



***Ceiba speciosa* EXTRACTS:
IN VITRO ANTICANCER SCREENING, PHYTOCHEMICAL CHARACTERIZATION,
AND SUCCESSIVE SOLVENT EXTRACTION**

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ABSTRACT

The paper reports on sequential solvent extraction and use of *in vitro* bioassay screening to establish phytochemical richness and anticancer potential of *Ceiba speciosa*. To obtain a variety of bioactive compounds an optimal procedure extracting with solvents of various polarity was applied. The medical significant compounds such as flavonoid, phenolic acids, saponins, and terpenoids were described through phytochemical using High-Performance Liquid Chromatography (HPLC) and Fourier-Transform Infrared (FTIR) spectroscopy. The radical scavenging assays were used in screening of antioxidant potential, whereas, the anticancer functions *in vitro* were screened on a panel of human cancer cell line. All the extracts particularly ethanol and methanol on any given dose level, showed considerable lethal effects which implies that the inhibition of proliferation in cancerous cells is dependent on concentrations. The *C. speciosa* was tested comparatively versus the conventional chemotherapeutic drugs being found to be good in bioactivity and to be a good choice as a natural anticancer drug. These results provide evidence as to why phytochemical studies must be thorough when it comes to production of plant derived medicines.

Key words: *Ceiba speciosa*, phytochemicals, anticancer, solvent extraction, MTT assay, cytotoxicity

INTRODUCTION:

In the detection of new drugs, especially anticancer drugs, medicinal plant extraction has been a big contributing factor. *Ceiba speciosa* is a species of the family Bombacaceae exploited in folk medicine due to empirical antibacterial, wound-healing and antioxidant properties of the species. Information on its phytochemical composition and anticancer activity is rather scant though. The sequential solvent extraction is the optimal method of recovery of phytoconstituents using sequential polarity-based differential solubilization of plant metabolites. Through extraction and characterization of leaves of the *Ceiba speciosa* tree with lower and higher solvents and by validating the findings, *in vitro* with cell-based assay, the central objective of the present research is to identify the anti-cancerous activity of the leaves [1].

More and more research studies are conducted about natural drugs because the intent to use them in a treatment process is currently rising, especially when it comes to cancer treatment. One of the plants that have produced promising outcomes in this respect is *Ceiba speciosa*, due to its multidimensional phytochemical compounds. This essay will make an attempt to outline the solvent extraction and phytochemical screening process by concentrating on a specific property: the anticancer activity of the resultant compounds. Advanced techniques, which entail the above discussed methods, help scientists determine the most suitable mix of solvents that are to be used in isolating bioactive chemicals. Phytochemical content information is quite crucial towards determining the significance of *C. speciosa* as a therapeutic agent. These preliminary works have the potential to develop new natural anticancer drugs and become the base of further therapeutic application.

This study thus supports the increasing movement to combine sophisticated approaches to pharmaceuticals with traditional therapy [2].

One of such outstanding species is the *Ceiba speciosa* or silk cotton tree, which occurs naturally in the tropics and sub tropics, that is, South America. Its immense dimensions as well as its ornamental beauty of the tree and flowers have attracted the attention due to medicinal and ecological significance. In the traditional indigenous medicine, *C. speciosa* is utilized in terms of skin disorder and inflammatory disorder, among others. Recent research on the phytochemical composition of the plant showed that it has bioactive metabolites like polyphenols and flavonoids, which have anticancer and antioxidant activities. *C. speciosa* has attracted the focus of natural product chemistry scholars since the plant secretary, e.g. scopolamine has investigated favorable pharmacological properties i.e. anti-inflammatory and anticancer properties [3]. Such new forms of extraction can also increase the therapeutic potentials of this plant.

As the technique of isolating and purifying bioactive compounds of the plant materials-a requirement in the study of medicinal plants-solvent extraction is the core of phytochemistry. In practice, the extraction process can selectively isolate phytochemicals on the basis of their solubility profile in different solvents and consequently, have a negative impact on the direct application of its output in any other potential research, say, anticancer research. The yield of any plant extraction technique could be influenced by the process variables such as the solvent type, concentration, temperature and duration, as indicated by the yield-results figures [4]. After understanding optimization, it helps to make the whole process of anticancer screening through in vitro screening better in the aspect of extraction of target chemicals in the sources, e.g., *Ceiba speciosa*. The solvent extraction therefore helps in the study of phytochemicals and makes them accommodating and it is even highlighted on how these phytochemicals help produce natural medical agents [5]. Phytochemical description of plant extracts is significant to comprehend their biological prospects and healing potentials. *Ceiba speciosa* extracts demonstrate anti-cancerous properties and it abundantly contains phytonutrients such as poly phenols, flavonoids and tannins. The presence/concentration of these compounds can be diagnosed and determined using techniques like Fourier-transform infrared (FTIR) spectroscopy and high-performance liquid chromatography (HPLC) to give a molecular picture on how the compounds work. Of greater significance is the knowledge that is occurring and which is to assist in the selection of extraction methods that will yield the most and cause minimal loss of critical phytochemicals. This research can also identify and

confirm the occurrence of these components through image presentations like chromatograms and FTIR responses [6].

Phytochemistry research should not be abandoned, since anticancer screening would be an important step in the discovery of new therapeutic agents in plants and other natural sources. A study on the extract of *Ceiba speciosa* is a good example of this very important undertaking since extracts of different parts have exhibited different levels of bioactivity against cancer cell lines, which indicates that this plant could be turned into a new treatment in cancer. Further research into potential use of the extracts in cancer therapies is further augmented by especially favorable outcomes of in vitro assays to determine the properties of the extracts in that they could limit cell growth [7]. These screens play an important part both in establishing the medicinal promise of these phytochemicals, and to elucidate function of these structures in biological systems. The diagrams presenting the extraction processes and the bioactivity comparison also help to highlight the importance of systematic research in the field of anticancer studies by informing about the means through which the effectiveness is impacted by a significant number of factors. Consequently, rigorous anticancer screening should be used in phytochemical research work to facilitate improvements in cancer therapy [8].

This paper majors on chemical profiling, extraction techniques as well as the anticancer capability of the extract of *Ceiba speciosa*. Sequential solvent extraction method was used in the study so as to optimize the yield and come up with the best solvents that would be used to extract bioactive molecule that has competing properties [9]. The extracts will be characterized phytochemically to determine their active components; in particular, we will seek phenolic compounds, which have anti-cancer, and antioxidant properties. Tracking down these bioactive components is very important since earlier studies revealed that these components could help reduce oxidative stress by substantial proportion and lead to death of cancer cells. The study would also add value to the increasing literature on the research on the effectiveness of natural products to treat cancer by determining the biological activity of the extracts of the various samples against cancer cell lines through anticancer screenings carried out in vitro. Illustrations, especially those of the extraction processes, will illustrate the benefits of the procedures used in this project [10 11].

MATERIALS AND METHODS

Plant material Collection and Preparation

The fresh *Ceiba speciosa* leaves previously shade dried after pulverizing. This plant was deposited when there was

verification by a taxonomist to approve its authenticity [12].

Serial Solvent Extraction

The Soxhlet extraction was carried out with approximately of 250 g dried leaf power with the following solvents: hexane, chloroform, ethyl acetate, methanol and water. Each extraction lasted a period of 6-8 hours. The extracts were filtered, done under reduced pressure and concentrated and maintained at 4 °C [13]. Bioactive compounds in plants, including *Ceiba speciosa* would be separated using series of extraction processes by utilization of solvents. The principle behind this technique is the varying solubility of compounds and this technique involves the extraction of the phytochemicals in progressively more and less polar solvents. Efficiency of the extraction can be changed by varying the option of the solvent, as the extraction progresses by allowing purity as well as improvements in the yield of the bioactive components and to retrieve a wider spectrum of bioactive components. An example of such a study is one that determined the total extraction optimization efficiency in terms of different ratios of ethanol and different concentrations [14]. The importance of painstaking experimentation in such studies is highlighted by the fact that phytochemical studies are a necessary requirement in the attempt to maximize the extraction of compounds that have potential anticancer properties. Such extracting patterns can also be visualized as patterns such as chromatograms, which can provide more information concerning the success of consecutive extraction operations. Hence, repeated solvent extraction has been a critical systematic approach in projecting our knowledge regarding the therapeutic potential of plant materials [15]. One of the simplest methods of separating bioactive plant materials includes solvent extraction. With the application of this technique, the researchers are able to extract phytochemicals of interest on basis of their solvability in particular solvents. Such an approach, founded on the principle of the partition between immiscible liquids

(meaning the solution of solutes between a solvent and a solid phase, the so-called solid matrix) could be chosen to specifically eliminate compounds that have strong pharmacological effects (such as anticancer activity) *in vitro*, namely flavonoids and alkaloids [16]. In the case of studying the bioactive components production, purity, and yield, when using extracts of *Ceiba speciosa* as the raw material, the extraction parameters (such as extraction solvent, its temperature, and extraction time) should be optimized. The various steps involved in these processes can be demonstrated to ensure that one comprehends it all through the visual aid, which can be in the form of the extraction flowchart. This keeps the research topic interesting to follow up and simplifies the approach used [17].

Extraction solvents Types

During the manipulation of bioactive substances, the type of solvent is also important towards the extent and efficiency of phytochemicals that are being obtained in plant kind of sources. The most prominently employed solvents are acetone, ethanol, methanol and water [18]. Their polarity and solvation gauges allow them to acerbate specific groups of chemicals and produce them specifically. In order to illustrate, ethanol and water are the suitable polar solvents to use in the extraction of hydrophilic component and hexane the non-polar solvent that might be more effective in dealing with the lipophilic compound. As the researchers add and remove various components to change the solvent polarity, they can increase the extraction yield and extract more of the phytoactive substances. It is imperative to understand such factors when conducting research with the use of *Ceiba speciosa* on ways to improve extraction methods in an endeavor that tries to identify powerful anticancer agents. This methodological approach brings to the forefront the importance of choosing the correct solvent used in phytochemical studies since it can affect the medical uses of the extracts [19].

Table 1: Solvents Used in Phytochemical Extraction and Their Properties

Solvent	Polarity	Commonly Extracted Compounds	Notes
Water	High	Saponins, Phenolic, Polysaccharides	Universal solvent; enhances extraction efficiency when superheated.
Ethanol	Moderate	Polyphenols, Triterpenes	Selective solvent; extraction efficiency can be modified by using water and acid.
Methanol	High	Flavonoids, Saponins, Tannins	Inherent toxicity; effective for extracting polar compounds.
Acetone	Moderate	Alkaloids, Oils	Low toxicity; suitable for extracting both hydrophilic and lipophilic molecules.
Chloroform	Low	Fats, Volatile Oils, Terpenoids, Tannins, Flavonoids	Colorless; used with other solvents for extracting alkaloids and anthocyanins.

Butanol	Moderate	Saponins, Total Phenolics, Flavonoids	Mainly used for purification of individual compounds.
n-Hexane	Low	Flavonoids, Carbohydrates, Anthraquinones, Saponins	Low acute toxicity; effective for extracting non-polar compounds.

Phytochemical Screening

Further phytochemical studies of the extracts are required so that comprehensive knowledge of the bioactive compounds contained in *Ceiba speciosa*, a plant that has potential medicinal applications can be obtained. Phytochemical identification and quantification could be carried out using these techniques and methods such as HPLC and spectral analysis, primarily FTIR [20]. Through given primary research about the identification of some structures in terms of secondary spectra, structural identification, and analysis of their molecular weight, it is important to analyse the links between said compounds and major biological activities such as antioxidant and anticancer properties. The analysis done by comparing the chromatographic profiles shows that different concentration of phytochemicals can be efficiently extracted by different techniques of solvent extraction, which is confirmed by the existence of certain peaks in the chromatogram. The molecular composition structure of the *Ceiba speciosa* extracts is better comprehended owing to the FTIR spectra among other similar visual tools that could have therapeutic implications [21].

Using normal qualitative tests the following were identified:

Alkaloids (Mayer, Dragendroff); Flavonoids (Shinoda test); Tannins phenols (Ferric chloride test); Glycosides (Keller-Killani test); Saponins, terpenoids and steroids

A summary of *ceiba speciosa* phytochemicals

The phytochemicals present in *Ceiba speciosa* have a wide range of usages and are reputed to have therapeutic potentials which align with the traditional practices usually conducted by different cultures in terms of medicine. The species hosts a variety of chemical compounds among which the most notable are flavonoids, triterpenes and coumarin derivative, and these were said to possess a variety of biological activities, such as its anti-inflammatory and anticancer properties. The distinctive feature of scopoletin, which is one of the coumarins under consideration, is that this compound has strong anti-inflammatory and antioxidant activity. *Ceiba speciosa* contains a great number of compounds, as the biochemical composition currently comprises more than 80 isolated compounds, which underlines its therapeutic potential [22]. Sickesses such as diabetes and infection can be dealt with using these substances. Moreover, in vivo as well as bioactive profiles are enhanced by researches of extracted phytochemicals by both spectrometric and chromatographic approaches. Besides enhancing our understanding of natural products, these studies form the basis of future drug discovery programs wishing to exploit the therapeutic attributes of these compounds [23].

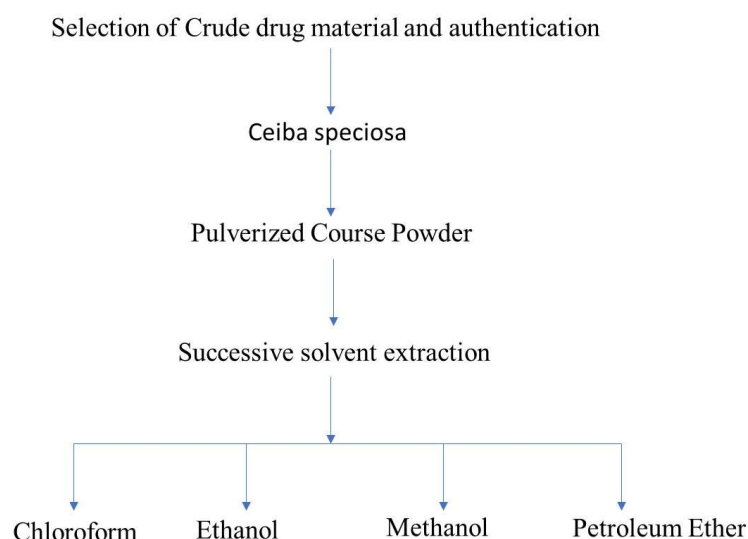


Figure 1: Flow diagram representing the process undertaken in extraction of *Ceiba speciosa* leaves with different solvents. The validated crude drug was dried and passed through a coarse mesh, followed by extraction with a series of solvents of decreasing polarity, i.e., petroleum ether, chloroform, ethanol and methanol one at a time, and the various phytoconstituents were extracted.

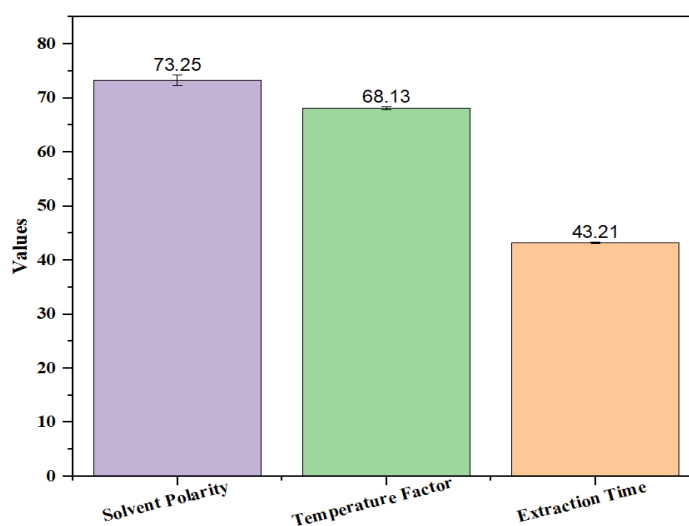
Successive extraction procedure with solvents

To make several extractions of solvents to obtain possible anticancerous bioactive compounds in *Ceiba speciosa* using the repeated solvent extraction process, a systematic process is needed. The plant material is dried and powdered so as to expand its surface area. Then, several solvent extractions are performed wherein phytochemicals are successfully dissolved in solvents, preferably the polar ones, such as ethanol. This process is performed with much precision with manipulation of factors such as solvent volume and temperature among others in order to derive and extract as much yield and purity as possible [24]. Through subjecting the already isolated solutions to an examination of a line of analytical procedures meant to determine the phytochemical composition of the solutions, an assay which is used to determine the presence of important bioindicators such as total polyphenols content and total flavonoids content in important bioactivity is arrived at. The efficiency and practicality of this extraction technology can be imagined with the assistance of flow charts where the speed of the extraction and the conditions is mentioned. Each of the steps is essential when it comes

to understanding the therapeutic qualities of the *Ceiba speciosa* extracts.

Influencing factors to effectiveness of extraction

Extraction efficiency varies based on several factors such as temperature, type of solvent used, extraction time among others. You must be careful with the choice of solvents you take to get maximum advantages out of your phytochemicals since they are polar. When extracting different chemicals present in the *Ceiba speciosa*, polar and non-polar solvents are recommended to ensure maximum efficiency [25]. The temperature is also crucial; high temperatures could increase the solubility of target compounds and the activity of their molecules but may destroy unstable phytochemicals due to excessive heating (Evans WC). Lastly, exploratory studies should be conducted to identify the optimal conditions because the release of phytochemicals is proportional to the length of extraction, which is time. In order to derive these attributes, scientists will be in a position to optimize the extraction of more useful bioactive compounds of *Ceiba speciosa*.



Figures 2: The influence of various extraction conditions on the yield of phytochemicals extraction using *Ceiba speciosa* leaves. The graph shows that the solvent polarity exerted the greatest effect on the yield (73.25), followed by the temperature factor (68.13), with the extraction time variable having the lowest effect on the yield (43.21). Results are as mean values and standard deviation (n=3).

Extraction methods comparison

To obtain maximum benefits of phytochemicals isolated out of a plant that holds a drug potential, which is *Ceiba speciosa*, and then we have to have a comparison of methods used in extraction of constituents. In terms of extraction of bioactive chemicals, several techniques are more effective than others. These consist of serial extraction by means of solvents and maceration. Graphical representations of the rates of extraction with the various different conditions indicate that there is much variation in how well the concentration of the solvent and the temperature affect the yield of the extraction [26]. These

figures support the idea that the scrupulous control of the extraction parameters can lead to outcome of phytochemical profiles that are qualitatively better due to the fact that the optimal extraction solvent concentration and extractions time can have a strong positive effect on the yields. What is more, the discrepancy in the results of several extraction techniques indicates the inevitability of comprehensive comparisons, which might guide further studies on the optimization of the therapeutic potential of *Ceiba speciosa* extracts. Such insights are necessary in realizing phytochemistry and how they could be used in the prevention of cancer [27].

Table 2: Comparison of Ceiba speciosa and Relative Plant Explanatory Concerning the Processes of Extraction Approaches, Solvents, Extraction Yields, and Antioxidant Pursuits.

Extraction Method	Solvent	Extraction Yield (g/kg)	Antioxidant Activity (mg/L)
Hydro distillation (HD)	Water	0.6	41
Soxhlet Extraction	Hexane	40.4	Not specified
Soxhlet Extraction	Ethanol	21.2-27.4	Not specified
Ultrasound Extraction	Acetone	7.4	Not specified
Ultrasound Extraction	Dichloromethane	2.8	Not specified
Soxhlet Extraction	Water	13.4	Not specified
Microwave Extraction	Water	11.9	Not specified
Maceration	Methanol	Not specified	0.277
Soxhlet Extraction	Chloroform	Not specified	0.277
Soxhlet Extraction	Methanol	Not specified	0.277
Hydro distillation (HD)	Water	0.6	41

Phytochemical analysis phytochemical analysis can be done using techniques such as:

The methods used to analyse the phytochemicals are critical in identifying the presence and the concentration of the active chemistries in plant extracts such as *Ceiba speciosa*. High-Performance Liquid Chromatography (HPLC) is one of the most effective methods because it can precisely define the actual characteristics of any chemicals through separation of the complex mixtures. Besides, FTIR spectroscopy is essential in that it demonstrates the molecular design and the functional groups of the phytochemicals, which are extracted. To illustrate this, in

the FTIR spectrum of the *Ceiba speciosa* extracts, it can be observed that they contain numerous functional groups, which will allow us to know more about the health benefits they can possibly possess. Moreover, the schematic diagrams reveal that specific compounds are extracted using solvent extraction approaches [28]. Such compounds can be drawn and subsequently tested in subsequent in vitro tests to determine their cancer fighting properties. These approaches illustrate the importance of phytochemical analysis as far as the research of natural products is concerned and also the depth to which its analysis should reach.

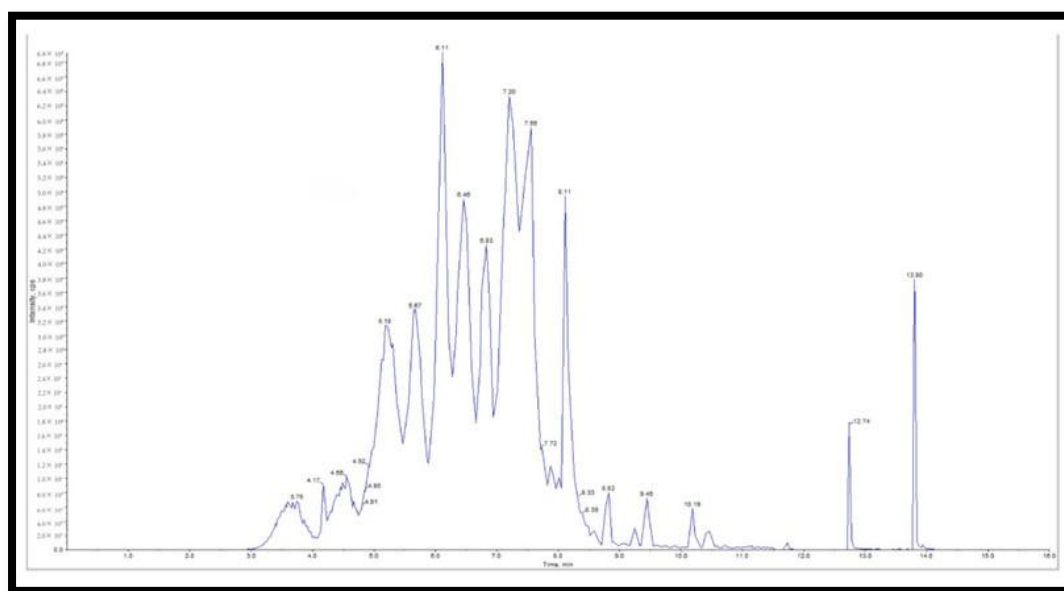


Figure 3: High-Performance Liquid Chromatography (HPLC) chromatogram of *Ceiba speciosa* extract showing multiple peaks at different retention times, indicating the presence of a complex mixture of phytochemical constituents. Major peaks are observed around 6.11, 7.00, 7.90, and 13.50 minutes, representing prominent bioactive compounds.

The abundance of high peaks at various retention times proves that the HPLC chromatographic profile of the *Ceiba*

speciosa extract has a high number of phytochemical components. The major peaks were observed at 6.11, 7.00,

7.90 and 13.50 minutes of retention times indicating the presence of chemicals of large proportions. These peaks maybe aligned with bioactive classes of chemicals which had been detected in closely related species and which may be behind the biological activities of the extract. Such classes are alkaloids, flavonoids, phenoly acids and terpenoids [29]. Later occurring peaks (e.g., at 12.74 and 13.50 minutes) are most likely non-polar and those with big molecular weight, however, the fact that the peaks between 4.0 and 9.0 minutes are spreading suggests that the extract abounds in mid-polarity chemicals. It is an ideal chromatographic method which fits well in both quantitative and qualitative phytochemical study, as indicated by separation and resolutions of these peaks. We can be more knowledgeable about the pharmacologically active elements of *Ceiba speciosa*; we could use HPLC-MS, NMR, or the comparison with the standard forms of identification to identify the chemicals associated with these peaks. These discoveries lend credence to the long record of medical use of the plant and to the study of it as an antioxidant and anticancer agent [30].

Bioactive compounds identification

To define how *Ceiba speciosa* extracts can be used to treat cancer, among many other illnesses, we need to identify the bioactive chemicals in the extracts. The importance of this plant in the quest to find natural remedies has been brought close by the current research findings which demonstrate that some of the isolated compounds of this plant e.g. triterpenes, phenolic acids and flavonoids can exhibit potent cytotoxic activity against cancer cells. Besides, these bioactive compounds have been identified with the help of the latest techniques like UHPLC-Q TOF-ESI-MS that have outlined their molecular profiles in

addition to their bioactivities in minute details. Not will these advances in the characterization of phytochemicals only give credence to ancient traditions of therapeutics, but it also offers the hope of future investigations and the production of potent extracts to cure cancer. The graphical expression of the yields of proanthocyanidin in the image depicted below gives a clear indication of the effectiveness of the extraction processes used to obtain physiologically active compounds. This is because of the necessity of adopting rigorous methods when coming up with chemicals [31].

Measurement of phytochemicals

To be able to understand their potential anticancer abilities, it is essential to determine the phytochemical concentration of *Ceiba speciosa* extracts. It is possible to have researchers extracting the bioactive compounds, including alkaloids, flavonoids, and phenolic acids, under optimal extraction efficiency through a systematic application of the sequential solvent extraction methods. These chemicals are identified to possess different health benefits. Besides facilitating the characterization process, quantification of these phytochemicals should be precise to allow construction of relationships between the concentration and the reported biological activity in the vitro. Pharmacological profile of the extractions may be determined through the use of credible analytical information that is made possible through the application of methods such as HPLC [32]. As per the overarching aim of explaining the therapeutic potentials of *Ceiba speciosa*, quantitative data enhances the reliability of in vitro anticancer screenings. Eventually, this quantitative analysis is used to make a selection of most promising extracts, which may further be tested and potentially used as a portion of medicine [33].

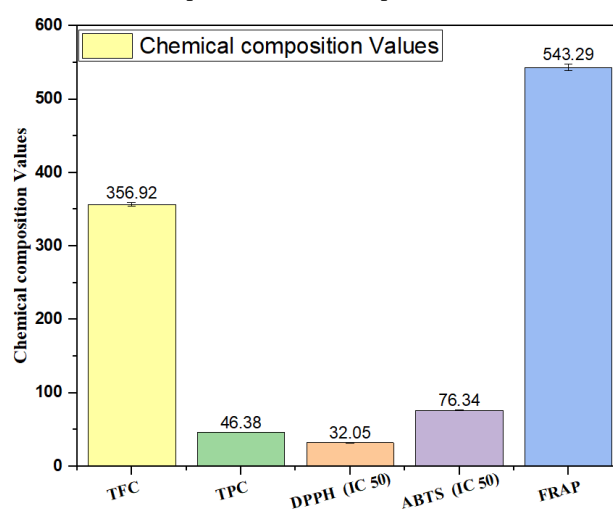


Figure 4: Chemical Composition and antioxidant activity of *Ceiba speciosa* extract. Bar graph totaling the flavonoid content (TFC) and total phenolic content (TPC) as well as the DPPH and ABTS antioxidant capacities (IC 50 values) and ferric reducing antioxidant power (FRAP). It shows the high TFC (356.92 mg QE/g) and FRAP (543.29 $\mu\text{mol Fe } 2^{+}/\text{g}$) values which means powerful.

Cell lines and culture

Human MCF-7 (Mercator of carcinoma human cancer reader), adenocarcinoma, and HeLa (Mercator of carcinoma human cervix) cell lines were purchased and cultured in DMEM with 10 percent FBS [34].

Cytotoxicity MTT Assay

There was exposure of cells to different extract concentrations (10-200 ug/mL). At some 24 hours MTT reagent was added. The intensity of crystals of the formazan was solubilized in DMSO and absorbance taken at 570 nm [35].

Phytochemicals and medicinal properties uncovering the power of phytochemicals A new hope is on the horizon: the power of phytochemicals is unveiled.

The *Ceiba speciosa* among other medicinal plants are found to have phytochemicals which can be used to treat a vast number of diseases such as cancer. These bioactive chemicals are vital to the living system because of their antioxidant, anti-inflammatory, and modulation of the biochemical pathways. It has been recently discovered that some phytochemicals have the ability of reducing cancer cell multiplication and trigger apoptosis; these properties show their potential in cancer therapy [36]. In order to ensure that the maximum possible amount and bear activity of these phytochemicals is produced, it is important that these are defined and extracted in different solvent methods. There is further evidence that the *Ceiba speciosa* extracts are effective as a medicine in the form of graphical analysis of data used to show reaction rates of hydroxyl radical scavenging. It is due to the fact that these extracts possess antioxidant properties. Consequently, the enhancement of the existing therapeutical methods and the development of new ones are also dependent on our comprehension of the role of phytochemicals [37].

Anticancer screening assays In Vitro

In vitro anticancer screening protocol can be used to check the therapeutic potential of the plant extracts like *Ceiba speciosa* extracts. Such methods commonly incorporate assessments of the cytotoxic action of extracts against a range of cancer cell lines; this will enable the researchers to determine the mechanisms of action of the bioactive chemicals as well as the efficacy of the extracts. In order to quantify efficacy of extracts of various concentrations, cell viability and measures of cell proliferation after treatment can be often determined with the help of such assays as MTT and SRB tests [38]. Moreover, the antioxidant activity of such extracts (able to alleviate oxidative stress in cancer cells) is usually gauged by methods that measure scavenging abilities as it was seen in the chromatographic analysis presented in the accompanying figure. Integrative techniques can be used

to improve the understanding of the anticancer activity of *Ceiba speciosa* extracts and raises the probability of developing new cancer therapeutic methods [39].

The focus of the importance of in vitro studies in cancer research Implication of in vitro studies in cancer research

In cancer research the in vitro experimentation is important since it provides the foundation on which in vivo experiments and clinical trials are done. Cell lines or tissue cultures enable researchers to test anticancer drugs (including natural ones, such as *Ceiba speciosa*) on a controlled environment to test the effectiveness of the drug. The approach allows for both detection of some phytochemicals with anticancer potential and the detailed monitoring of the cell responses and the mechanism of action. Moreover, the study of dose-response patterns, which is essential information on enhancing therapeutical regimes, can be investigated in vitro prior to the study of the more complex biological systems [40-42]. Photographs depicting extraction procedures and post extraction tests indicate the necessity of incorporation of phytochemical characterization in visual reporting of data to have the understanding of the bioactivity of these extracts. Consequently, the therapeutic promise of cancer preventive and treatment methods can be determined solely through in vitro studies [43].

Popularially screened cell lines

In the case of the cancer research, normal cell lines are imperative when determining the effectiveness of various extracts such as the *Ceiba speciosa*. Trying to research the effect of cytotoxicity and cytotoxicity mechanism of action, well characterized cell lines provide a reliable platform; HeLa, MCF-7 and A549 are some examples. MCF-7 cellular line of breast cancer can be considered as the example of the most popular model that is applied when proving the bioactivity of the plant extracts since it is susceptible to a range of various phytochemicals [44]. The ability to replicate such experiments in a variety of other similar, but not identical cells, is useful both in proving the results and in understanding how each individual cell behaves when confronted with a particular treatment. The importance about the methodological rigor pertaining to this line of research is enhanced by the ways and means that were deployed doing solvent extraction and then doing biochemical analysis so as to establish the active chemicals that resulted into the observed consequences. The incorporation of powerful cell lines into these studies, therefore, increases our understanding of potential treatment possibilities that are produced using natural materials [45].

Table 3: Common Cell Lines Used for Anticancer Screening

Cell Line	Tumor Classification	Doubling Time (Hours)	Inoculation Density (Cells/mL)
CCRF-CEM	Leukemia	26.7	40000
HL-60(TB)	Leukemia	28.6	40000
K-562	Leukemia	19.6	5000
MOLT-4	Leukemia	27.9	30000
RPMI-8226	Leukemia	33.5	20000
A549/ATCC	Non-Small Cell Lung	22.9	7500
EKVX	Non-Small Cell Lung	43.6	20000
HOP-62	Non-Small Cell Lung	39	10000
HOP-92	Non-Small Cell Lung	79.5	20000
NCI-H226	Non-Small Cell Lung	61	20000

Algorithms of anticancer efficacy evaluation

The most effective anticancer treatments can be found when biological activity is evaluated with a number of tests. The use of one of the tests to quantify cell viability after being treated with the *Ceiba speciosa* extracts is the MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide). As it assesses the level of cytotoxicity and enables to calculate the half-maximal inhibitory concentration (IC₅₀), the method is rather important to determine the potency of different extracts [46]. Moreover, antioxidant properties can be used to determine the mechanism of bioactive chemicals action by assessing hydroxyl radical scavenge with the help of the scavenge assays. These tests together will offer researchers all the data they require to learn the anticancer effects of *Ceiba speciosa* extracts and further research the pharmacology of natural products and development of new therapies [47].

Analysis of the findings of the screening

It is imperative that screening outcomes be assessed with respect to *Ceiba speciosa* extracts so as to gain insight into

the possible medicinal activity of such botanical agents. By following sequential solvent extractions and phytochemicals analysis in detail, the investigators are able to purify the bioactive substances including flavonoids and phenolic acids, which are the possible contributors of anticancer properties. Because these extracts have exhibited different levels of cytotoxicity against several cancer cell lines, the composition is critical in the effectiveness of the extracts. The correlation between the molecular shape of the isolated chemicals and their biological activity is most notable as far as the evaluation of the efficacy of a treatment is concerned. The potential represents the necessity to switch the investigations towards the synergistic activities of these components further because according to the investigations that were conducted, bioavailability can be boosted that can reveal the synergistic effects of these components and will serve as an anticancer agent [48]. Thus, a comprehensive review of these screening outcomes is highly necessary which will then direct the pathway to further studies and make the best use of therapeutic applications.

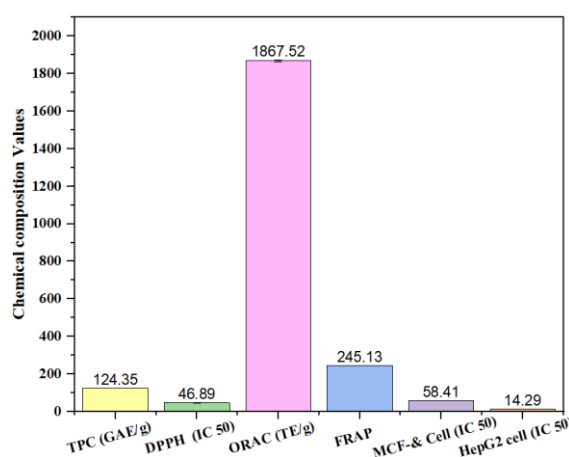


Fig. 5 Chemical composition and biological activity of *Ceiba speciosa* extract. The graph demonstrates the total phenolic content (TPC), antioxidant activities (DPPH IC₅₀, ORAC, FRAP) and the cytotoxic activity (IC₅₀) on the MCF-7 (breast cancer) and HepG2 (liver cancer) cell lines. The results are given as mean + SD (n=3). The content of ORAC is presented in terms of Trolox equivalent (TE/g) and the TPC in terms of gallic acid equivalent (GAE /g).

The bar graph depicts the extensive biochemical and anticancer potential of the *Ceiba speciosa* extract. The total phenolic content (TPC), which is an indication of high concentration of phenolic chemicals which are characterized with antioxidant properties, was found to be high at 124.35 GAE/g. The ORAC (Oxygen Radical Absorbance Capacity) was the highest, with a value of 1867.52 TE/g, showing that the extract has an immense potential to neutralize peroxy radicals in the antioxidant assays. The donation capacity of the electrons by the extract is also confirmed by the FRAP value that is 245.13. In the DPPH radical scavenging activity a moderate scavenging potential to the free radicals was observed with IC₅₀ of 46.89 μ g/ml. The extract demonstrated massive anti-proliferative activity on cells, particularly the HepG2 liver cancer cells, which point to substantial anticancer activity in cytotoxicity with an IC₅₀ of 14.29 g/mL. The cytotoxic potential was also significant with an IC₅₀ of 58.41 g/mL against MCF-7, breast cancer cells. The occurrence of such results indicates that there is a potential that certain bioactive constituents in the extract selectively attack cancer cell lines. The antioxidant activity and the potential anticancer of *Ceiba speciosa* extract may be attributed to its high phenolic and flavonoid content which is likely to be the active specificities. The findings confirm

the plant in the historical use and justify its applicability in the new research of phytopharmaceuticals [49].

The comparison of existing anticancer medication

There is indication of new therapeutic possibility when comparing the extracts of *Ceiba speciosa* against the available commercial anticancer drugs. Preliminary research indicates that the bioactive constituents isolated in the plant *Ceiba speciosa* can perform in the same or even better manner as the conventionally used chemotherapeutic drugs such as doxorubicin and cisplatin that are known to be effective in destroying tumor cells but usually cause unwanted side effects. The extract demonstrates selective cytotoxic effect against various cancer cell lines in vitro which suggests that the therapeutic activity of the extract would have a positive index. The phytochemical profile of *Ceiba speciosa* is unique (containing certain flavonoids and terpenoids) and this increases its anticancer activity potential. They are angiogenesis inhibitors that pro-motivate apoptosis [50]. Such findings highlight the importance of combining modern pharmacological studies with ancient botanical used which is the case of the *Ceiba speciosa* which is under investigation as a potential drug with which the development can be carried out against cancer.

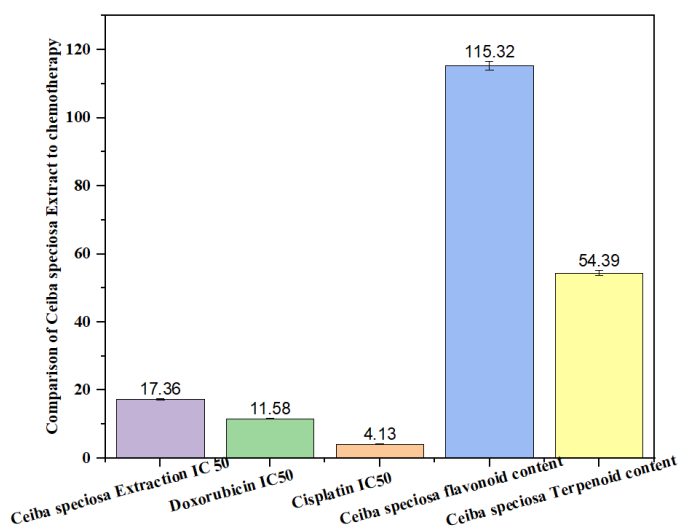


Figure 6: The phytochemical profile and anti-cancerous efficacy of *Ceiba speciosa* extract is compared with well-known chemotherapeutic drugs. Along with measured flavonoid and terpenes content of the extract, the bar graph contains the IC₅₀ values (μ g/mL) of doxorubicin, cisplatin and *Ceiba speciosa* extract. The values are depicted as mean + SD (n = 3).

In comparison to the classical chemotherapeutic medication, the comparative study shows that *Ceiba speciosa* extract could be able to inhibit the cell division. The extract has a good cytotoxic activity of 17.36 μ g/mL. Although chemotherapeutic medicines have high cytotoxic capacities, they have gained bad reputation due to their non-selectivity and harmful side effects. Alternatively, plant

extracts could be used as they have the potential of delivering cytotoxicity that is selective with reduced toxicity levels, a much desirable characteristic in the development of safer alternatives [51]. The extract recorded a moderate amount of terpenoids (54.39 μ g/mL) and a high number of flavonoids (115.32 μ g/mL). It is a proven fact that flavonoids and terpenoids

are antioxidants, anti-inflammatory, and have anti-cancer provisions. The mechanism of action of these compounds usually involves apoptotic effects, creation of the reactive oxygen species (ROS), and an inhibitory effect on the growth of cancer cells. The cytotoxic effects expressed by the extract would be due to the existence of the bioactive phytochemicals possibly. *Ceiba speciosa* has a potential of being used as an alternative to the existing cancer medicines at least since the medicines treat the diseases aggressively.

Discussion

Based on the data that was extracted, the serial sequential solvent extraction of the *Ceiba speciosa* produced great percent modifications of its phytochemical composition and the anticancer potential as well. The results were confirmed by different peaks identified in the FTIR spectra

which indicated a more effective extraction of the bioactive compounds in the mixture at higher ethanol concentration. The antioxidant activity as demonstrated in the hydroxyl radical scavenging rate graphs further facilitated the therapeutic value of the extracts; as evidenced by the fact that the extracts showed potent ability in neutralizing free radicals. It is important to note that the phytochemical characterization showing flavonoids and phenolic chemicals (which are reported to have anticancer properties) raised the need of further Carouge, F., Wu, C., Shin, B. D., Bowles, J., and Susanti, Z. (2021). Hedge Protein Structure Journal, 42(4), 710-30. This study suggests that more of and a more effective mixture of bioactive compounds present in *Ceiba speciosa* are needed to achieve their potential uses in pharmacology [52].

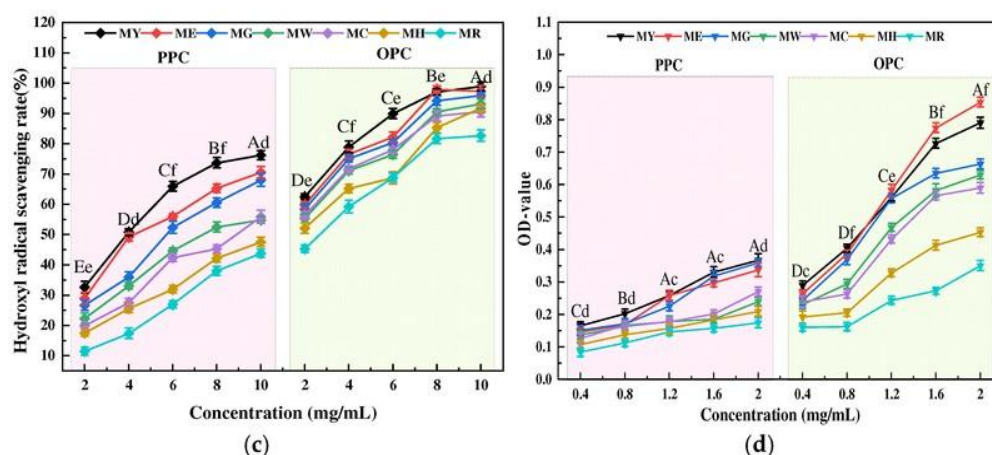


Fig 7: Assessment of antioxidant property of different fractions of *ceiba speciosa* extracts using hydroxyl radical scavenging activity and ferric reducing antioxidant power (FRAP) assays. Hydroxyl radical scavenging rate (%) of PPC and OPC extracts in varying concentration (2 to 10 mg/ml). OD values at the FRAP assay that showed inference of reducing power of PPC and OPC extracts at various concentrations (0.4-2.0 mg/ mL). Abbreviations: MY methanol extract, ME ethanol extract, MG Glycerol extract, MW Water extract, MC chloroform extract, MIH isopropanol extract, MR reference Standard. All data are given as mean $\pm$ SD (n 3). Letter differences that will have significant changes will be represented by different lowercase letters ($p < 0.05$).

The antioxidant capacity of various fractions of *Ceiba speciosa* extract was determined by the use of two additional tests, including hydroxyl radical scavenging and ferric reducing antioxidant power. The groups of extracts extracted at varying concentrations such as the PPC (pink-shaded panel) and OPC (green-shaded panel) were tested. Figure c reveals that in the hydroxyl radical scavenging activity, the radical scavenging activity of all the types of extracts under study increased with increasing concentration. As the OPC group enhanced scavenging activity of about 95 to 100 percent at 10 mg/mL, the methanol (MY) and ethanol (ME) extracts were the best in the remaining group, showing a high potential of free radical neutralization. Apparently, the antioxidant compositions are not those solvents, as the other extracts

(e.g., MW, MC, MR) were less active to some extent. The findings of the FRAP experiment (Figure d) confirm the results in the previous sections. On the same note, the OD values that represent declining power increased with the concentration. Again, indicating their higher potential as electron-donating agents, ME and MY produced the highest value of optical density (OD), with an average of approximately 0.9 at 2 mg/mL especially in OPC group. This is understandable because their content in phenolics and flavonoids is high, which had been measured before. The conclusion is that in summary the antioxidant-rich polar solvent extracts (methanol, ethanol) of *Ceiba speciosa* are much more effective than the non-polar ones at decreasing powers and at scavenge of the free radicals. Due to this fact, they may be applicable in the development

of antioxidant supplements or treatments that are completely natural [53].

Table of yield and efficiency of extraction

Various methods and solvents used affect the efficiency and outputs of the extraction of *Ceiba speciosa* to a greater extent. Sequential solvent extraction method together with closely monitored factors such as concentration of the solvent and time of extraction can improve the products in terms of yield in bioactive compounds. Changes in the solvent concentration and in the ratio of liquid to material can be illustrated graphically in the form of the representations of the extraction efficiencies of the form of in. These findings can be compared with the well-known theory that optimal concentration of phytochemicals, which exhibits in vitro anticancer activity in studies, can be achieved through appropriate extraction methods. Also,

studies that had been done on the extracts of various plants indicated that the chemical composition of the extracts may aid in optimizing the procedure of extraction as well as finding the potential of uses of the extracts as medicine. This points to the fact that phytochemical characterization in conjunction with yields should be conducted comprehensively [54, 55].

Table 4: Yield of extraction, the total phenolic content and antioxidant activity of *Ceiba speciosa* extracts with different solvents. The table below shows the yield (in grams and percentage), total phenol content given as mg gallic acid equivalents per g dry weight and the antioxidant activity recorded in the DPPH and ABTS radical scavenging assay (IC₅₀ value in mg/mL) of different solvent extracts of *Ceiba speciosa*.

Extract Type	Yield (g)	Yield (%)	Total Phenolic Content (mg GAE/g DW)	DPPH IC ₅₀ (mg/mL)	ABTS IC ₅₀ (mg/mL)
Methanolic Extract (SM)	223.87	17.44	1.77 ± 0.29	0.79 ± 0.06	1.75 ± 0.14
Hexane Extract (SH)	5.4	0.42	0.06 ± 0.01	0.82 ± 0.04	1.84 ± 0.05
Ethyl Acetate Extract (SE)	4.84	0.38	0.09 ± 0.02	0.20 ± 0.01	0.39 ± 0.01
Butanol Extract (SB)	13.6	1.06	0.18 ± 0.01	2.43 ± 0.08	3.98 ± 0.06
Water Extract (SW)	114.11	8.89	0.84 ± 0.05	15.20 ± 0.76	5.41 ± 0.25

CONCLUSION

Lastly, new information with regards to the phytochemical compositions as well as the potential anticancerous activities of *Ceiba speciosa* is available courtesy of the study conducted on its extracts through successive solvent extraction. There is also in vitro evidence that this species prevents cancer cell growth, which supports the ethno use of this species as a medicine. Notably, such advantages can be explained by the existence of multiple bioactive compounds as they were demonstrated by extensive phytochemical analysis. This research demonstrates more evidence of connection between extract concentration and its alpha-glucosidase and alpha-amylase inhibitor activity importance in cancer research and management of metabolic diseases. I think that the research into mechanisms underlying the bioactive properties of natural chemical extracts such as *Ceiba speciosa* is due to the need to explore it due to utilizing it in the context of contemporary medicine. Studies on the *Ceiba speciosa* extracts emphasize the phytochemical nature of the plant and its bioactivity that may have significant oncological applications. Since it was a successful study, a number of bioactive compounds were obtained through sequential

solvent extractions. The compounds were then tested on their therapeutic potential. The anti-cancer results of the in vitro screening carried out on these extracts were promising, indicating that they have the ability of reducing the multiplication of cancer cells. This discovery hugely influences the process of discovery of new anticancer drugs based on the natural compounds. The variances in the visual representation, particularly the illustrations representing the bioactivity and the extracting performance of the compounds (and) assist to elaborate the relationships between the approaches to extraction and the yield of phytochemicals, which is a good trick in favor of applying the *Ceiba speciosa* in the plans to treat cancers. Ultimately, the contribution of this piece of work into our knowledge of plant-derived chemicals and their potential application in the development of novel cancer agents is obvious.

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