

# In-Vitro Anticoagulant Study of Leaf Extract of *Couropita Guianensis*

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## RESEARCH ARTICLE

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### ABSTRACT

Anticoagulant activity of different extract of cannon ball tree was evaluated in-vitro. Medicinal plant acts as a metabolic bio factory, which can be accessed as therapeutic potential with lesser side effects. The phyto constituents of various medicinal plants can be correlated with pharmacological study. In vitro study was carried out with a vision to setup standards that could be beneficial for detecting authenticity of this medicinal plant. Numerical standards reported in this work could be useful for the compilation of suitable monograph of *Couropita guianensis*. Various phyto constituents such as alkaloids, tannins, saponins and glycosides are producing a better opportunity for testing various pharmacological activities. The results obtained from this research revealed that that the plant contains bioactive agents which are connected with anticoagulant activity in plants.

**Keywords:** Anticoagulant activity, flavonoids, extraction, chemotherapy

### 1. Introduction

The scope of herbal medicine is sometimes extended to include fungal and bee products, as well as minerals, shells and certain animal parts. Herbal medicine, use of natural plant substances (botanicals) to treat and prevent illness. The practice has existed since prehistoric times and flourishes today as the primary form of medicine for perhaps as much as 80% of the world's population. Over 80,000 species of plants are in use throughout the world. Along with acupuncture, herbal medicine is considered primary health care in China, where it has been in documented use for over 2,500 years. (1-5) The use of plants as medicines predates written human history. Many of the herbs and spices used by humans to season food also yield useful medicinal compounds. (6-7)

#### Plant profile

##### Scientific Name:

*Couropita guianensis* Aubl.

##### Common Names:

Cannon ball tree, Sal tree, Ayauma tree

##### Vernacular Names:

Hindi – Shivaling Ayahuma, Kailashpathi

Tamil – Nagalingam

Telugu – Mallikarjuna, Nagamalli

##### Habit

*Couropita guianensis* is a large evergreen tree growing to a height of 20 meters. Leaves are alternate, oblong-obovate, up to 20 centimeters long, entire to slightly serrate and hairy on the veins beneath. (8-10)

##### Habitat

*Couropita guianensis* is native to tropical northern South America, especially the Amazon rainforest and the southern Caribbean. Also occurs in India, where it is probably native, and Thailand. (11-13)

##### Ethnomedicinal uses

Leaves of *C. guianensis* are widely used as analgesics by the Brazilian rural population. Juice made from the leaves is used to treat skin disorders and the Shamans of South America have even utilized tree parts for curing malaria. (14-17)

##### Objective

Through lots of investigation from local areas, information from tribal people, village people, from some of book shaving ethnomedicinal documentation. The research was performed systematically with the following schedule.

Phytochemical evaluation of *Couropita guianensis* leaves

- Collection and extraction of leaves
- Qualitative tests of chloroform and aqueous extracts

Pharmacological evaluation of the extracts

Evaluation of anti-coagulant activity of the chloroform and aqueous extracts of the leaves of *Couroupita guianensis*.

## 2. Materials and Methods

### Collection of blood samples

The blood samples were obtained from normal individuals by using sterile syringes, withdrawn from vein of right arm of each individual and placed separately in containers containing tri-sodium citrate to prevent the clotting process. Centrifugation (15 minutes at rate 3000 rpm) was carried out to separate the blood cells from plasma in order to obtain pure platelet plasma (ppp) for prothrombin time test. The obtained plasma sample of each individual were poured separately in plane containers using automatic pipette and stored at room temperature.

### Collection of blood and Plasma re-calcification

0.2 ml plasma, 0.1 ml of crude extract of different concentration and different volume of  $\text{CaCl}_2$  (25 ml) were added together in a clean fusion tube and incubated at  $37^\circ\text{C}$  in water bath. For control experiment extract, solution was replaced by same volume of 0.9% saline

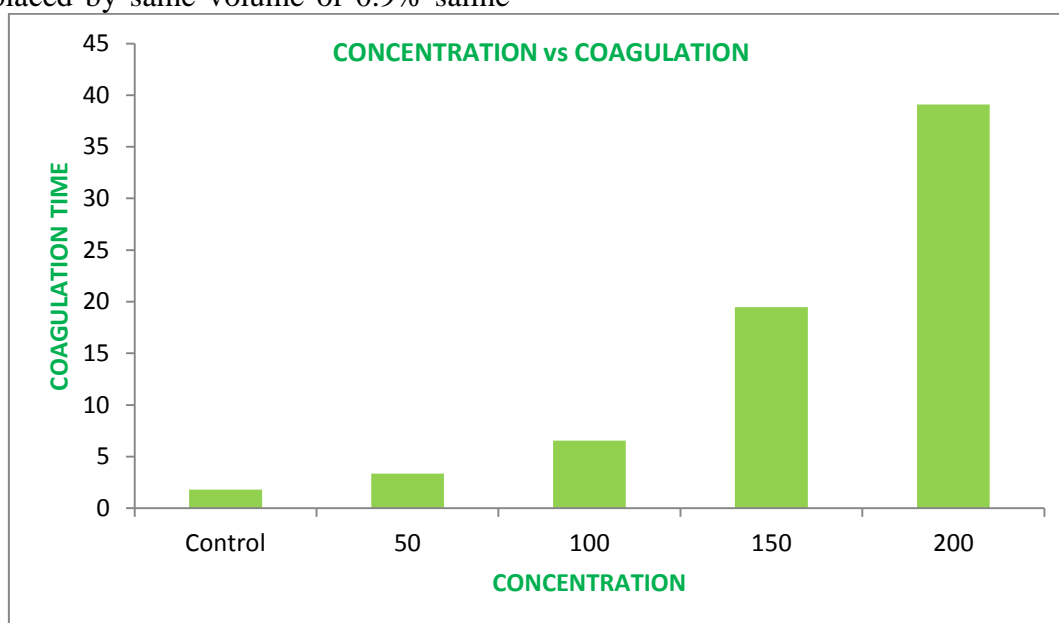
water. The clotting time was recorded with stopwatch by tilting the test tubes every 5 seconds. This time is called the prothrombin time.

### Blood coagulation study

Blood samples were collected from healthy volunteers, using a disposable polypropylene syringe, and then anti-coagulated using 3.8% tri-sodium citrate in a polypropylene container (9 parts of blood to 1 part of tri-sodium citrate solution). It was immediately centrifuged at  $4000 \times g$  for 15 min, and plasma was separated and pooled. The freshly prepared plasma was stored at  $4^\circ\text{C}$  until its use. In a test tube 0.1 ml test plasma and EDTA were added and shaken briefly to mix the reagent and plasma. The tube was placed at  $37^\circ\text{C}$  for 20 min for incubation. After the incubation, 0.1ml pre-warmed calcium chloride solution was forcibly added into the mixture of plasma and reagent. To this, one ml of chloroform & aqueous extracts were added separately in different concentrations and kept at  $37^\circ\text{C}$ . A stopwatch was started to record the coagulation time in seconds. The tube was shaken to mix the contents and it was stopped as soon as the clot formation began.

## 3. Result and discussion

Anti-coagulant effect of leaf extracts of *Couroupita guianensis*



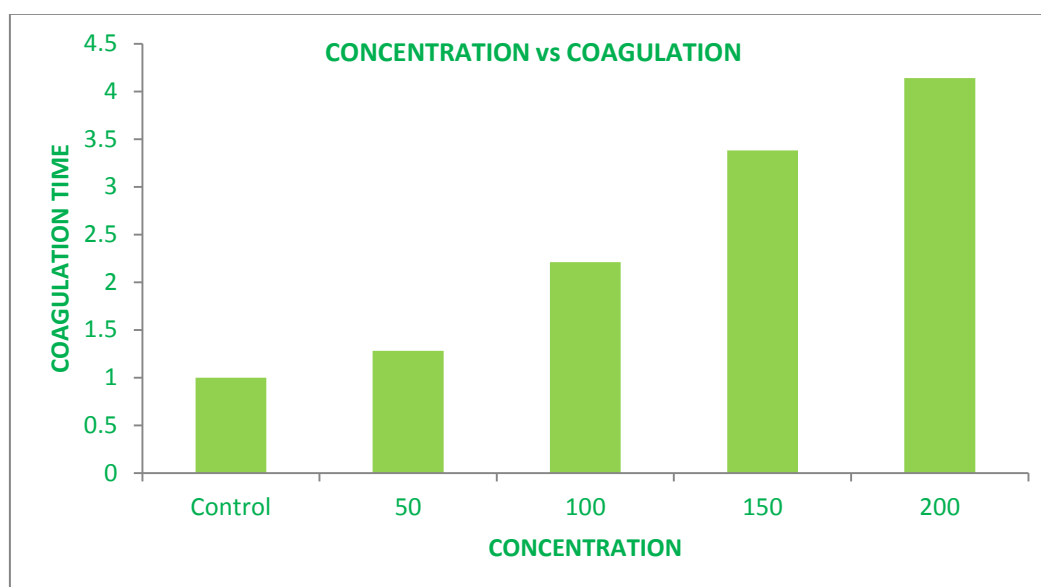
**Figure 1.** Showing comparison with Concentration Vs Coagulation with Chloroform extract

**Table 1.** Anticoagulant effect of Chloroform extract of *Couroupita guianensis*

| Concentrations (µg/ml) | Amount of plasma | Amount of extract | Calcium chloride solution | Time of coagulation(mins) |
|------------------------|------------------|-------------------|---------------------------|---------------------------|
| Control                | 0.2ml            | 0.1ml             | 0.3ml                     | 1.4                       |
| 50                     | 0.2ml            | 0.1ml             | 0.3ml                     | 3.36                      |
| 100                    | 0.2ml            | 0.1ml             | 0.3ml                     | 6.54                      |
| 150                    | 0.2ml            | 0.1ml             | 0.3ml                     | 19.48                     |
| 200                    | 0.2ml            | 0.1ml             | 0.3ml                     | 39.11                     |

**Table 2.** Anticoagulant effect of aqueous extract of *Couroupita guianensis*

| Concentrations (µg/ml) | Amount of plasma | Amount of extract | Calcium chloride solution | Time of coagulation (min) |
|------------------------|------------------|-------------------|---------------------------|---------------------------|
| Control                | 0.2ml            | 0.1ml             | 0.5ml                     | 1                         |
| 50                     | 0.2ml            | 0.1ml             | 0.5ml                     | 1.28                      |
| 100                    | 0.2ml            | 0.1ml             | 0.5ml                     | 2.21                      |
| 150                    | 0.2ml            | 0.1ml             | 0.5ml                     | 3.38                      |
| 200                    | 0.2ml            | 0.1ml             | 0.5ml                     | 4.14                      |

**Figure 2.** Showing comparison with Concentration Vs Coagulation with aqueous extract

#### 4. Conclusion

Anti-coagulant activities of chloroform and aqueous extract of *Couroupita guianensis* were carried out. From the present study it is proved that both the extract have remarkable anti-coagulant activity than the control solution. The phytochemical constituents like Tannins, glycosides present in the couroupita guianensis leaves shows potent anticoagulant activity in

the present studies. Further studies using in vivo models and to isolate active constituents from extract are required to carry out and established the effectiveness and pharmacological rational for the use of couroupita guianensis as an anticoagulant drug.

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### Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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