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Phytochemical and acute toxicity study of *Cajanus cajan* fabaceae

[1] Zokoré Zazou Ange Kévin, [2] Yapo Adou Francis, [3] Okpekon Aboua Timothée, [4] Miezan Bilé Aka Patrice, [5] Droucoula Guillaume Cyril, [6] Koua Kadio Brou Donald, [7] Kouakou Siransy Gisèle

[1, 2, 4, 5, 6] Laboratory of Biochemical Pharmacodynamics, UFR Biosciences, Felix Houphouet Boigny University, Abidjan, Côte d'Ivoire

[3] Laboratory of Organic Chemistry and Natural Substances, UFR Sciences of Structures of Matter and Technology, Felix Houphouet Boigny University, Abidjan, Côte d'Ivoire

[7] Laboratory of Pharmacology, Clinical and Therapeutic Pharmacy, UFR Pharmaceutical and Biologic Sciences, Felix Houphouet Boigny University, Abidjan, Côte d'Ivoire

Abstract
Cajanus cajan, a plant of the Fabaceae family, is used in food and traditional medicine to treat various diseases. However, there is little information on its chemical composition and the toxicity of its leaves. Thus this study was undertaken to identify the chemical groups of *Cajanus cajan* leaves which justify its use in traditional medicine and to determine the toxicological parameters. Standard methods of characterization and thin layer chromatography (TLC) were used for phytochemical screening. The acute toxicity study of *Cajanus cajan* was carried out according to guideline OECD 423 using Wistar female rats. Phytochemical screening indicated the presence of flavonoids, sterols and polyterpenes, polyphenols, catechin tannins, quinone substances and saponosides. The acute toxicity study of the extracts, managed by oral way at Wistar rats, was used to determine 50 % lethal dose (LD_{50}) values of aqueous (LD_{50} = 3715.35 mg/kg body weight) and hydroethanolic extracts. (LD_{50} = 1174.90 mg/kg body weight). Also the maximum tolerated dose (MTD) values were 2500 mg/kg and 400 mg/kg body weight respectively for the aqueous and hydroethanolic extracts. The presence of these secondary metabolites would justify the many pharmacological properties of *Cajanus cajan*. Also, the toxicological data allow to conclude that the aqueous and hydroethanolic extracts are slightly toxic.

Keywords: *Cajanus cajan*, phytochemical screening, acute toxicity

1. Introduction
The use of plants in traditional medicine has played an important role in almost every culture on earth, including Asia, Africa, Europe and America. The plants are known as medicinal owing because they contain chemicals substances which can promote health and alleviate disease [1]. *Cajanus cajan* is a perennial species of the Fabaceae family. Also, known as pigeon pea, pea congo or ambrevado, this plant is a leguminous plant with seeds cultivated in rainfed agriculture in the semi-arid tropical areas, mainly in India [2]. *Cajanus cajan* is also found in Mexico, South America, West and East Africa [3]. It is used for feeding and treating various diseases such as swelling, pain, malaria [2]. However, in Côte d'Ivoire, there is little information on the chemical composition and acute toxicity of *Cajanus cajan* leaves. This study is part of the research of chemical compounds and evaluation of acute toxicity.

2. Material and Methods
Plant Material
The plant material used consists of *Cajanus Cajan* leaves. These leaves were collected in the area of Azaguié in the department of Agboville and were identified at the Centre National de Floristique (CNF) of Félix Houphouët Boigny University.

Animals
Adult Wistar rates weighing between 160 and 183 g were used for the evaluation of the acute toxicity of the extracts of *Cajanus cajan* by oral route. These animals were acclimated at room temperature of 26±1 °C with a relative humidity of 50±5 % and a 12 h light-dark cycle. The animals were placed in spacious hygienic plastic cages containing chips during the experimental period. These animals were fed with pellets from the company FACI® (Fabrication d'Aliments Composés Ivoiriens) and drank tap water.

Methods
Extracts Preparation : The leaves of *Cajanus cajan* were dried out of the sun at room temperature for about 2 weeks. The crushed dry leaves gave a greenish-colored powder.

Aqueous extract
100 g of *Canajus cajan* leaves powder were added to 1 liter of distilled water and the mixture was boiled for twenty minutes (20 min). The decoction was filtered twice on white cotton and once on filter paper Whatman N° 3. The filtrate was oven-dried at 40 °C for 2 days [4].

Hydroethanolic extract

100 g of *Canajus cajan* leaves powder were macerated for 24 hours in 1 liter of ethanol-water 70 %. The macerate obtained was then filtered twice on white cotton and once on filter paper Whatman N° 3. The filtrate was concentrated using a rotary evaporator and then oven-dried at 40 °C. for 2 days [5].

Phytochemical screening

The phytochemical study of aqueous and hydroethanolic extracts 70 % of *Cajanus cajan* was performed using the standard reactions described by Trease and Evans (2002) [6]. The presence of the different chemical groups was confirmed by the thin layer chromatography (TLC) method described by Wagner *et al*. (1984) [7].

The chemical composition of the reagents is a function of the nature of the phytochemical compound to be characterized.

The presence of the alkaloids in the extracts was highlighted by the reagents of Bouchardat, Dragendorff and Valser-Mayer.

The presence of flavonoids in the extracts was shown through the Chinoda test and gallic tannins through the ferric chloride solution.

The presence of quinones was demonstrated by means of the Borntraëger's reagent and the saponosides by the physical test of the foam.

Sterols and polyphenols were demonstrated using Liebermann's reagent, while catechin tannins were revealed using Stiasny's reagent.

For the performance of TLC, the same chemical reagents were used as indication reagents. DiphenylBorinate + Polyethylene Glycol was used for flavonoids, giving a observable yellow color in the visible. For polyterpenes and sterols, sulphuric vanillin was used giving a green coloring for polyterpenes and a coloring purplished for sterols at 254 nm UV. After preparation of the plate, the reagent is squirted and the plate is heated, the saponosides are indicated in red-purple compared to the reference (escin). Moreover, detection of alkaloids was performed by spraying the Dragendorff reagent giving the orange spots. For polyphenols, Fast Blue was used giving a blue color at 254 nm UV. For quinone compounds the potassium hydroxyl was used giving a blue color for Coumarins and yellow for Anthrones at 365 nm UV.

Acute toxicity study

The acute toxicity study was carried out according to the guideline OECD 423 [8]. Distilled water was used for the preparation of different concentrations of *Cajanus cajan* extracts. The concentrations of the extracts aqueous and hydroethanolic 70 % were prepared taking into account body weight and quantity of product to be administered. The mean weight of the rats was 166 ±3.54 g. The rats were fasted for 24 hours before administering the different doses of extracts of *Cajanus cajan*. For the oral dose of 100 mg/kg of body weight (mg/kg/bw), 1 mL of the distilled water is added to 16.6 g of extract corresponding to 16.6 mg/mL for an animal.

Aqueous extract

Nine (9) rats were divided into 3 lots of 3 comprising a control lot (lot 1). Each animal in the control group received 1 mL of distilled water orally. The rats from lots 2 and 3 received concentrations of aqueous extract by the oral way starting with the maximum dose of 5000 mg/kg body weight according to:

- Lot 3 → dose of 5000 mg/kg of body weight
- Lot 2 → dose of 2000 mg/kg of body weight

Thus, animals treated were subjected to continuous observation for 14 days with particular attention during the first 24 hours in order to identify the clinical signs and deaths in each group.

Hydroethanolic extract

Twelve (12) rats were divided into 4 lots of 3 comprising a control group (lot 1). Each sanimal in the control group received 1 mL of distilled water by oral way. The rats from lots 2, 3 and 4 received various concentrations of hydroethanolic extract 70 % orally starting with the maximum dose of 5000 mg/kg bw based on:

- Lot 4 → dose of 5000 mg/kg body weight
- Lot 3 → dose of 2000 mg/kg body weight
- Lot 2 → dose of 300 mg/kg body weight

The animals, thus treated, were subjected to continuous observation for 14 days with particular attention during the first 24 hours in order to identify the clinical signs and deaths in each group.

3. Results

The triphytochemistry of aqueous and hydroethanolic extracts of *Cajanus cajan* leaves revealed the presence of various phytochemicals. flavonoids, sterols and polyterpenes, polyphenols, the catechin tannins and quinone substances were present in both extracts but in more abundant quantities in the hydroethanolic extract. Alkaloids and gallic tannins missed in both extracts while the saponosides were present in the aqueous extract but absent in the hydroethanolic extract. Thin layer chromatography confirmed the results obtained by the tube reaction. Thus, the sterols and polyterpenes, the polyphenols and the quinones were present in the two extracts but in more abundant quantities in the hydroethanolic extract. As for the flavonoids, they were present in the hydroethanolic extract but absent in the aqueous extract (Table 1).

Acute toxicity

Just after administration of the extracts aqueous and hydroethanolic 70 % of *Cajanus cajan* to the female rats, clinical signs were observed. For the aqueous extract, at the dose of 5000 mg/kg body weight, we observed abdominal constrictions, a fast breathing, an immobility, a loss of appetite in the first 24 hours. These same signs were observed at the level of the hydroethanolic extract at doses of 5000 and 2000 mg/kg of body weight (Table 2).

Deaths were observed during the first 24 hours with the dose of 5000 mg/kg of body weight for the aqueous extract and with the doses of 5000 and 2000 mg/kg of body weight for the hydroethanol extract. However there were no deaths at doses of 2000 and 300 mg/kg body weight respectively for the aqueous and hydroethanolic extracts (Table 3). The data in Table 2 made it possible to plot the evolution curves of the mortality of the rats according to the logarithm of the doses of the aqueous and hydroethanolic extracts of *Cajanus cajan*. The LD_{50} values were 3715.35 and 1174.90 mg/kg bw

respectively for the aqueous and hydroethanolic extracts (Figures 1 and 2).

4. Discussion

The phytochemical study of aqueous and hydroethanolic extracts of *Cajanus cajan* leaves revealed the presence of flavonoids, sterols and polyterpenes, polyphenols, catechin tannins and quinone substances. Similar results have been reported in Nigeria by Oke [9] and Adaobi [10]. Oke showed the presence of flavonoids, terpenes, tannins, saponosides and alkaloids in the ethanolic extract of *Cajanus cajan* leaves. As for Adaobi [10] it highlighted the presence of saponins, tannins, terpenoids and resins but an absence of flavonoids and alkaloids in the methanolic extract of the leaves of *Cajanus cajan*. There is therefore a difference in secondary metabolites obtained between our tests and those of Oke [9] and Adaobi [10]. We note a presence of flavonoids from our results and those of Oke [9] but absent from those of Adaobi and a presence of alkaloids only from those of Oke [9].

In fact, the composition of a plant in secondary metabolites could vary according to the geographical situation, the organ sampled, the sampling period and the storage conditions [11]. These secondary metabolites would be responsible for the pharmacological properties of plants which could justify their use in traditional therapy. Thus according to Mulinnacci et al. [12], polyphenols have an antioxidant effect. Also, the polyphenols have hypotensive and antihypertensive properties shown by several authors such as Arora *et al*. [13], Rodrigo *et al*. [14]. According to Evans and Trease [6], terpenes have antimalarial activity and tannins known for their diuretic, antibacterial, antitumor, antiviral activities. The flavonoids also have diuretic properties [15] and antiallergic, antibacterial, anticancer, antiinflammatory properties [6]. According to Ngbede *et al*. [16], saponosides reduce hypercholesterol, hyperglycemia, and have an activity inflammatory. Quinones have antimicrobial, anticoagulant, antispasmodic [17] and antihypertensive properties [18].

The acute toxicity study by gavage determined the toxicological parameters of the aqueous and hydroethanolic extracts of *Cajanus cajan*. The aqueous extract had a maximum tolerated dose (MTD) of 2500 mg/kg body weight and an LD_{50} of between 2000 and 5000 mg/kg bw. For the hydroethanolic extract, the maximum tolerated dose (MTD) was 400 mg/kg and the LD_{50} is between 300 and 2000 mg/kg bw. The globally harmonized classification system makes it possible to classify the aqueous extract of *Cajanus cajan* leaves in category 5 and the hydroethanolic extract of category 4 and are defined as slightly toxic substances according to the Hodge and stener scale [19]. Our results were not consistent with those of Adaobi *et al*. [10] who obtained an LD_{50} above 5000 mg/kg orally for the methanolic extract of *Cajanus cajan* leaves on female rats. . This could be explained by the fact that the toxicity is related to the methods of preparation and administration of the phytomedicine [20].

Table 1 : Phytochemical Screening

		Sterols and polyterpenes	Polyphenols	Flavonoids	Tannins		Quinones Substances	Alkaloids		Saponoside
					Gal	Cat		D	B	
Tubes	HE	+ +	++	++	–	++	+	–	–	–
	AE	+	++	++	–	++	++	–	–	+
TLC	HE	++	++	++	ND	ND	++	ND	ND	ND
	AE	+	+	–	ND	ND	+	ND	ND	ND

AE: Aqueous extract ; HE: Hydroethanolic extract ; ND: Not detected ; Gal : gallic ; Cat : catechin ; B : Bouchardat ; D : Dragendorff
TLC : Thin Layer Chromatography ; - Absence ; + low presence ; ++ High presence

Table 2: Clinical signs observed during the first 24 hours after oral administration of the total aqueous and hydroethanolic extracts of *Cajanus cajan*

	Hydroethanolic extract				Aqueous extract		
Lots/Clinical signs	**Lot 1 (0 mg/kg bw)**	**Lot 2 (300 mg/kg bw)**	**Lot 3 (2000 mg/kg bw)**	**Lot 4 (5000 mg/kg bw)**	**Lot 1 (0 mg/kg bw)**	**Lot 2 (2000 mg/kg bw)**	**Lot 3 (5000 mg/kg bw)**
Abdominal constrictions	–	–	+	+	–	–	+
Immobility	–	–	+	+	–	–	+
Fast breathing	–	–	+	+	–	–	+
Difficult displacement	–	–	+	+	–	–	+
Food	–	+	–	–	+	+	–

+ Presence of signs ; - Absence of signs ; bw : body weight

Table 3 : Determination of the acute toxicity parameters in rats treated with aqueous and hydroethanolic extracts of *Cajanus cajan*

	Hydroethanolic extract				Aqueous extract		
Lots	**Lot 1 (témoin)**	**Lot 2**	**Lot 3**	**Lot 4**	**Lot 1 (témoin)**	**Lot 2**	**Lot 3**
Rat number	3	3	3	3	3	3	3
Injected doses (mg/kg bw)	0	300	2000	5000	0	2000	5000
Logarithm of injected doses		2.47	3.30	3.69		3.30	3.69
Dead rats number	0	0	2	2	0	0	2
Mortality (%)	0	0	66.66	66.66	0	0	66.66

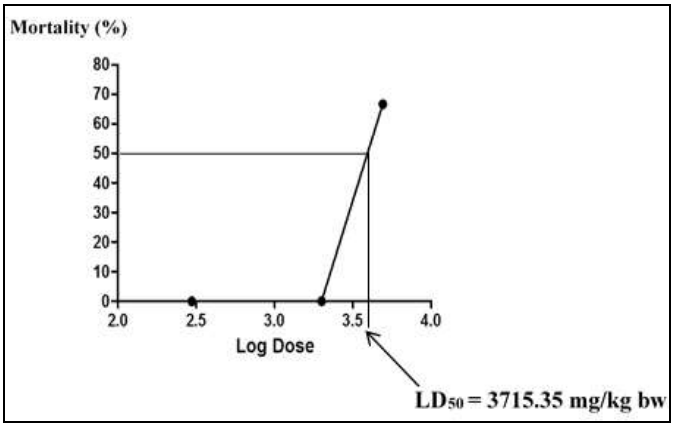

Fig 1: Rats mortality versus doses of aqueous extract leaves of *Cajanus cajan*

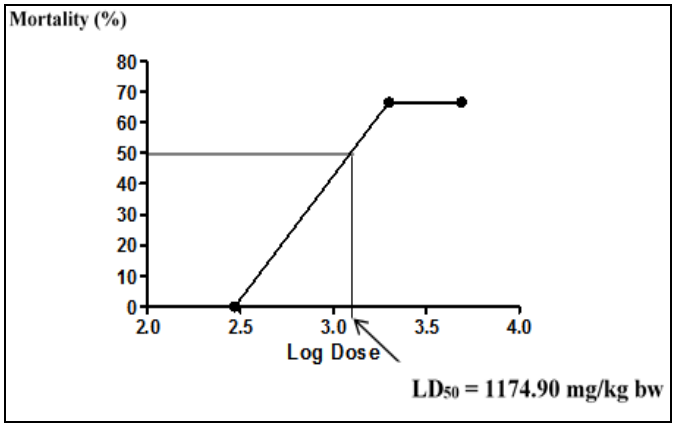

Fig 2: Rats mortality versus doses of hydroethanolic extract leaves of *Cajanus cajan*

5. Conclusion

This study identified the chemical groups such as flavonoids, sterols and polyterpenes, polyphenols, catechin tannins and quinone substances. Also, the study of the acute toxicity showed that the aqueous and hydroethanolic extracts are slightly toxic. Due to phytochemical and acute toxicity data, leaves of *Cajanus cajan* could justify its use in traditional medicine for the treatment of certain pathologies.

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7. Competing interests

Authors have declared that no competing interests exist.

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