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Original Article

Investigations on the Anticancer Properties of Bark Extract of *Couroupita guianensis* against Breast Cancer

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ABSTRACT

Background: One of the major causes of death in women is breast cancer worldwide. There are a lot of shortcomings in the proper diagnosis of breast cancer at early stages in the medical field. Unremitting research is going on for detecting breast cancer in the early stages as the possibility of a cure is bright. **Objectives:** The present study on the *C. guianensis* plant aims to demonstrate the bioactive principles of methanol and aqueous bark extract and their potential cytotoxic properties. **Materials and Methods:** The bark extracts of *C. guianensis* were subjected for sequential extraction using various solvents of increasing polarity for therapeutic analysis such as antioxidant, anti-inflammatory, antiangiogenesis and cytotoxicity. **Results:** The bioactive compounds might have acted synergistically making these extracts potent and can be used as antioxidant and anti-inflammatory agents. Antiangiogenesis assay was performed by CAM assay showcasing antiangiogenesis and it showed the aqueous methanol extract of *C. guianensis* successfully demonstrated anticancer activity against MDA-MB-231 breast cancer Cell lines. **Conclusion:** The present plant extracts may have potential bioactive compounds which can be utilized for therapeutic applications.

Keywords: Angiogenesis, Breast Cancer, *C. guianensis*, Cytotoxicity, Phytochemicals.

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INTRODUCTION

In recent times, one of the major causes of death and a hindrance to high life expectancy is cancer (Sung *et al*., 2021). It is the word reported that, over 19.3 million new cancer diagnosis instances and about 10 million mortality cases in 2020, (Debela *et al*., 2021) By the year 2030, this rate might go upto 21.4 million fresh cases and 13.2 million deaths (Gupta *et al*., 2015). Small molecule inhibitors, monoclonal antibodies and immunotoxins are preliminary categories of targeted cancer therapy (Baudino *et al*., 2015). In females, most prevalent and second greatest death cause among the world population is breast cancer. Among all the cancers, breast cancer is the second most common cause of death and it is mostly found in the females of age group 45 and 55 years. The frequency of breast cancer is almost 1 in 8 women, necessitating various treatments including surgery, chemotherapy, radiotherapy, and hormone therapy (Baudino *et al*., 2015). Chemotherapy is one of the ways to treat this disease and the developments in anticancer remedies have enhanced patient care. Regrettably, conformist chemical drugs also are the reason for adversative side effects on native cells/tissue, such as inhibition of bone marrow function, vomiting, queasiness, and alopecia (Baskar *et al*., 2014). Recent studies have suggested that natural antioxidants and many phytochemicals could be effective adjuvant therapies for cancer treatment due to their anti-proliferative and pro-apoptotic properties (Baudino *et al*., 2015). Consequently, there is a constant quest to identify anticancer compounds from plants to find safer substitutions and reduce the side effects of chemotherapy, given the numerous advantages of natural herbal medicines (Singh *et al*., 2016). The use of herbal medicines to address various ailments dates to ancient times, such as in Ayurveda, Unani, and Siddha, and has become deeply ingrained in human culture (Yin *et al*., 2013). Furthermore, phyto-based medicines are the basis of contemporary pharmaceuticals and exploration for novel and efficient extracts from medicinal plants remains in central attraction in recent years because of the presence of diverse bioactive compounds such as alkaloids, terpenoids, glycosides, steroids, flavonoids, and phenolic compounds (Kumar *et al*., 2006). Medicinal plants are considered as the basis for the development of various pharmacopiel and non pharmacopiel drug formulations. Apart from that plants are essential for the development of human cultures around the world (Ouerghemmi

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et al., 2016). *Couroupita guianensis* belonging to *Lecythidaceae* family is well recognized for its medicinal and ornamental values. The plant is commonly called a Cannonball tree because of the cannonball appearance of its fruits (Sheba *et al.*, 2020; Garg *et al.*, 2021). The chemical constituents present in the plant is α-amirin, β-amirin, β-sitosterol, nerol, tryptanthrine, indigo, indirubin, isatin, linoleic acid, carotenoids and sterols (Wong *et al.*, 2011). Hence, the primary objective of the present study was to evaluate the cytotoxic potential of extracts of *Couroupita guianensis* against Breast cancer cell line.

MATERIALS AND METHODS

Collection of plant material

The *C. guanensis* plant material (bark) was collected from Hubli, Karnataka, India in the month of May 2025. After being cleaned off the dust particles by running tap water, the tree bark was dried under the shade for 15-20 days, powdered coarsely, and stored in cool and dry place using airtight containers.

Solvent phytoextraction

The phytochemicals were extracted using Soxhlet apparatus with different solvents such as chloroform, acetone, methanol and water in the ratio of 1:10. After the extraction, they were dried using flash evaporatorand stored in airtight containers.

Qualitative phytochemical analysis

Detection and qualitative estimation of phyto chemicals of all the plant extracts was carried out by standard methods (Tiwari *et al.*, 2011).

GCMS profiling

GCMS analysis was performed for the methanol extract of *C. guianensis* plant using GCMS model GCMS-QP2010S. Fused silica used as closed column and Helium gas used as mobile phase for separation of active components present in the test sample. The identification of components was based on the comparison of their mass spectra with those of NIST 11 mass spectral library (Davies, 1990; Massada, 1996).

In vitro antioxidant activity by DPPH radical scavenging assay

This assay was performed with all four extracts of *C. guianensis* is conferring to the method given by (RiceEvans *et al.*, 1997). Ascorbic acid was used as a positive control. The DPPH scavenging activity of each sample was calculated using the following equation:

$$\text{DPPH scavenging activity (\%)} = Ac - At / Ac \times 100$$

where Ac is the absorbance of the control reaction (100 µL of ethanol with 100 µL of the DPPH solution) and At is the absorbance of the test sample. The whole experimental setup was performed in triplicates. The results are expressed as Mean±Standard Deviation (SD).

Evaluation of anti -inflammatory activity by Protein denaturation method

In vitro antiinflammatory activity was executed by using protein denaturation assay. This activity was performed following the method developed by (Padmanabhan *et al.*, 2012). Diclofenac sodium was used as a standard (Reactin100, Cipla Pharmaceuticals). Phosphate Buffered Saline (PBS) was used to take the control readings. The experimental setup was performed in triplicate. The results are expressed as Mean±Standard Deviation (SD).

The inhibition percentage was measured by the formula:

$$\% \text{ inhibition} = At - Ac / Ac \times 100$$

Where Ac is the absorbance of the control and At is the absorbance of the test sample.

DNA Cleavage Study

The DNA cleavage study of the *C. guianensis* bark extract was determined by agarose gel electrophoresis using the yeast DNA as a target. The sample of 100 µL was mixed with the target DNA. The combination was then incubated at 37°C for 2hr. Subsequently, the DNA and the sample were mixed with the tracking dye bromophenol blue (1:1). It was then loaded into 1% agarose wells along with one control well and was electrophoresed at 50V for 30 min using Tris-EDTA buffer (pH 8.0). The bands were visualized under UV Light and photographed for analysis (Jebiti *et al.*, 2015).

In vivo Anti angiogenesis activities of *Couroupita guianensis* extract.

In vivo antiangiogenic activity was performed by Chick Chorioallantoic Membrane (CAM) assay. The CAM assay was performed according to the method of Lokman *et al.*, 2012. The CAM assay does not require any ethical approvals to perform the experiment.

Evaluation of cytotoxic activity on MDA-MB-231 cell line

In vitro cytotoxicity assay was carried out by MTT assay on MDA-MB-231 breast cancer cell lines. The percentage inhibition of growth was calculated, after subtracting the background and the blank. The concentration of the test drug needed to inhibit cell growth by 50% (IC_{50}) was produced from the dose-response curve for the cell line (Alley *et al.*, 1986).

RESULTS

Phytochemical analysis

The preliminary phytochemical investigation disclosed the existence of 'alkaloids, phenols, flavonoids, tannins, anthraquinones, glycosides, lignins' and sterols in various extracts such as chloroform, acetone, methanol, and water. Specifically, the chloroform extract contained alkaloids, the acetone extract contained alkaloids, terpenoids, and saponins, and the methanol extract contained alkaloids, flavonoids, glycosides, terpenoids, and carbohydrates. The aqueous extract showed the presence of alkaloids, flavonoids, glycosides, terpenoids, and carbohydrates.

GCMS profiling

Methanol extract was subjected to GC MS profiling, phytochemicals such as 2-Methoxy-4-Vinylphenol, Tetradecanoic acid, 1,2-Benzenedicarboxylic Acid, Bis(2-Methylpropyl) Ester, Methyl EsterHexadecanoic Acid, Hexadecanoic Acid, Octadecanoic Acid Methyl Ester, Octadecanoic Acid 1-Hexadecanol, acetate, 9-Octadecenamide, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, Octadecanoic acid 2,3-dihydroxypropyl ester, beta-Amyrin were found in NIST library. The aqueous extract was subjected to GCMS profiling, phytochemicals such as 5-Hydroxymethylfurfural, Hexadecanoic acid, methyl ester, Hexadecenoic acid, Octadecanoic acid, beta-Amyrin were found in NIST library (Table 1).

Table 1: GCMS Profiling of Methanol and Aqueous Extract of *Couroupita Guianensis*.

Methanol Extract		
Peak#	**Name**	**Base m/z**
1	2-Methoxy-4-Vinylphenol	150.05
2	Tetradecanoic Acid	73.00
3	1,2-Benzenedicarboxylic Acid, Bis(2-Methylpropyl) Ester	149.05
4	Hexadecanoic Acid, Methyl Ester	74.00
5	Hexadecanoic Acid	73.05
6	Octadecanoic Acid, Methyl Ester	74.00
7	Octadecanoic Acid	73.05
8	1-Hexadecanol, Acetate	83.10
9	9-Octadecenamide	59.00
10	Hexadecanoic Acid, 2-Hydroxy-1-(Hydroxymethyl)Ethyl Ester	98.10
11	Octadecanoic Acid, 2,3-Dihydroxypropyl Ester	98.10
12	9-Octadecenamide	59.00
13	.Beta.-Amyrin	218.15
Aqueous Extract		
Peak#	**Name**	**Base m/z**
1	5-Hydroxymethyl furfural	97.05
2	Hexadecanoic acid, methyl ester	74.00
3	Hexadecanoic Acid	73.00
4	Octadecanoic acid	73.00

In vitro Antioxidant activity by 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) assay

DPPH assay was executed with four different extracts and ascorbic as a standard. Aqueous extract showed maximum antioxidant activity at 100 µg/mL (58.51±11.87), followed by that, methanolic extract at 80 µg/mL (58.02±5.10) showed maximum antioxidant activity. The results are expressed as Mean±Standard Deviation (SD) (Figure 1).

In vitro anti-inflammatory activity

The different concentrations of the chloroform, acetone, methanol and aqueous extracts of *Couroupita guianensis* were subjected to an anti-inflammatory activity assay by protein denaturation method with Reactin 100 (Cipla Pharmaceuticals Ltd.,) as the standard drug. The assay revealed a significant anti-inflammatory property of the extracts. The aqueous extract possesses maximum anti-inflammatory activity at 250 µg/mL (85.58±9.31%), followed by the methanolic extract at 200 µg/mL (81.95±2.35%).

DNA cleavage of *Couroupita guianensis* extract

The DNA Cleavage of *Couroupita guianensis* extract was analysed as there were very faint bands seen in lane 2 (DNA+Methanol extract) and lane 3(DNA+Aqueous extract) of the gel plate, suggesting that the extract possesses DNA cleavage activity.

In vivo Anti-angiogenesis activities of *Couroupita guianensis* extract by CAM (chick Chorioallantoic membrane) assay

The present study carried out on the *C. guianensis* revealed that the plant possesses anti-angiogenesis properties with methanol and aqueous extracts. The methanol and aqueous extract were injected and eggs were observed on the 6th day of incubation, showing the potential of narrowed blood vessels with methanol (50 µg/mL) and aqueous (50 µg/mL) extracts (Figure 2).

In vitro assay for cytotoxic activity on MDA-MB-231 cell line

Cytotoxicity results of *Couroupita guianensis* methanol extract showed an enhanced effect with increasing concentration exhibiting IC_{50}=185.64 µg/mL, as the concentration of *Couroupita guianensis* methanol extract increases the cell viability decreases (Figure 3). Cytotoxicity results of the aqueous extract showed enhanced effect with increasing concentration exhibiting IC_{50}=202.35 µg/mL, as the concentration of aqueous extract increases the cell viability decreases (Figure 3).

DISCUSSION

Plants possess various medicinal properties is known since the ancient time because of this property there is an high interest of using plants belonging to different families in treating and curing several deceases, (Patti *et al.*, 2019). Several research unveiled that *C. guianensis* has various medicinal properties due to the presence of phytochemicals. Phytochemicals such as alkaloids, glycocides, flavonoids, saponins, phenols, phytosterols, tannins, etc, has many beneficial property whose presence in the plants make them quintessential in the therapeutic usage.

GCMS profiling of methanolic and aqueous extracts revealed phytochemicals such as 2-Methoxy-4-Vinylphenol, which can induce cell cycle arrest (Jeong and Jeong, 2010), Tetradecanoic acid showcases antiurease, antielastase and antioxidant properties (Sokmen *et al.*, 2014), 1,2-Benzenedicarboxylic Acid has cytotoxic and antimicrobial properties (Krishnan *et al.*, 2014), Bis(2-Methylpropyl) Ester possesses antimicrobial activity (Peng *et al.*, 2017), Methyl Ester, Hexadecanoic Acid exhibits antioxidant, hypocholesterolemic, nematicide, and pesticidal activity (Mazumder *et al.*, 2020), Octadecanoic Acid Methyl Ester showcase anti-inflammatory and antiviral activity (Reagan *et al.*, 2013)1-Hexadecanol acetate, 9-Octadecenamide possess anticancer, antibacterial and anti-inflammatory activities (Cheng *et al.*, 2010), 2-hydroxy-1-(hydroxymethyl)ethyl ester exhibits antioxidant, nematicidal and hypocholesteremic activities (Tyagi and Agarwal, 2017), 2,3-dihydroxypropyl ester shows antimicrobial activity (Canli *et al.*, 2023), beta.-Amyrin shows anti-inflammaatory and antitumor activities (Viet *et al.*, 2021).

The results of the DPPH assay may be due to the synergistic activity of the phytochemicals revealed by GCMS analysis, which were present in the extracts and possess radical scavenging properties against DPPH free radicals.

An ailment known as inflammation occurs when various immune cells gather at the site of damage. These inflammatory responses could be either long-term or short-term. The non-steroidal drugs that are employed presently pose side effects in the form of gastric problems. Protein denaturation is a well-studied root of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, the capacity of phytoextract to constrain protein denaturation was evaluated. The methanol

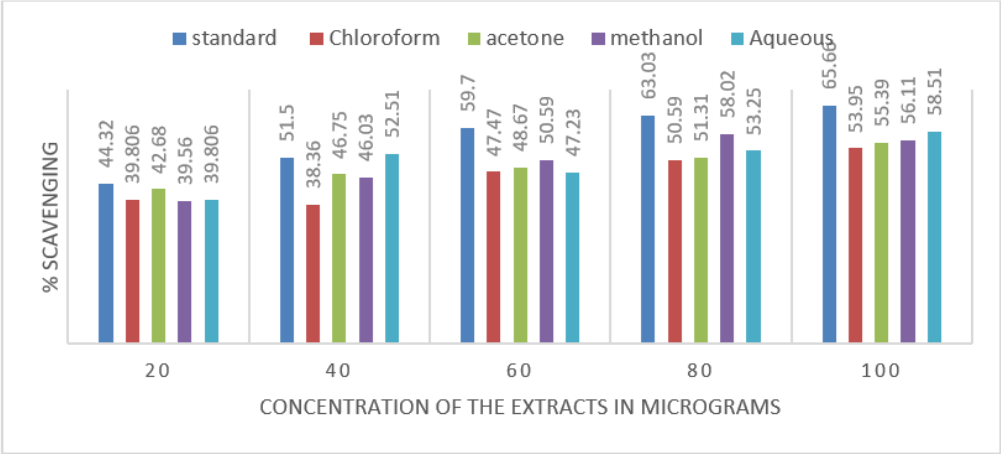

Figure 1: *In vitro* antioxidant activity of *C. guianensis* extracts by DPPH Radical Scavenging Assay.

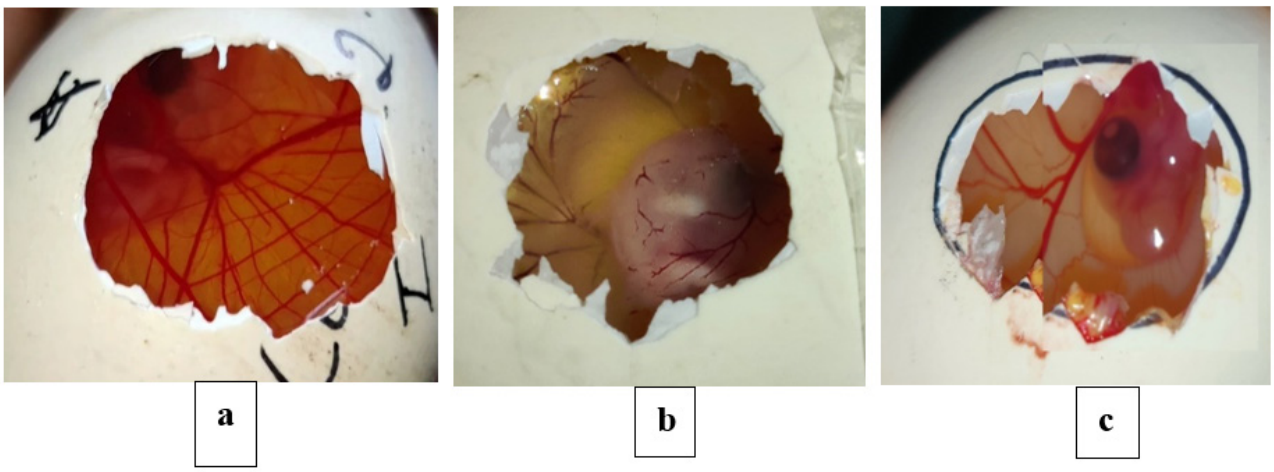

Figure 2: *In vivo* antiangiogenesis activities of *C. guianensis* by CAM Assay. a) Control, b) Aqueous Extract (50 µg/mL), c) Methanol Extract (50 µg/mL).

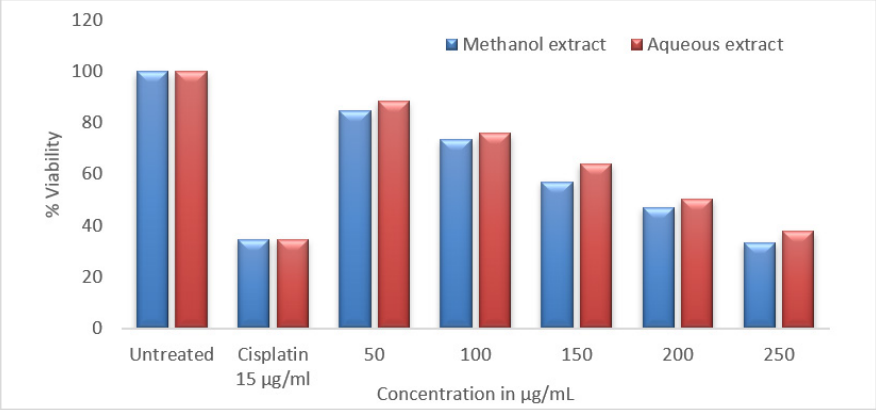

Figure 3: *In vitro* cytotoxicity assay of *C. guianensis* methanol and aqueous extracts on MDA-MB-231 Cell lines.

extract showcased the highest anti-inflammatory activity, which indicates least protein denaturation at the site of inflammation (Kulkarni *et al*., 2019).

Angiogenesis is the formation of new blood vessels to deliver oxygen and nutrients to body tissues. So anti-angiogenesis drugs are used to stop tumors from growing their blood vessels. The phytochemicals present in this plant extracts may be preventing transcription of the angiogenesis factors of VEGF and HIF1α (Hoseinkhani *et al*., 2020).

The MTT assay results of the extracts of *C. guianensis* methanol and aqueous extracts revealed that the cytotoxic effect was dose-dependent. Among both the extracts methanol extract was found to be more cytotoxic to MDA-MB-231 cells compared to aqueous extract. This is due to the presence of a large number of phytoconstituents in methanol extract when compared to aqueous extract. These phytoconstituents might have acted synergistically to reduce the number of active cells.

CONCLUSION

The present study carried out on the *C. guianensis* plant successfully demonstrated that the methanol and aqueous bark extract consist of various bioactive compounds. These bioactive compounds might have acted synergistically, making these extracts potent and can be used as antioxidant and anti-inflammatory agents. The aqueous and methanol extracts of *C. guianensis* successfully demonstrated the anticancer activity against the MDA-MB-231 cell lines. Thus, from the present study, it is concluded that the plant *C. guianensis* may be used to treat the disease that is caused due to oxidative stress and inflammation and it might be a potent source in treating cancer.

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ABBREVIATIONS

µL: Micro litre; **GC:** Gas Chromatography; **MS:** Mass Spectrometry; **DPPH:** 2,2- diphenyl-2- picrylhydrazyl; **IC:** Inhibitory Concentration; **CAM:** Chick Choliallontoic Membrane; **NIST:** National Institute of Standards and Technology; **EDTA:** Ethylene diamine tetraacetic acid; **DNA:** Deoxyribonucleic Acid; **UV:** Ultra Violet; **MTT:** 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **m/z**: mass to charge ratio; **SD:** Standard Deviation.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Nisha Ramesh Dubashi was involved in planning the research, Plant collection and data generation. Prashant Dodamani and Madan Desai conducted Cell culture studies and statistical analysis of the data. Neelam Mishra and Ramakrishna were involved in manuscript preparation and plagiarism check. Badarinath Druvarao Kulkarni- overall planning and execution of the research and manuscript preparation and communications.

FUNDING

We declare that the present work was not funded by any agency or organization.

SUMMARY

- Plant collection, phytoextraction, and preliminary phytochemical analysis of bark extracts of *Couroupita guianensis.*
- Characterization of phytochemicals by using GCMS technique.
- Evaluation of antioxidant and anti-inflammatory activity of bark extract of *Couroupita guianensis*.

- *In vitro* cytotoxicity studies of extracts of *Couroupita guianensis* bark on breast cancer cell lines.
- Evaluation of DNA damage and anti-angiogenesis (*In Vivo*) activities of extracts of *Couroupita guianensis*.

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