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Evaluation of Anti-Coagulant Activity of the Chloroform and Aqueous Extracts of the Leaves of *Couroupita guianensis*

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ABSTRACT

Objective: To investigate the anti-coagulant activity of the chloroform and aqueous extracts of the leaves of *Couroupita guianensis.* **Methods:** The anti-coagulant activity of the chloroform and aqueous extracts of the leaves of *Couroupita guianensis* and the results were compared for the both extracts. **Results:** The aqueous and chloroform extracts of leaves of *Couroupita guianensis* showed better anti-coagulant activity at a concentration of 0.1 ml. The anti-coagulant effect of extracts is comparable with that of the effect produced. **Conclusion:** From the above finding it can be concluded that the plant possesses significant anti-coagulant activity at 0.1ml concentration measured by time taken for coagulation. The current investigation leads to conclusion that the leaves of *Couroupita guianensis* have potent anticoagulant activity when compared with the conventionally used drug.

INTRODUCTION

Plants have been used for medicinal purposes for as long as history has been recorded. Despite the progress in orthodox medicine, interest in alternative medicine, including herbalism, is on the increase in the West and for 80% of the world herbal medicine is still the only kind to which ordinary persons have ready access. A great variety of plants are used for medicinal treatments. Either the dried plant, or a specific part of it (root, leaves, fruit, flowers, seeds), is formulated into suitable preparations — compressed as tablets or made into pills, used to make infusions (teas), extracts, tinctures, etc., or mixed with excipients to make lotions, ointments, creams, etc.

Similarly, many consider that since plants are natural materials they are safer and will produce fewer side effects than synthetic drugs. There is little substance or reason in either of these claims. For example, comfrey (*Symphytum officinale*) is considered a safe herb and is used as a demulcent. However, it contains pyrrolizidine alkaloids, which are toxic to the liver and can cause liver cancer. Herbal medicine (or "herbalism") is the study and use of medicinal properties of plants. Studies show that in tropical climates where pathogens are the most abundant, recipes are the most highly spiced. Further, the spices with the most potent anti-microbial activity tend to be selected. In all cultures, vegetables are spiced less than meat, presumably because they are more resistant to spoilage.

Among the more popular remedies used are ginseng, to increase stamina and as a mild sedative; St.-John's-wort, for mild depression; echinacea, to aid the immune system and alleviate colds; kava, to calm anxiety and treat insomnia; saw palmetto, for enlarged prostate; and ginkgo biloba, to improve short-term memory.

This widespread use has prompted demands that herbal remedies be regulated as drugs to ensure quality standards. The U.S. Food and Drug Administration (FDA) can require a clinical trial on any herb that has a health claim on its label, but medical testing, which is geared toward observing a particular active component, is difficult to apply to herbs, which may have many interacting ingredients.

Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as

insects, fungi and herbivorous mammals. Many of these phytochemicals have beneficial effects on long-term health when consumed by humans, and can be used to effectively treat human diseases. The World Health Organization (WHO) estimates that 80 percent of the population of some Asian and African countries presently uses herbal medicine for some aspect of primary health care.

Couroupita guianensis is a deciduous tree belonging to the family Lecythidaceae. It is native to South India and Malaysia. Various part of the tree have been reported to contain oils, ketosteroids, glycosides, couroupitine, indirubin, isatin and phenolic substances The pulp of the fruits oxidizes bluish-green when exposed to air and is extremely malodorous probably because of sulphur compounds in the fruits. The fruit contains small seeds in a white, unpleasant smelling edible jelly. The large fruit, which is woody and very spherical, measuring up to 25 centimeters wide, gives the species the common name "cannonball tree". A smaller fruit contains perhaps 65 seeds, while a large one can have 550. One tree can bear 150 fruits. The fruit takes up to a year to mature in most areas, sometimes as long as 18 months. This plant is used for treating mange and other skin conditions. The pulp of the fruit of the cannon ball tree is rubbed on the infected skin of mange dog. It is claimed that when the dog licks its skin, this medicine will also work internally. The flowers are used to cure cold, intestinal gas formation and stomachache.

PLANT PROFILE

Scientific Name:

Couroupita guianensis Aubl.

Common Names:

Cannonball tree, Sal tree, Ayauma tree

Ethnomedicinal uses:

- Leaves of *C. guianensis* are widely used as an 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

➢ *Couroupita guianensis* infusions or teas obtained from different parts of the tree used traditionally to treat hypertension, tumours, pain and inflammatory processes

➢ *Couroupita guianensis* flowers are used to cure cold, intestinal gas formation and stomach ache

➢ In Orissa, decoction of flowers has been used to boost the immune system to fight number of diseases

➢ The fruit pulp of *Couroupita guianensis* is used to disinfect wounds

MATERIALS AND METHODS

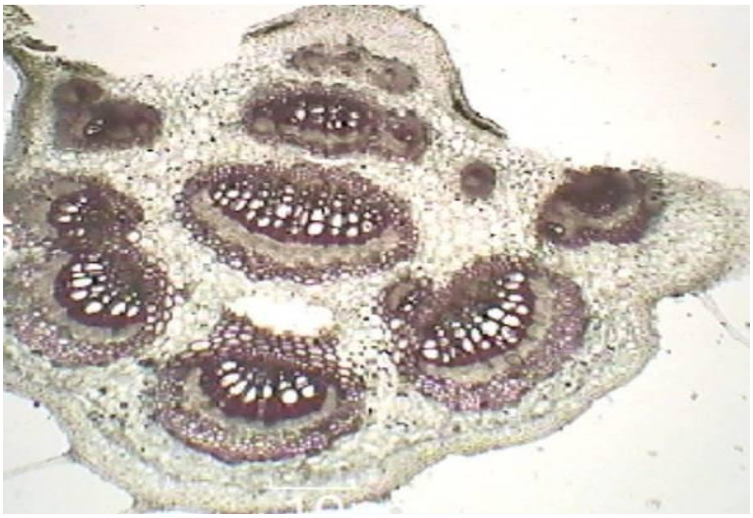
The plant was collected from Bobbili region, Vizianagaram, in the month of December. The leaves were washed thoroughly with sterile distilled water in order to remove any dirt or filthy particles present on the surface and were shade dried at room temperature for about 10 days. The shade dried leaves were pulverized in mixer grinder to form fine powder and passed through mesh size 100.

Three slides are prepared accordingly with different reagents as following and seen under compound microscope

1. T.S.+Glycerin

2. T.S.+Iodine solution+Glycerin

3. T.S+Phloroglucinol+Con.HCl+Glycerin



The transverse section of the leaf of *Couroupita guianensis* shows the presence of:

- Starch grains
- Anomocytic stomata
- Prism type of calcium oxalate crystals
- Covering trichomes
- Xylem vessels

Preparation of Extracts by successive solvent extraction

The finely powdered leaf drug of *Couroupita guianensis* about 80gm was extracted with chloroform (50-55^{0}C) for 72 hours by continuous hot percolation method using soxhlet apparatus. Then it was evaporated to form a dry mass of chloroform extract.

Aqueous extract is prepared by dissolving 500 ml distilled water in 500 gm of finely powdered leaf drug of *Couroupita guianensis* and kept for 36 hrs.Then it is filtered and evaporated

EVALUATION OF ANTI-COAGULANT ACTIVITY OF LEAF EXTRACTS OF *Couroupita guianensis*

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 was poured separately in plane containers using automatic pipette and stored at room temperature.

Collection of blood and Plasma recalcification

0.2 ml plasma, 0.1 ml of crude extract of different concentration and different volume of $CaCl_2$ (25 ml) were added together in a clean fusion tube and incubated at 37ºC 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 × g for 15 min, and plasma was separated and pooled. The freshly prepared plasma was stored at $4^{o}C$ 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 $37^{o}C$ 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 $37^{o}C$. 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. The activity is expressed in term of clotting time ratio in relation to control. The steps were repeated three times for each sample, and average of the test value was noted. Normal saline was used in place of the extracts for the negative control, and 50 mg/ml of EDTA for the positive control. Effect of chloroform and aqueous extracts on Prothrombin time (PT) was calculated by using stopwatch.

RESULTS

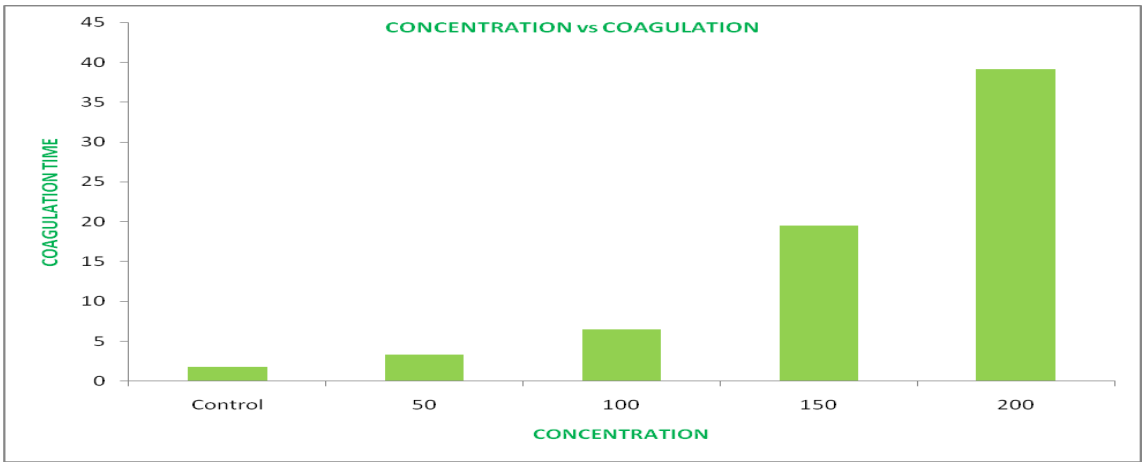
QUALITATIVE ANALYSIS OF BIOACTIVE COMPOUNDS IN DIFFERENT SOLVENT EXTRACTS OF *Couroupita guianensis* LEAVES

Test name	Chloroform extract	Aqueous extract
Mayer's	++	+
Wagner's	++	+
Dragendroff's	++	+
Tannins	+	+
Phlobatannins	++	+
Glycosides	++	++
Sterols	++	+
Resins	++	+++
Phenols	+	+
Anthraquinones	++	+
Carbohydrates	++	++
Cardiac glycosides	-	_
Steroids	+	+
Terpenoids	++	++
Alkaline reagent Test	+	+

ANTI-COAGULANT EFFECT OF LEAF EXTRACTS OF *Couroupita guianensis*

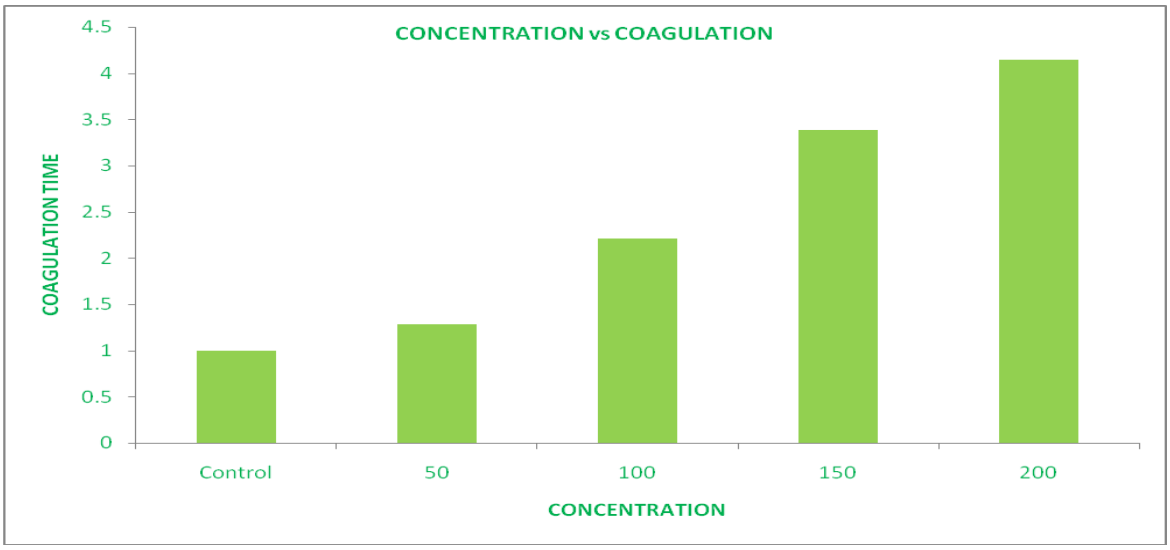
Determination of Coagulation time using Chloroform extract

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



Determination of Coagulation time using aqueous extract

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



DISCUSSION

Preliminary phytochemical analysis of chloroform extract & aqueous extract showed the presence of Flavonoids, Glycosides, Alkaloids, Steroids and Triterpenoids whereas the aqueous extract revealed the Tannins, Glycosides & Alkaloids as active phytochemical constituents. The data revealed that the 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. Screening and proper evaluation of alternatives that may be both sustainable and environmentally acceptable. The results of this study have shown promising anti-coagulant activity suggesting the possible use of extracts in haematology.

CONCLUSION

The phytochemical constituents like tannins, glycosides present in the *Couroupita guianensis* leaves shows potent anti-coagulant 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 rationale for the use of *Couroupita guianensis* as an anti-coagulant drug.

The results of the present study clearly indicated that the crude chloroform and aqueous extracts of *Couroupita guianensis* produce anti-coagulant activity. The plant possesses significant anti-coagulant activity at 0.1ml concentration measured by time taken for coagulation. The current investigation leads to conclusion that the leaves of *Couroupita guianensis* have potent anti-coagulant activity when compared with the conventionally used drug. Further studies using *in vivo* models and to isolate active constituents from extract are required to carry out and established the effectiveness and pharmacological rationale for the use of *Couroupita guianensis* as an anti-coagulant drug. There is growing interest in correlating the phytochemical constituents of a medicinal plant with its pharmacological activities.

The present study was carried out with a vision to set up standards that could be beneficial for detecting the authenticity of this vital medicinal plant. Numerical standards reported in this work could be useful for the compilation of a suitable monograph of *Couroupita guianensis*. As the plant produce secondary metabolites in order to protect themselves from microorganism,

herbivores and insects, thus anti-microbial effect is somehow expected from plants namely flavonoids, alkaloids, tannins, saponins and glycosides are producing a better opportunity for testing wide range of microorganism. The results obtained from this work revealed that the plants contained bioactive agents which are connected with anti-coagulant properties in plants.

Since *Couroupita guianensis* is easily available and well tolerated, it can be incorporated into medications for anti-coagulant agent. However, further studies for its incorporation into oral preparations, safety and cost- effectiveness has to be conducted.

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