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Research article

Assessment of anticancer potential in *Caesalpinia Bondoc*: a comparison between leaf and kernel extracts

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ABSTRACT

This study presents a comparative evaluation of the anticancer activity of leaf and seed extracts of Caesalpinia bonduc. Ethyl acetate extracts were prepared using the Soxhlet extraction technique to isolate the plant's bioactive constituents. C. bonduc was selected due to its long history in traditional medicine and its reported pharmacological potential. The cytotoxic effects of the extracts were assessed against MCF-7 breast cancer cells, a widely used cell line in breast cancer research.The experimental results indicate that both leaf and seed extracts possess notable anticancer properties, demonstrated by their ability to reduce cell viability and inhibit the proliferation of MCF-7 cells. These findings suggest that C. bonduc contains potent bioactive compounds capable of inducing growth inhibition or cell death in cancer cells. Further investigation into the specific phytochemicals responsible for these effects and their underlying mechanisms may support the development of novel plant-based anticancer agents. Overall, this study adds to the growing body of evidence supporting the value of medicinal plants in cancer research and highlights the significance of traditional knowledge in guiding modern therapeutic discoveries.

Keywords: Caesalpinia bonduc, Soxhlet extraction, Ethyl acetate, MTT assay, Anticancer activity, MCF-7 cells, Breast cancer, Bioactive compounds, Cytotoxicity, Natural products, Traditional medicine, Plant-based therapies, Cell viability, Cancer treatment.

INTRODUCTION

Breast cancer is among the most widespread and challenging forms of cancer globally, making it a major public health concern. Despite advances in treatment strategies, it is still important to investigate new antiviral drugs to increase effectiveness, reduce side effects, and prevent drug reactions. In this context, natural compounds obtained from medicinal plants have attracted great attention because they have many pharmacological properties and can be anticancer agents [1].

Caesalpinia bonduc is a tropical medicinal plant widely recognised in traditional healing systems. Several parts of the plant—particularly the leaves and seeds—have been valued for their therapeutic properties. Among these, the anti-inflammatory potential of the leaves and seeds has attracted considerable scientific interest, positioning the plant as a promising candidate for further pharmacological investigation.

In phytochemical research, Soxhlet extraction remains one of the most reliable techniques for isolating bioactive compounds from plant materials. Its main advantages include high extraction efficiency and the ability to obtain multiple classes of secondary metabolites from a single sample. When paired with modern analytical tools, this method greatly enhances the identification and characterisation of biologically active constituents. To evaluate the biological activity of plant extracts, the MTT (2,5-diphenyltetrazolium bromide) assay is commonly employed. This assay measures the metabolic reduction of MTT by mitochondrial enzymes in viable cells, providing quantitative insight into cell viability, cytotoxicity, and proliferation. In this study, the cytotoxic effects of C. bonduc leaf and seed extracts were tested against MCF-7 breast cancer cells, allowing assessment of their potential anticancer activity. By examining both cytotoxic and antiproliferative effects, this work contributes to a broader understanding of plant-derived compounds and their possible therapeutic roles in breast cancer management. The findings aim to integrate traditional knowledge with contemporary research approaches, supporting the future development of plant-based anticancer agents [2].

MATERIALS AND METHODS

Fresh and dried leaves and kernels of Caesalpinia bonduc were collected, cleaned, and shade-dried before being ground into a fine powder. Soxhlet extraction was carried out using ethyl acetate as the solvent, and the obtained extracts were concentrated through rotary evaporation and dried under reduced pressure.

MCF-7 cells were cultured in DMEM supplemented with fetal bovine serum, antibiotics and maintained in a CO_2 incubator. Cells were divided into control and treatment groups, with different concentrations of leaf and kernel extracts prepared in DMSO. For the MTT assay, cells were seeded into 96-well plates, exposed to extracts, incubated with MTT reagent, and analysed spectrophotometrically to determine cell viability. IC_{50} values were calculated through nonlinear regression. All experiments were performed in triplicate, using aseptic techniques, and doxorubicin served as the positive control. Ethical clearance was obtained from the Institutional Ethics Committee [3].

Collection and preparation of *Caesalpinia bonduc* leaves and kernels

Plant materials were first authenticated through morphological examination, with assistance from a botanist when required. Mature, disease-free leaves and seed pods were harvested using clean, sharp tools to avoid contamination or damage. After collection, the materials were cleaned to remove debris and insects, and the leaves were separated from the kernels. Drying was carried out in a well-ventilated, shaded area using racks or trays to ensure proper air circulation and prevent direct contact with the ground. Once dried, grind the leaves and kernels separately into a fine powder to enhance extraction efficiency. Store the powdered material in airtight containers labelled with relevant information, ensuring protection from moisture, light, and pests [4].

Soxhlet extraction procedure for obtaining plant extracts

The Soxhlet extraction method is commonly used to obtain plant extracts, especially for compounds that are less soluble in traditional solvents.

Materials and Equipment:

Sample Description:

Sample – Caesalpinia bonduc kernels

Sample –Caesalpinia bonduc Leaves

Sample Form: Solid powder

Activity: Ethyl acetate Extraction of a herbal plant drug.

Procedure

Approximately 20–25 g of the powdered plant material was accurately weighed and wrapped in a filter paper thimble. The thimble was then placed inside the extraction chamber of the Soxhlet [5].

apparatus. The empty round-bottom flask was weighed separately and connected to the apparatus. The condenser was attached properly, ensuring that the cooling water entered from the lower inlet and exited from the upper outlet, which helps maintain uniform condensation and prevents air pockets [6].

Ethyl acetate was poured into the Soxhlet chamber until the siphon arm was partially filled. Once the assembly was placed on a water bath, heating was initiated, maintaining the bath temperature at 70–75°C. During extraction, the solvent repeatedly evaporated, condensed, and siphoned back, allowing continuous washing of the plant material. The extraction was allowed to run for about 7 hours, or until the solvent in the siphon became almost clear, indicating completion of the extraction. After the process, the thimble containing the exhausted plant sample was removed, and the solvent from the extract in the flask was recovered. Both the flask and the filter-paper thimble were kept in a hot air oven for approximately 3 hours to eliminate any residual solvent. The absence of solvent odour confirmed complete drying. Finally, both components were reweighed, and the amount of extract obtained was calculated from the weight difference [7].

Calculation

From flask. The empty weight of the flask is “x” after the sample extraction weight of the flask in “y”.

% of= ((y-x)/weight of sample) *100 From Filter paper.

Take gross weight of sample (filter + sample) = y. After extraction and drying (filter + sample) = x.

Note: % should be the same from filter paper & flask calculation, ±2 variation may be. % of= ((y-x)/weight of sample) *100.

Take gross weight of sample (filter + sample) (y)- 17.546 gm. After extraction and drying (filter + sample) (x).

Sample – Caesalpinia Bondoc kernels – 7.7 Sample –Caesalpinia Bondoc Leaves – 6.8 % yield w/w of= ((y-x)/weight of sample) ×100 [8].

RESULTS

The extraction was carried out by Soxhlet extraction (Continuous extraction method) using ethyl acetate.

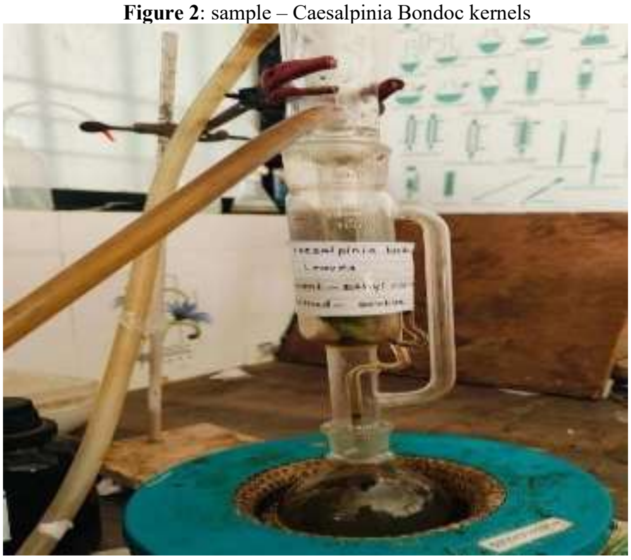
The yield was obtained: Sample – 1) Caesalpinia Bondoc kernels-7.7gm 2) Sample –Caesalpinia Bondoc Leaves-6.8gm

Table 1: Observation

Plant Names	Part	Colour	Nature	% yield w/w
Sample – Caesalpinia bonduc kernels	Powder	Dark Green	Solid	69.2
Sample Caesalpinia bonduc kernel	Powder	Dark Green	Solid	58.8

Figure 1: Sample –Caesalpinia Bondoc Leaf

Figure 2: sample – Caesalpinia Bondoc kernels



MTT assay

MTT Assay

Sample Description

Caesalpinia Bondoc Kernels extract

Caesalpinia Bonduc Leaves Extract

Sample Form: Solid

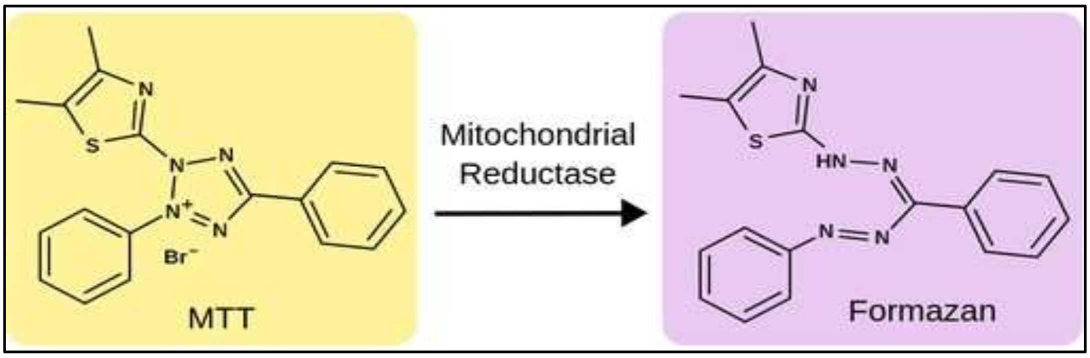
Requirements

The in vitro cytotoxicity study was carried out using the MCF-7 human breast cancer cell line. Cells were maintained in high-glucose DMEM supplemented with fetal bovine serum (Gibco) and a 100× antibiotic–antimycotic solution to ensure contamination-free growth. All cell-handling procedures were performed inside a certified biosafety cabinet, and the cultures were incubated in a CO_2 incubator to maintain optimal physiological conditions. For the viability experiment, cells were seeded into a 96-well microplate and treated with the test samples. After completion of the assay, absorbance values were recorded using an ELISA plate reader (Benesphera E21) to determine the percentage of surviving cells.

Principle

The MTT assay works on a colour-based reaction in which only living, metabolically active cells participate. Functional mitochondria contain the enzyme succinate dehydrogenase, which converts the yellow, water-soluble MTT reagent—chemically known as 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide—into dark purple, insoluble formazan crystals. This transformation happens exclusively in viable cells, making the amount of formazan formed directly proportional to cell survival. The accumulated formazan is then dissolved and quantified using a spectrophotometer, providing an indirect measurement of cell viability [20].

Figure 3: Reaction



Procedure

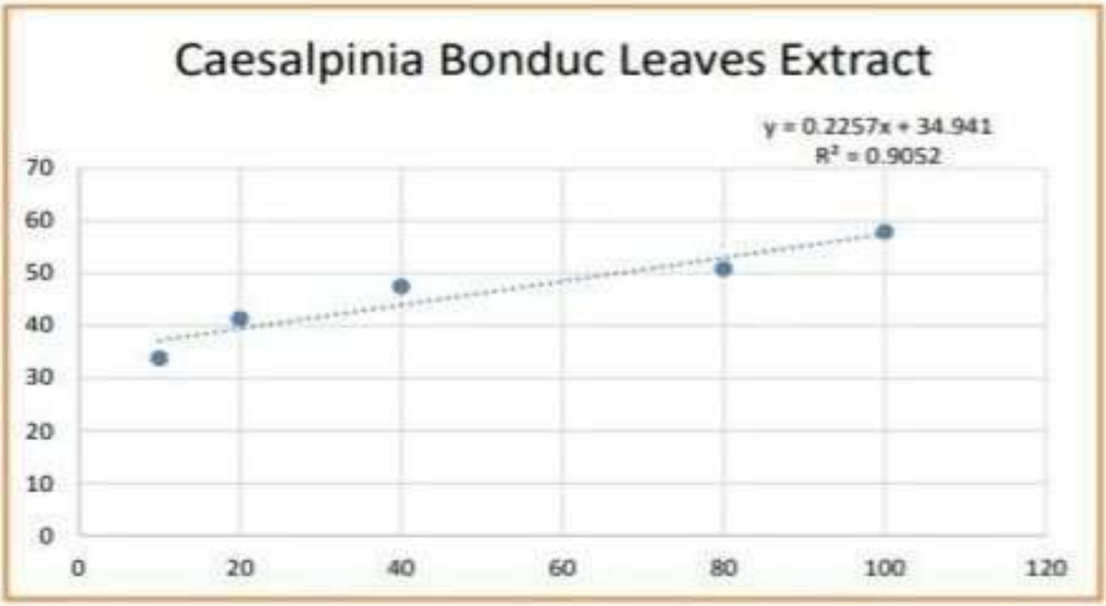
The Soxhlet extraction procedure began with the proper assembly of the apparatus by attaching the extractor to a round-bottom flask filled with ethyl acetate below the siphon level and connecting it to a condenser. A measured quantity (40–50 g) of dried and powdered Caesalpinia Bondoc leaves or kernels was placed into a cellulose thimble and inserted into the extractor. The solvent in the round-bottom flask was then heated using a heating mantle, allowing the vapors to rise, pass through the plant material, and carry dissolved compounds upward into the condenser. As the vapors cooled, the solvent condensed and dripped back into the extractor, creating a continuous cycle of extraction and recirculation. This process was maintained for 6–24 hours, depending on the solubility and extraction efficiency required. After completion, the solvent–extract mixture was collected from the flask and filtered to remove residual plant particles. When necessary, the filtrate was concentrated using a rotary evaporator to remove excess solvent under reduced pressure, leaving behind the crude plant extract. The final extract was stored in a clean, airtight, dark container and appropriately labeled with details such as plant part, extraction method, solvent used, and date of preparation.

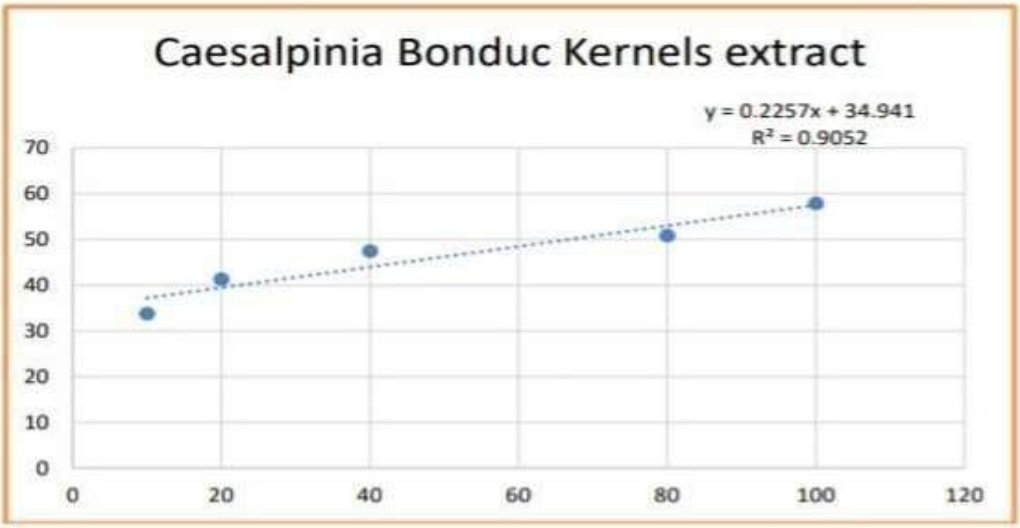
Table 2: Association between age, sex, and treatment outcomes

concentration (µg/ml)	Absorbance (O D)				Cell viability (%)	IC50 (µg/ml)
	1	2	3	Average		
5FU 10	0.678	0.682	0.712	0.690667	40.17	13.38
20	0.501	0.582	0.526	0.536333	37.09	
40	0.394	0.465	0.393	0.417333	35.4674221	
80	0.48	0.376	0.34	0.398667	33.88101983	
100	0.333	0.325	0.396	0.351333	29.85835694	

Table 03: Comparative Cytotoxic Effects of Caesalpinia bonduc Leaf and Kernel Extracts on MCF-7 Breast Cancer Cells (MTT Assay)

concentration (µg/ml)	Absorbance (O D)				Cell viability (%)	Percent inhibition	IC50 (µg/ml)
	1	2	3	Average			
Control	1.174	1.168	1.188	1.176			
Caesalpinia Bonduc Kernels extract 10	0.621	0.625	0.605	0.617	52.46599	47.53401	21.01
20	0.587	0.577	0.581	0.581667	49.46145	50.53855	
40	0.546	0.566	0.554	0.555333	47.22222	52.77778	
80	0.435	0.433	0.439	0.435667	37.04649	62.95351	
100	0.409	0.423	0.411	0.414333	35.23243	64.76757	
Caesalpinia Bonduc Leaves Extract 10	0.788	0.768	0.781	0.779	66.2415	33.7585	66.93
20	0.699	0.678	0.694	0.690333	58.70181	41.29819	
40	0.612	0.622	0.621	0.618333	52.57937	47.4206 3	
80	0.587	0.579	0.569	0.578333	49.178	50.822	
100	0.487	0.498	0.502	0.495667	42.14853	57.85147	





The extraction process yielded a greater quantity of extract from the leaves compared with the kernels, and the leaf extract was found to contain a higher concentration of phytochemicals. Both the leaf and kernel extracts demonstrated dose-dependent cytotoxicity against MCF-7 breast cancer cells. Notably, the leaf extract exhibited a lower IC_{50} value, indicating stronger anticancer activity [9].

DISCUSSION

The enhanced anticancer potency of the leaf extract may be attributed to its richer profile of bioactive phytochemicals, which are known to induce apoptosis and suppress cancer cell proliferation. The pronounced cytotoxic effect observed in the leaf extract suggests that Caesalpinia bonduc leaves may possess more therapeutically active constituents than the kernels. Overall, these findings underscore the potential of this plant as a promising natural source for developing anticancer agents. However, further in vivo investigations and detailed mechanistic studies are essential to fully understand the pathways involved and validate its clinical relevance [10].

CONCLUSION

The anticancer evaluation of extracts RS1 and RS2 at concentrations ranging from 10–100 µg/mL revealed that RS1 exhibited stronger cytotoxic effects against the MCF-7 breast cancer cell line. When compared with the standard drug 5-fluorouracil, RS1 demonstrated notable activity across all tested concentrations, indicating its potential as a promising candidate for future plant-based anticancer research.

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