



Research Article

Phytochemical Screening And Immunomodulatory Activity Evaluation Of The *Couroupita Guianensis* Aubl. Flowers

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ABSTRACT

The study shows the presence of phytochemicals and the immunomodulatory potential of *Couroupita guianensis* Aubl. flowers. Phytochemical analysis analyzed and showed many biological substances, including alkaloids, flavonoids, phenols, tannins, and saponins, these phytochemicals are the key substances for immunomodulation. Elemental analysis was done for the assessment of the composition of elements present in it to support the study with evidence of the presence of Chromium, Copper, Iron, and Manganese which contributed to immunomodulatory activities. For nutrient composition assessment of *Couroupita guianensis* Aubl. Flowers, Proximate analysis was performed which also showed the presence of moisture content, ash, protein, fat, and nitrogen-free extract (NFE). Further, for the assessment of immunomodulatory activity in-vitro techniques such as the MTT assay was performed which correlated based on cell immunomodulation will have an impact on cell viability and cytotoxicity. The results were promising and the floral extract had strong immunomodulatory effects, such as increased cell proliferation. These studies suggest that *Couroupita guianensis* flowers exhibit immunomodulatory abilities, which could be linked to the numerous chemical components present in it. This study demonstrates the therapeutic potential of *Couroupita guianensis* flowers in strengthening the immune system and supports further research into the creation of novel immunomodulators.

INTRODUCTION

Couroupita guianensis, known as Kailash Pati tree in Maharashtra and its common name is cannonball tree which is commonly found in India, belongs to the order Ericales and the family Lecythidaceae¹. It has the curative potential and also used to treat various diseases like stomach

aches, wounds, toothache, and common cold and is also it has got anti-inflammatory properties¹. It also an effective antifungal, antimycotic, and analgesic¹. It has anti-rheumatic, rubefacient properties, it also helps to cure intestinal gas formation, the fragrance also helps in the treatment

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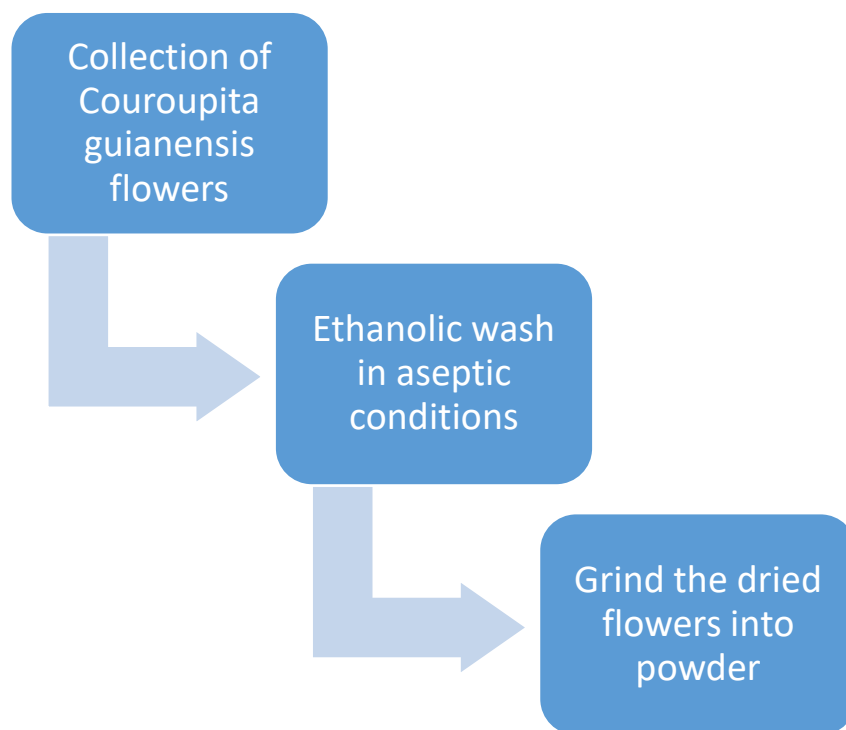
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of asthma. Antioxidants are also present, which aid to reduce lipid oxidation and scavenge free radicals., the aroma or smell of the flower is used to treat asthma². According to previous studies, there is a presence of linoleic acid, linalool, sterols, carotenoids, indigo, indirubin, eugenol, and α , β -

amyryns which makes it as bioactive component essential for multiple medicinal uses³. The studies done until now inspired us to do further investigation of immunomodulatory activities in *Couroupita Guianensis* Aubl. flowers.

MATERIALS AND METHODOLOGY:



(Flow chart no.1)

Flowers of *Couroupita guianensis* were procured from Umargam, Gujrat state. Direct plucking of flowers was done to avoid soil contamination. These flowers were dipped out in absolute ethanol before air drying to avoid spoilage and kept in laminar air flow for drying purposes. The dried flowers were ground into powder which was used for further applications.

Characterizations:

Elemental Analysis:

Elemental analysis is a technique used for determining the elemental composition of a *Courupita Guianensis*, it helps in the assessment of quantitative aspects. In this study, elemental analysis helps in for an assessment of elements like Chromium, Iron, Manganese, and Copper which

enhance the therapeutic properties of the flower and reduces the toxicity.

Proximate Analysis:

It is the technique basically used for the analysis of moisture content, carbohydrate content ash, fat content, ash content, and nitrogen-free extract (NFE)¹.

Phytochemical analysis:

Phytochemicals are the components which occurs naturally in the plants. These phytochemicals boost up the several activities and also helps in showing up it gives the essential information of the therapeutic potential and its applications².

In-vitro studies (MTT assay):

The MTT assay is a in-vitro technique which is performed for assessing immunomodulatory activity by measuring cytotoxicity, cell viability

and proliferation³. It helps in understanding the working mechanism of immunomodulation properties upon the cells.

RESULTS:

Elemental Analysis:

A sample weighing about 0.1 grams was used.

Used acid mixture: 3 mL HCL, 1 mL HNO₃, and

2 mL HF. The samples were heated in a microwave for 25 minutes at 190 degrees, with a 15-minute ramp. After cooling the sample to room temperature, distilled water was added to make up to 25 ml.

Elements	Concentrations in ppm
Chromium	13.152
Copper	34.194
Iron	393.592
Manganese	5.022
Cadmium	ND (less than 0.01ppm)

Couropita guianensis flowers contain trace elements that have potential immunomodulatory effects. With 13.152 ppm of chromium, 34.194 ppm of copper, 393.592 ppm of iron, and 5.022 ppm of manganese, they contain a wide range of micronutrients known to alter immunological function. Chromium, it also causes toxicological concerns⁴. But when it is present in small amounts it enhances immunological response⁵, but copper

and iron are required for immune cell formation and function. Manganese contributes to antioxidant defense systems, which are essential for immunological control. The absence of cadmium (less than 0.01 ppm) reduces potential harmful consequences. The presence of these trace elements signifies that Couropita guianensis flowers may have immunomodulatory effects.

Proximate Analysis:

Parameters	Mean Value (% w/w)
Total ash	24.38
Water insoluble ash	9.21
Acid insoluble ash	0.24
Water soluble extractive value	11.28
Alcohol soluble extractive value	10.55
Loss on Drying	8.98

Proximate analysis of Couropita guianensis reveals a considerable ash concentration (24.38% w/w), with water-insoluble ash (9.21% w/w) and acid-insoluble ash (0.94% w/w) indicating mineral composition. Its water-soluble extractive (11.28% w/w) and acid-soluble extractive (10.44% w/w) values point to soluble bioactive chemicals. These elements are most likely responsible for its possible immunomodulatory effect. Extract also

have compounds such as alkaloids, phenolics, and flavonoids that upregulate the immunological responses by cytokine synthesis modulation and increased immune cell activity⁶. Furthermore, the loss on drying (8.98% w/w) indicates moisture content, which influences the stability of bioactive chemicals important for immunomodulation⁷. Couropita guianensis shows promise as a natural immunomodulatory agent.

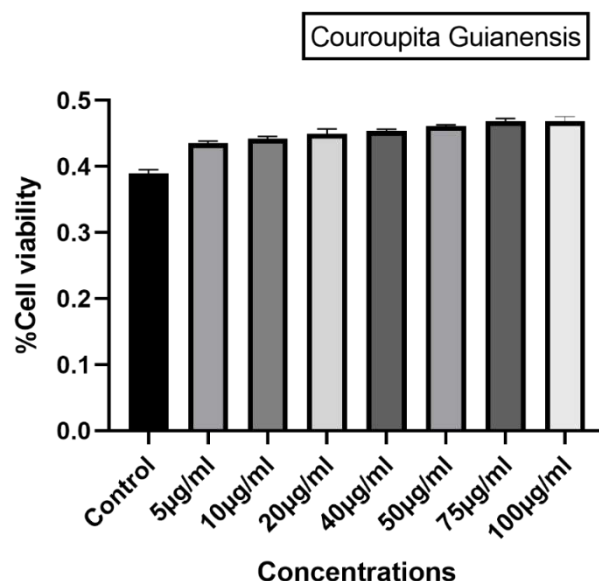


Phytochemical analysis:**Table no.3 Phytochemical analysis of Couropita guianensis flowers**

Serial no	Name of Test	Solvent Extracts of Couropita guianensisflowers						
		H ₂ O	CH ₃ OH	Ethanol	EtAc	CHCl ₃	Hexane	Pet ether
a	Alkaloids							
	1. Dragendorff's Test	A	P	P	A	A	A	A
	2. Mayer's Test	P	P	A	A	A	A	A
	3. Hager's Test	P	P	P	A	A	A	A
b	Saponins	P	P	P	A	A	A	A
c	Flavonoids							
	1. Shinoda Test	A	P	P	P	A	A	A
	2. Slakaline reagent Test	A	P	P	P	A	A	A
d	Steroids							
	1. Salkowski Test	A	A	A	A	A	A	A
	Libermann burchard Test	A	A	A	A	A	A	A
e	Tannins							
	1. Lead acetate Test	A	A	A	A	A	A	A
	2. Ferric chloride Test	A	A	A	A	A	A	A
	3. Potassium dichromate Test	A	A	A	A	A	A	A
f	Phenols							
	Ferric chloride Test	P	P	P	P	A	A	A
g	Glycosides							
	Legal's Test	A	P	A	A	A	A	P

The above table shows the phytochemical studies carried out on Courupita Guianensis flowers. Alkaloids found in methanol and ethanol extracts demonstrate that these solvents are able to solubilize immunomodulatory compounds. These data show promise for the potential utility of water, methanol, and ethanol extracts as immunomodulators - a finding consistent with the presence of saponins². Methanol, ethanol, and ethyl acetate extracts were rich in flavonoids, and these flavonoids have antioxidant, anti-inflammatory, and immunomodulatory activities,

which underline their potential to modulate immune responses. The fact that steroids were absent from all extracts argues against these extracts possessing an immunosuppressive action of the kind seen in steroids. The latter suggests that the tannins present in these extracts may not have the same immunomodulatory impact. The effect of glycosides on immune functions can be exerted through various related pathways. Both methanol and petroleum ether extracts show the presence of glycosides which indicates that these extracts may display immune-modulatory properties⁸.

MTT assay**(Graph no. 1 of MTT assay cell viability)**

It is a molecular biological in-vitro technique that helps in the assessment of immunomodulatory studies as it shows the cellular metabolic activity as shows the direct impact of components or the samples on the proliferation of the cells which helps to correlate the immunomodulatory activity with cell proliferation assay⁹. The assay was performed by using the mononuclear cells as they are the important components of immune systems. The yellow tetrazolium dye dissolved in PBS is taken up by the cells during metabolism and forms purple formazan crystals³. The absorbance shows the cell proliferation rate for respected concentration. The absorbance rate is higher in the samples as compared to the control group as it shows that the concentrations from 5µg/ml to 100µg/ml show the immunostimulant for the cells present.

CONCLUSIONS:

The prepared powder of Courupita Guianensis Aubl flowers was assessed for its immunomodulatory activities by characterizations. The elemental analysis showed the presence of trace elements in the flower which is core essential for the growth and development

of an individual. The proximate analysis showed the presence of alkaloids, phenolics, and flavonoids that can stimulate immune responses by altering cytokine production and enhancing immune cell activity. An MTT assay using mononuclear cells showed higher cell proliferation in all the concentrations which depicts it as an immunostimulant.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation.

ETHICAL CONSIDERATIONS:

No ethical permissions were needed.

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REFERENCES:

1. Bukunmi Aborisade A. Phytochemical and Proximate Analysis of Some Medicinal Leaves. *Clin Med Res.* 2017;6(6):209.
2. Ferdous N, Rahman M, Alamgir A. Investigation on phytochemical, cytotoxic and antimicrobial properties of ethanolic extracts of *Centella asiatica* (L.) Urban. *J Med Plants Stud.* 2017;5(2):186–8.
3. Naeini A, Khosravi A. Evaluation of the immunostimulatory activity of *Z iziphora tenuior* extracts. 2010;459–63.
4. Mishra V, Tomar S, Yadav P, Vishwakarma S, Singh MP. Elemental Analysis, Phytochemical Screening and Evaluation of Antioxidant, Antibacterial and Anticancer Activity of *Pleurotus ostreatus* through In Vitro and In Silico Approaches. *Metabolites.* 2022;12(9).
5. Shrivastava R, Upreti RK, Seth PK, Chaturvedi UC. Effects of chromium on the immune system. *FEMS Immunol Med Microbiol.* 2002;34(1):1–7.
6. Chopade BA, Ghosh S, Ahire M, Patil S, Jabgunde A, Bhat Dusane M, et al. Antidiabetic activity of *gnidia glauca* and *dioscorea bulbifera*: Potent amylase and glucosidase inhibitors. *Evidence-based Complement Altern Med.* 2012;2012.
7. Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. *Medicines.* 2018;5(3):93.
8. Pokhrel S, Neupane P. Phytochemical analysis, antioxidant and antibacterial efficacy of methanol and hexane extract of *Centella asiatica*. *Bibechana.* 2021;18(2):18–25.
9. Stockert JC, Horobin RW, Colombo LL, Blázquez-Castro A. Tetrazolium salts and formazan products in Cell Biology: Viability assessment, fluorescence imaging, and labeling perspectives. Vol. 120, *Acta Histochemica.* Elsevier GmbH; 2018. p. 159–67.

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