroalcoholic extracts isolated and resolved spots with Chloroform, Ethyl Acetate, Methanol, and Glacial Acetic Acid (9:8:2:1).
- Under UV light (254 nm), spots developed in various solvent systems were analyzed, and their Rf values were calculated.

Analysis of the Hydroalcoholicextract of Couroupita guianensis leaves (HAECGL) using high-performance thin layer chromatography (HPTLC)

An adaptation of thin layer chromatography that enhances versatility, separation efficiency, and detection limits, high performance thin layer chromatography (HPTLC) is a modern version of the technique. Plant extracts can be identified using HPLC since each species produces a unique chromatogram. For quality control, test samples and their standard chemical markers can be compared using chromatograms of different lots. The HPTLC method is reliable for quantifying nanograms even when they are present in

complex forms. It is possible to identify a drug, monitor its purity, detect adulterants, determine if the material is botanically derived, and identify constituents by HPLC fingerprint analysis (Mythili et al., 2013).

Instrument	: CAMAG make HPTLC with win CATS 1.4.3software
Stationary phase	: TLC plate silica gel 60 F 254 pre coated layer(10x5 cm)
Thickness	: 0.2mm
Mobile phase	: Chloroform : Ethyl acetate : Methanol : Glacial acetic acid (9:8:2.1)
Standard	: Quercetin
Sample	: Hydroalcoholic extract of Couroupit aguianensisleaves
Solubility	: Ethanol
Sample applicator	: Linomat 5
Standard concentration	: 100µg/ml
Sample concentration	: Hydroalcoholic extract of *C. guianensis*(1000 µg/ml)
Sample applied	: Sample and standard were applied as 8mm bands.
Track 1 and 2	: Standard (4µl and 8 µl)
Track 3 and 4	: Extract (4µl and 8 µl)
Development chamber	: Twin trough chamber (20cm x 10cm)
Development mode	: Ascending mode
Distance run	: 75m
Lamp	: D2 slit
Dimensions	: 4.00x0.30mm, Micro
Measurement mode	: Absorbance
Detection	: At 254 nm and at 366 nm using densitometry TLCscanner

Preparation of the plates

The plates used for HPTLC was silica gel 60 F 254 (10x5cm), thickness 0.2mm. 0.1mg/ml of the standard quercetin solution and 1 mg/ml of hydroalcoholic extract of Couroupita guianensis solution was applied. The mobile phase used was Chloroform : Ethyl acetate : Methanol : Glacial acetic acid (9:8:2.1).Then 4 µl and 8 µl of sample and standard were applied in the form of band (8mm) using LINOMAT 5 sample applicator in the pre coated plate. Then the chromatogram was developed for 15 mins, dried at room temperature and scanned at 254 nm and 366 nm in TLC densitometry scanner. Then the Rf value and the peak area of the standard and the extract were noted at 254 nm and 366 nm. The overly spectrum was

recorded.

Estimation of quercetin in hydroalcoholic extract of leaves of Couroupita guianensis

The area under the curve for the sample and standard were noted and the content of quarcetin was calculated using the formula. Amount of quercetin present in the sample were calculated a sample.

***In-vitro* antioxidant activity**

Hydrogen Peroxide Scavenging Assay

3.8 mL of 0.1 M phosphate buffer (pH 7.4) was prepared, and 1 mL of each test solution at different concentrations was added, then 0.2 mL hydrogen peroxide solution was added. As soon as the reaction has finished for 10 minutes, the absorbance is measured at 230 nm. A blank reaction mixture is used without any sample. It is also possible to prepare sample blanks without reagents. In the process of standardization, ascorbic acid is used.

Here is a formula for determining hydrogen peroxide inhibition percentage,

% inhibition = [(Control–Test) / Control] × 100

***In-vitro* Anticancer Study in HCT-116 Cell Lines**

The in-vitro anticancer activity of *Couroupita guianensis* leaf extract was evaluated using the HCT-116 human colorectal carcinoma cell line over a 7-day study period. The HCT-116 cells, obtained from the National Centre for Cell Science (NCCS), Pune, India, were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 100 units/mL penicillin, and 100 µg/mL streptomycin. The cells were maintained in a humidified incubator at 37°C with 5% CO_2 atmosphere to support optimal growth conditions. Initially, the cells were propagated in 75 cm^2 culture flasks containing 15 mL of DMEM, and upon reaching sufficient proliferation through multiple passages, they were seeded into 96-well microplates for experimental analysis.

For the cytotoxicity assay, HCT-116 cells were plated at a density of 1×10^4 cells per well in the 96-well plate format. After an initial 24-hour incubation period, the culture medium was replaced with 100 µL of medium containing various concentrations of the hydroalcoholic leaf extract (1, 5, 10, 20, 40, 80, 150, 300, 500, and 1000 µg/mL). Untreated cells were maintained under the same conditions and served as the negative control group. Following 72 hours of treatment, cell viability was assessed using the MTT assay, a colorimetric method that measures the metabolic activity of viable cells through mitochondrial dehydrogenase activity.

In this assay, MTT solution (5 mg/mL in PBS) was added to each well, and the plates were further incubated at 37°C in 5% CO_2 for 2–4 hours to allow the formation of purple formazan crystals. After incubation, the medium was discarded and the insoluble formazan crystals were dissolved in 150 µL of DMSO. The absorbance of each well was then measured at 570 nm using a microplate reader, providing a quantitative estimation of cell viability. The percentage of viable cells was calculated using the following formula:

% Viability = (Ab_1 / Ab_0) × 100

where Ab_1 is the absorbance of treated cells and Ab_0 is the absorbance of control (untreated) cells. This procedure enabled the assessment of dose-dependent cytotoxicity of the leaf extract, helping to establish its potential anticancer efficacy against human colorectal cancer cells.

Anti-mutagenic Assay

The anti-mutagenic potential of the hydroalcoholic extract of Couroupita guianensis leaves (HAECGL) was evaluated over a period of 7 days using the Salmonella typhimurium TA100 strain, a histidine-dependent mutant commonly employed in Ames test for detecting base-pair substitution mutations. The bacterial strain, which harbors the hisG46 mutation, was obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), CSIR-IMTECH, Chandigarh, India, and stored at –80°C until use. Prior to experimentation, the genotype was verified for its histidine auxotrophy as described by Tejs et al. (2008). The bacteria were cultured in nutrient broth medium under sterile conditions.

The study followed the plate incorporation method, a standard procedure for detecting mutagenic and anti-mutagenic properties. For each experiment, a frozen stock culture of S. typhimurium TA100 was revived and incubated in nutrient broth for 16 hours at 37°C to achieve optimal bacterial density (approximately 2–5 × 10^8 cells/mL). In sterile test tubes, 100 µL of the overnight bacterial culture, 50 µL of HAECGL at varying concentrations (1, 5, 10, and 20 µg/plate, dissolved in 15% DMSO), and 500 µL of phosphate buffer were added to 2 mL of top agar containing 0.5 mM L-histidine and 0.5 mM D-biotin. The entire mixture was poured onto pre-prepared minimal agar plates, which served as the nutrient-limiting medium to facilitate revertant colony formation.

The plates were then incubated at 36 ± 1°C for 36 hours, after which the number of histidine-independent revertant colonies (his^+ revertants) was counted. The results were expressed as colony-forming units per plate (CFU/plate) and recorded as mean ± standard deviation (SD) from triplicate trials conducted for each concentration. The positive control consisted of sodium azide (NaN_3) at a concentration of 1.0 µg/plate, which was freshly prepared in distilled water and used to induce mutagenicity. The negative control used 15% DMSO, which served as the solvent for the extract.

The anti-mutagenic effect of the extract was calculated using the following formula:

% Anti-mutagenicity = [(R - S_1) / (R - S_0)] × 100

Where:

R = Number of revertants/plate induced by the mutagen alone (NaN_3),

S_0 = Number of spontaneous revertants (without mutagen or extract),

S_1 = Number of revertants/plate in the presence of both extract and mutagen.

This assay helped to determine the potential of HAECGL to inhibit chemically induced mutations, thereby supporting its possible role as a natural chemopreventive agent.

Results & Discussion

Pharmacognostical studies

Macroscopic Characterization of *Couroupita guianensis* Leaf

The leaves of Couroupita guianensis Aubl., commonly known as the Cannonball tree, were collected and subjected to macroscopic examination to document key morphological features relevant for pharmacognostical identification. Fig. 1(A) represents the twig bearing multiple simple, oblong-lanceolate leaves arranged alternately. The leaves are clustered at the ends of branches, giving a whorled appearance. Each leaf blade is elongated with an entire margin, an acute apex, and a cuneate to attenuate base. The surface is glabrous and smooth to the touch. Fig. 1(B) displays the dorsal view of a mature leaf. The upper surface is dark green, glossy, and shows a prominent midrib with weakly visible lateral veins. The lamina is stiff, coriaceous, and shows no pubescence. Leaf length typically ranges from 15–20 cm, with a width of 5–8 cm. The midrib is raised and centrally aligned, suggesting reticulate venation. Fig. 1(C) illustrates the ventral view of the same leaf. The lower surface is slightly lighter in color with a more prominently visible venation pattern. Lateral veins diverge from the midrib at regular intervals, forming an intricate network, which is characteristic of dicotyledonous plants. Petioles are stout and measure around 2–4 cm in length, supporting the large leaf lamina. These macroscopic features serve as essential diagnostic tools for authenticating the plant material and differentiating Couroupita guianensis from closely related species. Accurate morphological identification ensures the integrity of pharmacological studies and herbal preparations derived from this species.

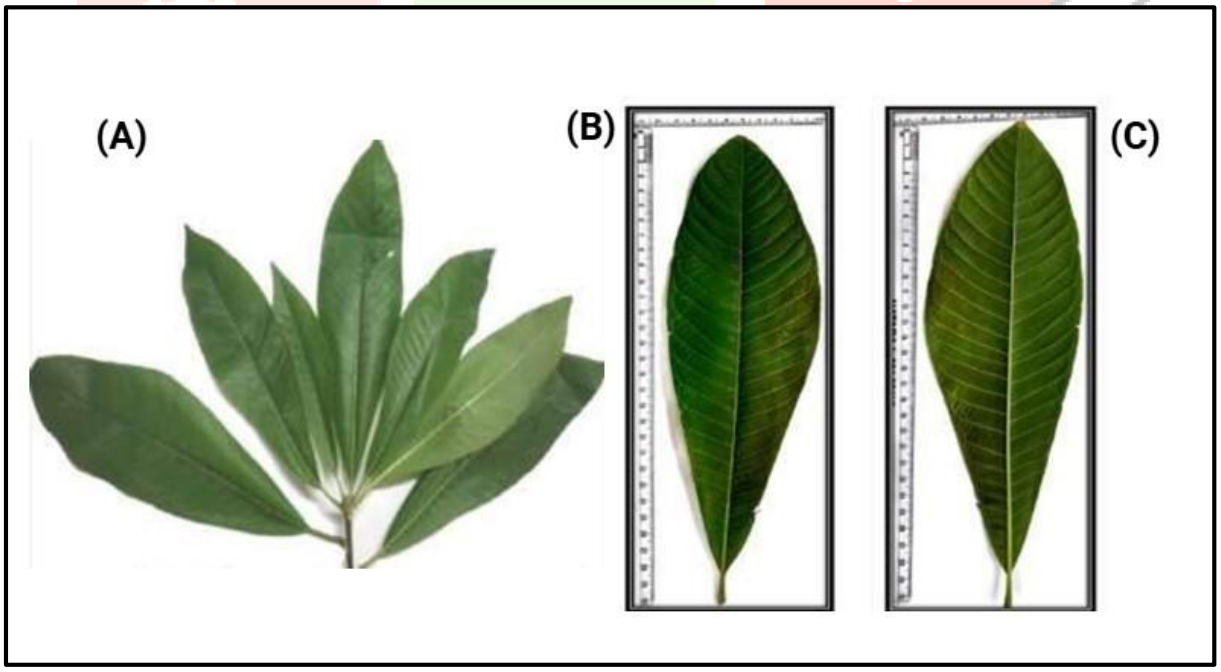

Fig.1. (A) Macroscopy twig, (B) and (C) Dorsal and ventral view.

Microscopic Examination of *Couroupita guianensis* Petiole

Transverse sections (TS) of the petiole of Couroupita guianensis were stained and analyzed to evaluate its internal anatomical features. The petiole exhibited a typical dicotyledonous structure with several noteworthy tissue arrangements, as depicted in Figure 2. Fig. 2(A) shows the upper side portion of the petiole under magnification. The epidermis (E) is single-layered, composed of compactly arranged cells covered with a thin cuticle. Beneath the epidermis lies the collenchymatous tissue (Col), providing mechanical support. Multiple vascular bundles of varying sizes are visible, scattered across the ground tissue. Fig. 2(B) presents the upper central portion of the petiole in an enlarged view. The centrally located vascular bundles (VB) are conjoint, collateral, and open, surrounded by a sclerenchymatous sheath. The phloem (Ph) lies external to the xylem (Xy), with distinct protoxylem and metaxylem vessels. Pericyclic fibers (Pcf) and resin canals (RC) are observed interspersed within the ground tissue. Fig. 2(C) illustrates the middle region of the petiole. Here, vascular bundles are more regularly spaced and encircled by parenchymatous ground tissue (GT). A continuous ring of sclerenchyma (Sc) is evident near the vascular tissues, and resin ducts (RD) are prominently seen. The presence of large parenchymatous cells suggests significant storage function in the petiole. Fig. 2(D) captures the lower portion of the petiole, highlighting the consistent arrangement of vascular bundles and ground tissue. The collenchymatous region continues to provide structural support, while scattered resin canals and calcium oxalate crystals (COC) can be identified in the parenchymatous ground tissue. The vascular bundles remain collateral and well developed, ensuring efficient transport. The transverse section study confirms the anatomical complexity of the petiole in Couroupita guianensis, which supports its identification and pharmacognostic standardization. The presence of resin canals, calcium oxalate crystals, and well-organized vascular bundles are characteristic anatomical markers.

S.No	Morphological characters	Observation
1	Color	Dark green
2	Odor	No odor
3	Taste	No taste
4	Surface	Coriaceous
5	Shape	Obvate-lanceolate
6	Length	14-25cm
7	Width	5-9cm
8	Apex	Acute
9	Base	Acute
10	Margin	Entire
11	Petiole	10-30mm long
12	Lateral nerves	alternate

Table.1. Macroscopical characters of Couroupita guianensis (leaf)

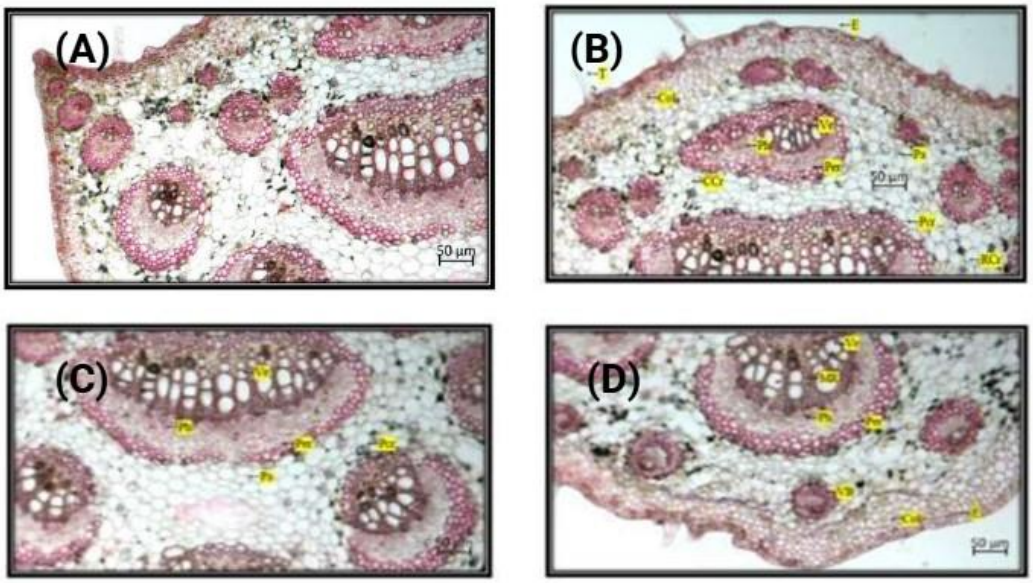

Fig.2. (A). TS of petiole - Upper side portion enlarged, (B). TS of petiole - Upper centre portion enlarged. (C). TS of petiole - Middle portion enlarged and (D). TS of petiole - Lower portion enlarged

Quantitative microscopy

Quantitative microscopic parameters provide valuable diagnostic features for pharmacognostical standardization of plant materials. The analysis of Couroupita guianensis leaves revealed the following data (Table 2), focusing on the upper and lower epidermis, stomatal characteristics, and venation pattern:

Parameters	**Upper epidermis**	**Lower epidermis**
Epidermal number	785-910	815-1005
Stomatal number	-	110-165
Stomatal index	-	11.99
Palisade ratio	2-5	
Vein islet number	3	
Vein termination number	8	

Table 2 : Quantitative microscopy of *Couroupita guianensis* leaf

The epidermal cell count was slightly higher on the lower epidermis compared to the upper, suggesting denser cellular arrangement. Stomata were absent on the upper surface and confined to the lower epidermis, classifying the leaf as hypostomatic. The stomatal number ranged from 110 to 165, with a calculated stomatal index of 11.99%, indicating moderate stomatal density. The palisade ratio, measured from transverse sections, ranged from 2 to 5, suggesting variation in palisade cell arrangement beneath each epidermal cell. Venation pattern analysis showed a vein islet number of 3 and a vein termination number of 8 per unit area. These parameters are crucial in confirming the identity of Couroupita guianensis leaves and aid in ensuring quality control for pharmacognostical and phytochemical studies.

Physico-Chemical Analysis of Couroupita guianensis Leaf Powder

Physico-chemical parameters are critical for the standardization and quality control of herbal drugs. The powdered leaves of Couroupita guianensis were subjected to various analyses, including ash content, moisture content (loss on drying), and extractive values using solvents of varying polarity. These values help assess the purity, identity, and chemical stability of the crude drug. The findings are summarized in Table 3.

S.No	Physico-chemical constants	Results
1.	Foreign organic matter	Nil
2	Loss on drying	3.45±0.10
3	Extractive value	
	Petrolium ether	2.3±0.11
	Chloroform	3.79±0.056
	Diethyl ether	1.8±0.172
	Aqueous	2.54±0.25
	Ethanolic	6.5±0.114
	Hydroalcoholic value	11.5±0.36
	Acetone	2.8±0.02
4	Ash value	
	Total ash	10.45±0.225
	Water soluble	4.08±0.170
	Acid Insoluble	0.59±0.001

Table 3: Physico-chemical constants of Couroupita guianensis leaf powder.

The absence of foreign organic matter confirms the purity of the drug sample. The loss on drying value (3.45%) indicates low moisture content, reducing the risk of microbial contamination. The ethanolic and hydroalcoholic extracts showed the highest extractive values (6.5% and 11.5% respectively), suggesting the presence of a high proportion of polar phytoconstituents. The total ash value was 10.45%, within acceptable limits, indicating minimal inorganic contamination. Water soluble and acid insoluble ash values further substantiate the quality and authenticity of the plant material.

Preliminary Phytochemical Screening of Couroupita guianensis Leaves

Phytochemical screening is a crucial step in identifying the broad classes of bioactive constituents present in medicinal plants. The hydroalcoholic extract of Couroupita guianensis leaves was subjected to standard qualitative tests to determine the presence or absence of various secondary metabolites. The results are summarized in Table 4.

S.No	Secondary constituents	Presence or absence
1	Alkaloids	Presence
2	Carbohydrates	Presence
3	Glycosides	Presence
3a	Cardiac glycosides	Absence
4	Sterols	Presence
5	Flavonoids	Presence
6	Proteins	Presence
7	Aminoacids	Presence
8	Terpenoids	Presence
8a	Triterpenoids	Presence
9	Gum	Absence
10	Mucilage	Absence
11	Saponins	Absence
12	Tannins	Presence
13	Fats and oils	Absence
14	Coumarins	Absence

Table 4: Preliminary phytochemical screening of hydroalcoholic extract of Couroupita guianensis leaves.

The extract tested positive for a wide range of phytoconstituents including alkaloids, carbohydrates, glycosides, sterols, flavonoids, proteins, amino acids, terpenoids (including triterpenoids), and tannins, all of which are known to exhibit various pharmacological activities. However, the extract was negative for cardiac glycosides, gums, mucilage, saponins, fats and oils, and coumarins. The presence of these constituents supports the traditional uses of Couroupita guianensis and provides a basis for further pharmacological and phytochemical investigations.

Quantitative Estimation of Total Phenolic Content

Phenolic compounds are important phytoconstituents contributing to the antioxidant potential of medicinal plants. The total phenolic content (TPC) of the hydroalcoholic extract of Couroupita guianensis leaves (HAECGL) was quantified using the Folin-Ciocalteu reagent method, with gallic acid as the standard. The absorbance values for varying concentrations of both gallic acid and the extract are presented in Table 5.

S.No	Concentration (Gallic acid and HAECGL)	Absorbance (Mean±SEM)	
		Gallic acid	HAECG
1	10	0.016±0.005	0.011±0.006
2	20	0.044±0.004	0.025±0.004
3	30	0.061±0.0038	0.054±0.007
4	40	0.088±0.008	0.081±0.008
5	50	0.119±0.008	0.106±0.004
		GAE	92.08mg/g

Table 5: Total phenolic content of HAECGL compared to standard gallic acid.

Using the standard calibration curve of gallic acid, the total phenolic content in the extract was found to be **92.08 mg GAE/g** of dry extract. This high phenolic content suggests that *Couroupita guianensis* leaves may possess strong antioxidant potential, supporting their traditional medicinal use.

Total Flavonoid Content (TFC)

The flavonoid content of the HAECGL was determined by the aluminum chloride colorimetric method using quercetin as a standard. Absorbance values were recorded at 415 nm for a series of standard quercetin solutions and extract concentrations. The results are presented in Table 6.

S.No	Concentration (Quercetin and HAECGL)	Absorbance (Mean±SEM)	
		Quercetin	HAECGL
1	10	0.074±0.0057	0.049±0.006
2	20	0.145±0.0032	0.087±0.004
3	30	0.198±0.0012	0.104±0.007
4	40	0.251±0.0011	0.175±0.008
5	50	0.311±0.0031	0.198±0.004
		QE	61.50mg/g

Using the linear regression from the quercetin standard curve, the total flavonoid content was calculated to be 61.50 mg quercetin equivalent (QE)/g of extract. This moderate level of flavonoids suggests potential antioxidant, anti-inflammatory, and other bioactive effects.

TLC profile

Thin-layer chromatography (TLC) emerges as a straightforward yet widely employed technique in the examination of secondary metabolites. In this study, TLC was conducted utilizing silica gel G as the stationary phase and a mobile phase composed of chloroform: ethylacetate : methanol: glacial acetic acid in the ratio of 9:8:2:1. The hydroalcoholic extract of *Couroupita guianensis* leaves (HAECGL) and quercetin served as the sample and standard, respectively. Subsequently, the Rf

(retention factor) value was computed, and the outcomes are delineated in Table 7.

Fig.3.: Thin layer chromatography of standard Quercetin and HAECGL

S.No	Mobile Phase	Sample	R_F value
1	Chloroform : ethyl acetate : methanol : glacial acetic acid (9:8:2:1)	Standard	0.55
2		Test	0.52

Table 7. TLC of Quercetin and HAECGL

Thin-layer chromatography (TLC) was performed on the hydroalcoholic extract of *Couroupita guianensis* leaves (HAECGL) using a solvent system comprising Chloroform: Ethylacetate : Methanol : Glacial acetic acid (9:8:2:1). The TLC analysis of HAECGL conclusively revealed the presence of quercetin.

HPTLC Profile

High-performance thin-layer chromatography (HPTLC) was conducted for the hydroalcoholic extract of *Couroupita guianensis* leaves (HAECGL). Subsequently, the Rf value and peak area of both the standard and the extract were recorded at wavelengths of 254 nm and 366 nm. An overlay spectrum was generated by superimposing the peak of standard quercetin with the corresponding peak of the sample.

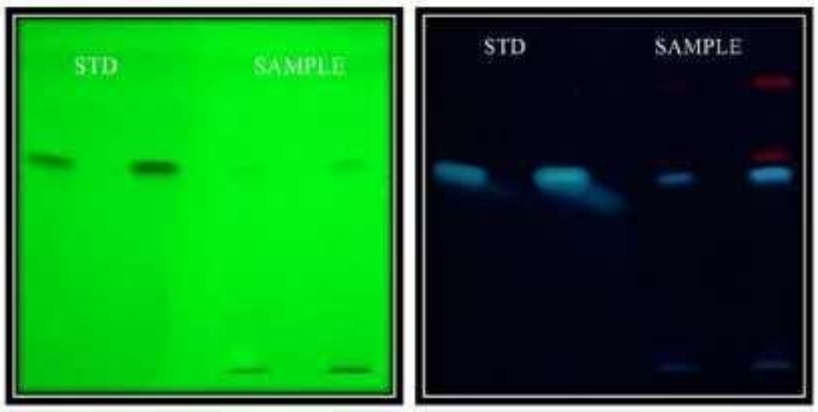

Fig.4. Detection of standard and sample at different wavelength (254 nm and 366nm)

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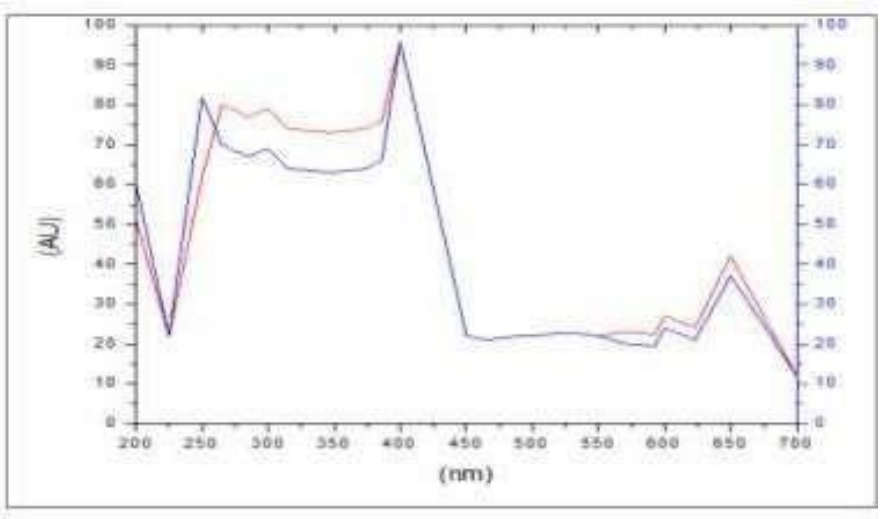

Fig.5. Overly spectrum for Quercetin and HAECGL Screening at 254 nm

Table 8 : Rf value at 254nm

S.No	HAECG		Quercetin	
	Rf value	Area	Rf value	Area
1	0.21	6155.14	-	-
2	0.52	8345.75	0.55	13449.11

Screening at 366 nm Table: Rf value at 366nm

S.No	HAECG		Quercetin	
	Rf value	Area	Rf value	Area
1	0.22	1241.33	-	-
2	0.29	1575.44	-	-
3	0.30	1831.22	-	
4	0.52	2451.33	0.55	
5	0.62	2841.12	-	-
6	0.81	3791.11	-	-

The quantity of quercetin contained in the sample was determined by comparing the areas of the standard peak and the corresponding sample peak relative to the concentrations of the standard and the sample. Through this calculation, the quercetin content in the hydroalcoholic extract of *Couroupita guianensis* leaves (HAECGL) was determined to range from 4.51 mg/g to 5.14 mg/g.

In vitro Antioxidant Activity

The antioxidant potential of the hydroalcoholic extract of Couroupita guianensis leaves (HAECGL) was evaluated using the hydrogen peroxide (H_2O_2) scavenging assay, with ascorbic acid serving as the standard reference. This assay is a well-established method to determine the ability of bioactive compounds to neutralize H_2O_2, a reactive oxygen species that can contribute to oxidative stress and cellular damage. The extract demonstrated a concentration-dependent increase in hydrogen peroxide scavenging activity. The percentage inhibition values at different concentrations (20–100 µg/mL) are presented in Table 9.

S.No	Concentration of Ascorbic acid and HAECGL	% inhibition of Ascorbic acid	% inhibition of HAECGL
1	20	10.6±2.87	11.08±3.847
2	40	33.04±1.222	34.14±3.054
3	60	57.54±2.151	54.77±2.085
4	80	75.04±2.321	66.34±2.047
5	100	91.88±1.458	74.35±1.44
	IC_{50}	**45.94 µg/ml**	**48.43 µg/ml**

Table 10: Hydrogen peroxide scavenging activity of HAECGL compared with standard ascorbic acid.

The IC_{50} value—the concentration required to inhibit 50% of hydrogen peroxide—was calculated for both the extract and the standard. HAECGL showed an IC_{50} of 48.43 µg/mL, which was only slightly higher than that of ascorbic acid (45.94 µg/mL), indicating significant antioxidant potential. These results suggest that the hydroalcoholic leaf extract of Couroupita guianensis possesses potent free radical scavenging ability, likely due to its rich content of polyphenolic and flavonoid compounds, as previously confirmed in the phytochemical analysis. The ability of the extract to neutralize hydrogen peroxide indicates its potential role in reducing oxidative stress and preventing oxidative damage, which is associated with aging and several chronic diseases. The antioxidant property of HAECGL supports its traditional use in herbal medicine and encourages further investigation into its therapeutic potential in oxidative stress-related disorders.

In vitro Anticancer Activity

The cytotoxic potential of the hydroalcoholic extract of Couroupita guianensis leaves (HAECGL)

was evaluated against human colorectal carcinoma HCT-116 cell lines using the MTT assay. This colorimetric assay measures mitochondrial activity as an indicator of cell viability, proliferation, and cytotoxicity. The extract was tested at concentrations ranging from 1 to 1000 µg/mL, and the percentage of viable cells was determined after 24 hours of treatment. The results, summarized in Table 10, show a concentration-dependent decrease in cell viability.

Concentration in µg per ml	% Cell viability
1	99.117±2.081
5	98.054±2.74
10	95.151±2.77
20	90.47±2.81
40	82.11±2.86
80	64.24±1.85
150	40.74±1.65
300	12.22±0.88
500	6.41±0.69
1000	1.05±0.108
IC_{50}	**109.5 µg/ml**

Table 11: Percentage of HCT-116 cell viability after treatment with varying concentrations of HAECGL.

The IC_{50} value—the concentration at which 50% of the cells were inhibited—was found to be 109.5 µg/mL, indicating moderate cytotoxicity. The decrease in cell viability at higher concentrations was statistically significant and suggests dose-dependent antiproliferative activity. The anticancer potential of HAECGL may be attributed to its rich content of bioactive phytoconstituents, including flavonoids, phenolics, and terpenoids, which are known for inducing apoptosis, disrupting cancer cell metabolism, and inhibiting tumor growth. These findings support the ethnomedicinal use of Couroupita guianensis and warrant further investigation into its mechanism of action, as well as potential synergistic effects with existing chemotherapeutic agents.

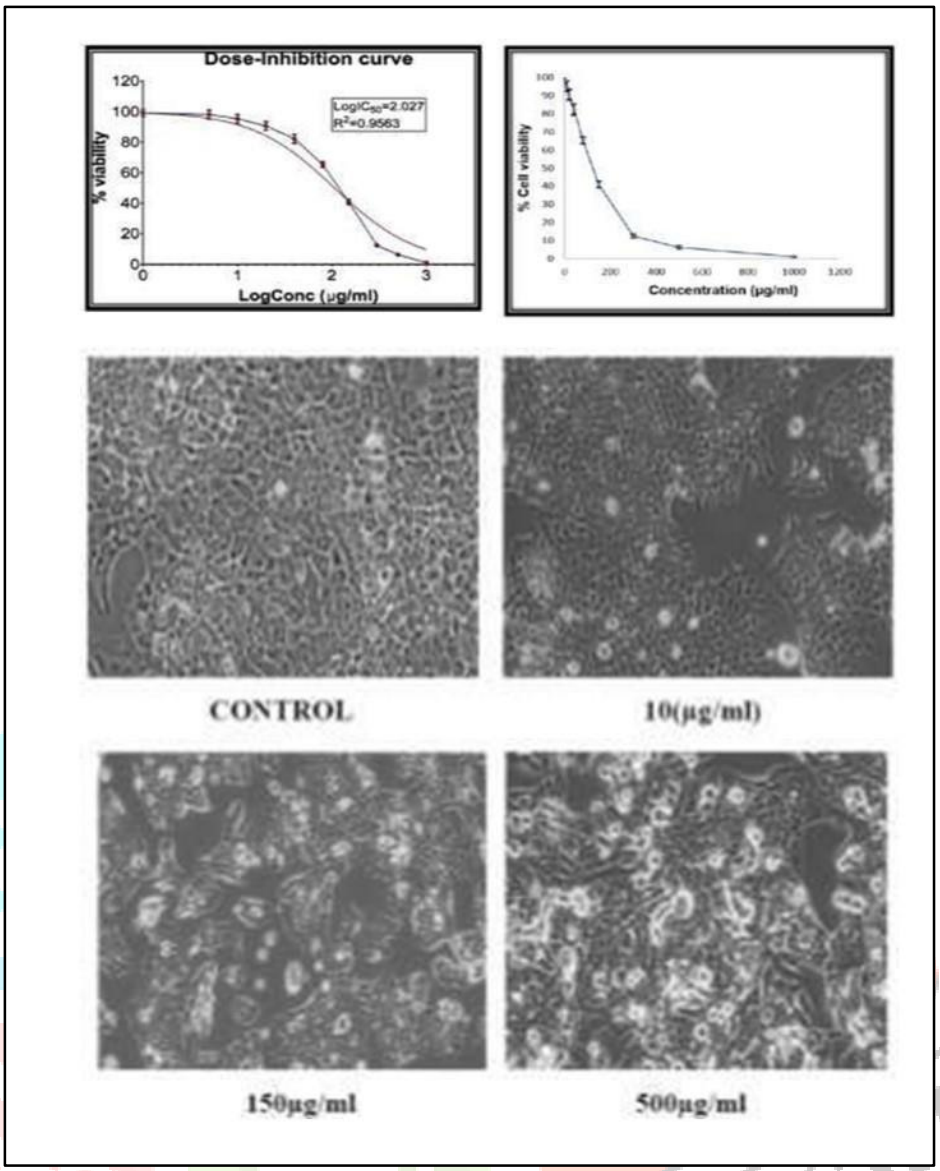

Fig.6. Effect of Control and HAECGL on HCT -116 Cell lines

Anti-mutagenic activity of HAECGL

The antimutagenic potency of the HAECGL was studied using TA 100 tester histidine-dependent strains of *Salmonella entrica typhimurium* against induced mutagenicity of sodium azide (NaN3). The results were shown in the Table.11

S.No	Group	S.typhimurium TA 100 (Mean ± SD)	
		Reverent colonies(CFU/Plate)	% Inhibition
1	Control (15%DMSO)	44.3±2.3	-
2	Test (1µg/ml)	48.6±3.4	-
3	Test (5µg/ml)	51.4±3.6	-
4	Test (10µg/ml)	46.4±2.1	-
5	Test (20µg/ml)	45.1±3.1	-
6	NaN_3 (1µg/plate)	432.4±40.1**	-
7	Test (1µg/ml) + NaN_3	396.4±30.5*[a]	8.1±0.7
8	Test (5µg/ml) + NaN_3	355.4±29.4*[a]	21.04±1.3
9	Test (10µg/ml) + NaN_3	311.1±27.1**[a]	28.4±1.4
10	Test (20µg/ml) + NaN_3	281.4±24.4**[a]	37.4±2.6

Table 11: % Inhibition of HAECGL on non-mutated and NaN3 mutation induced strains of *S.typhimurium*

Discussion

The comprehensive analysis of Couroupita guianensis leaves in this study highlights their rich phytochemical composition, notably the presence of phenolics, flavonoids, and tannins, which are well-known for their antioxidant and cytotoxic effects (1-10). These bioactive compounds likely underpin the observed pharmacological activities, such as significant antioxidant capacity and antimutagenic potential, reinforcing the therapeutic value of this plant (11-18). The extraction with hydroalcoholic solvents yielded higher extractive values, suggesting that such solvents are more efficient in isolating a broad spectrum of phytochemicals, which is consistent with findings in similar medicinal plants (19-27). Microscopical characterization offers valuable diagnostic features, essential for the correct botanical identification and quality control of the leaf material, which is critical for ensuring reproducibility in herbal drug development (28). The demonstrated antioxidant and antimutagenic activities align with previous ethnomedicinal uses of C. guianensis, but this study further quantifies these effects, thus providing a scientific basis for its traditional use. This study is among the first to integrate detailed microscopical analysis with phytochemical profiling and pharmacological evaluation specifically focused on Couroupita guianensis leaves. By correlating these aspects, the research not only confirms the therapeutic potential of the leaves but also establishes reliable microscopic markers that can support future pharmacognostic studies and quality assurance in herbal formulations.

Conclusion

The study establishes that Couroupita guianensis leaves possess a rich array of bioactive phytochemicals, notably phenolics, flavonoids, and tannins, which contribute significantly to their antioxidant and antimutagenic activities. The high extractive yield from hydroalcoholic solvents and detailed microscopical features provide essential parameters for plant authentication and quality control. These findings substantiate the medicinal value of the leaves and their potential application in developing natural antioxidant and cytotoxic agents. The novelty of this research lies in its combined pharmacognostic and pharmacological approach, offering new insights into the bioactive potential and microscopical identification of C. guianensis leaves, thereby laying a foundation for their standardized use in herbal medicine.

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