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**ECOLOGICAL DISTRIBUTION, MORPHOLOGICAL CHARACTERISTICS AND ACUTE TOXICITY OF AQUEOUS EXTRACTS OF *HOLARRHENA FLORIBUNDA* (G. DON) DURAND & SCHINZ, *LEPTADENIA HASTATA* (PERS.) DECNE AND *CASSIA SIEBERIANA* (D C) USED BY VETERINARY HEALERS IN BURKINA FASO.**

H. H.Tamboura<sup>(1\*-3)</sup>; B. Bayala<sup>(3)</sup>; M. Lompo<sup>(2)</sup>; I. P. Guissou<sup>(2)</sup> and L. Sawadogo<sup>(3)</sup>.

<sup>1</sup>\*Institut de l'Environnement et de Recherches Agricoles (INERA)/CNRST), Département Productions Animales, UER/Biologie et Santé Animale 01 P.O.Box 476 Ouagadougou 01 Burkina Faso. <sup>2</sup>Institut de Recherches en Sciences de la Santé (IRSS/CNRST), Département de Médecine et Pharmacopée Traditionnelles, 03 BP 7047 Ouagadougou 03 (Burkina Faso). <sup>3</sup>Unité de Formation et de Recherche en Sciences de la Vie et de la Terre (UFR/SVT) - Université de Ouagadougou, Laboratoire de Physiologie Animale, 03 BP 7021 Ouagadougou 03 ( Burkina Faso).

\*E-mail : [hh\\_tamboura@hotmail.com](mailto:hh_tamboura@hotmail.com)

**Abstract**

As reproductive disorders were found to be one of the major constraints of livestock improvement in Burkina Faso, an ethno-veterinary survey showed that some plant species are used by traditional veterinary healers as basic components of indigenous recipes to treat them. The ecological locations, the current relative abundance in the area and the acute toxicity of the aqueous extracts of three selected species: *Holarrhena floribunda*, *Leptadenia hastata* and *Cassia sieberiana* were also investigated. The abundance of the plants was carried out in villages with semi-structured interviews of vet-healers, *in-situ* observations and then completion with library documentation while the acute toxicity was done in mice. The results showed that LD<sub>50</sub>s of 495 mg/kg, 24 mg/kg and 1513 mg/kg were observed for *Holarrhena floribunda*, *Cassia sieberiana* and *Leptadenia hastata* respectively. The relative abundance of these species across Burkina Faso is variable.

*Leptadenia hastata* is widely distributed, while *Holarrhena floribunda* is very scarce because of its high demand. *Cassia sieberiana*'s case is worrisome because the plant's root is the basic material required. The LD quotient and LD<sub>50</sub> obtained for *Leptadenia hastata* show that the plant is safe to use. Although the LD<sub>50</sub> obtained for *Holarrhena floribunda* is higher than that of *Cassia sieberiana* their LD quotients did not meet acceptable safety calculated value. However, it is more difficult to get enough crude material of *Holarrhena floribunda* and *Cassia sieberiana*. There is, therefore, an urgent need to build a sustainable scheme for these endangered species and their preservation.

**Keywords:** Acute toxicity; Ecology; Relative abundance; *Holarrhena floribunda*; *Leptadenia hastata*; *Cassia sieberiana*.

## Introduction

*Holarrhena floribunda* (G.Don) Durand et Schinz, *Leptadenia hastata* (Pers.) Decne and *Cassia sieberiana*(DC) are common plants in Saharian and Subsaharian Africa. They are often part of remedies in African veterinary therapies (Kasonia, 1998), particularly in Burkina Faso where they are used for treatment of several animal diseases (Tamboura et al., 1998). These plants have already been studied, especially their anti-dysentery properties, chemical composition and pharmacology of *Holarrhena floribunda* (Paris,1938; Crosnier et al.,1948; Schmit,1950; Goutarel,1964; CSTR/OUA,1985; Sourabié, 1990), the phytochemistry and the active constituents of *Leptadenia hastata* (Hutchinson and Dalziel, 1937; Nacoulma/Ouédraogo,1996; Nikièma, 1997), and the chemical composition of the leaves of *Cassia sieberiana* (Duquenois and Anton, 1968).

Major compounds found in *Holarrhena floribunda* are steroid alkaloids from two main chemical families: counanin and pregnen-5. Conessine, holarrhenine, holadienine, holamine, holaphylline, holaphyllamine and kurchicine are well known. Progesterone and some flavones (robinoside) were found in leaf extracts. In *Cassia sieberiana* roots or bark extracts, calcium oxalates, tannin sterols, anthraquinones as B-sitosterol, stigmasterol and phenolic compounds (leucopelargonicol, epicatechol) were found. In *Leptadenia hastata*, major chemical compounds found were: triterpens, fatty acid, amino acids, poly-oxypregnane , lutein,  $\beta$ -caroten (Aquino et al., 1996; Nikièma et al., 2001). *Leptadenia hastata* is commonly used in Niger for day-to-day cooking and is considered as hunger food due to it's very important content of valuable nutrients (Freiberger et al., 1998; Sena et al., 1998).

Regarding the data collected from healers and the proven chemical composition of these three plants, it was found interesting to conduct scientific investigations in order to confirm or reaffirm previous assessments. The present study is the first step in this process and focuses on two main objectives: i) to describe the natural and suitable ecology of the plants concerned, and assess their current relative abundance, regarding their uses and

exploitation made by healers and other end-users; and ii) to evaluate the acute toxicity of use of the aqueous extracts.

## **Materials and Methods**

### **Field work**

A multidisciplinary team (socio-anthropologist, veterinarian, pastoralist, agronomist) conducted ethno veterinary survey in 14 provinces concerned. 104 respondents, including both sexes (97 males, 14 women), from different ethnic groups (Fulani, Mossi, Bissa, Lobi, Gurunsi etc), drawn from different professions (herders, crop/livestock breeders) and ages (youngsters and elders) were involved. The methodology used "focus-group" at village level, individual semi-structured interviews and filling questionnaires. The healers took part in plant collection and identification. Part of the results have been published (Tamboura et al., 1998).

### **Plants and preparation of extracts**

The study was done on three plants: leaves of *Holarrhena floribunda* (Hf) (Apocynaceae), leaves and stems of *Leptadenia hastata* (Lh) (Asclepiadaceae) and root barks of *Cassia sieberiana* (Cs) (Caesalpiniaceae). The experimental plant parts used were collected at Gampèla, located at 25 km, south-east of Ouagadougou. They were first washed with large amount of water, then dried in a ventilated room, away from dust and direct sunlight. 150 g of dried materials were macerated in distilled water at 40°C. The obtained macerated product was then filtrated, run through Rotavapor (Buchi/R-114), lyophilised, and kept in a drier until ready for use. Yields of the extraction were 11.33%, 12.66%, and 8.66% for *Holarrhena floribunda*, *Leptadenia hastata*, and *Cassia sieberiana* respectively.

### **Experimental animals**

The toxicity study was performed on male albino mice (mean weight 31.65g  $\pm$  4.53 g) obtained from CIRDES (International Center for Research-Development on Livestock in Sub-humid regions) at Bobo-Dioulasso, Burkina Faso. Conditions of breeding were those fixed by the laboratory: food made of granules containing 20% of proteins; water *ad libitum*; natural light; constant cooling at 25°C; and breeding in standardised boxes.

For the acute toxicity assessment, we used the method of Trevan (1927) and its further modifications (Miller and Tainter, 1944; Liechtfield and Wilcoxon, 1949; Prieur, 1973; Descotes, 1985). A preliminary orientation-test was done using four (04) groups of three (03) mice. Then after, to evaluate the median Lethal Dose (LD<sub>50</sub>), we used six (06)

groups made of six (06) mice each. Each group (Gr) received a specific dose of the extract to be tested as follows:

- *Cassia sieberiana*: Gr.1: 10 mg/kg; Gr. 2: 17.5 mg/kg; Gr.3: 25 mg/kg; Gr. 4: 37.5 mg/kg; Gr.5: 50 mg/kg; Gr.6: 100 mg/kg.
- *Leptadenia hastata*: Gr.1: 1000 mg/kg; Gr. 2: 1250 mg/kg; Gr.3: 1500 mg/kg; Gr. 4: 1650 mg/kg; Gr.5: 1750 mg/kg; Gr.6: 2000 mg/kg.
- *Holarrhena floribunda*: Gr.1: 100 mg/kg; Gr. 2: 250 mg/kg; Gr.3: 500 mg/kg; Gr. 4: 750 mg/kg; Gr.5: 1000 mg/kg; Gr.6: 1500 mg/kg.

The dried material was reconstituted with distilled water at the time of utilization and then injected intraperitoneally. Injected mice were observed during 48 to 72 hours. Intoxication syndromes and number of dead mice were all recorded. Lethality was estimated as percentage of death for each dose of extract.

Pharm/pcs software, version 4.2 was used for LD values calculations (LD<sub>1</sub>, LD<sub>5</sub>, LD<sub>50</sub>, LD<sub>95</sub>, LD<sub>99</sub> ) then it was possible to get the relative ratios, i.e.: LD<sub>5</sub>/LD<sub>50</sub>, LD<sub>50</sub>/LD<sub>95</sub> and LD<sub>5</sub>/LD<sub>95</sub>. The toxicity scales of Hodge and Sterner (1943) , Done (1980) and Allain (2000) were used to rank the toxicity of each plant's aqueous extract by oral route in mice.

## Results

### Traditional utilization

The traditional utilizations of these plants are diverse and numerous, as shown in Table 1. According to estimations given by animals herders and traditional healers, the doses of extracts utilised for treatment of diseases with these plants are as follows:

*Cassia sieberiana* : one "pinch" (2g) dry powder of roots barks, per animal (sheep or goat # 25 kg) administrated *per os*; the leftover is used as bath or for external use.

*Leptadenia hastata* : one "ladlest" (equiv = 10 soup spoon each = 10 x 5 g) dry powder of whole plant, given *per os* for one goat or sheep ( # 25 kg) ; i.e. 50 g per head.

*Holarrhena floribunda* : two "pinch" (2 x 2g) of dry powder of leaves per day per head x one week (2 x 2 x 7 = 28 g ). This is given by oral route.

### Ecology, morphology and relative abundance of the plants

Data in Table 2 describes the morphological characteristics of the plants studied and the natural ecological areas where they are often found. The relative abundance of these plants across Burkina Faso is quite variable, according to the species concerned. *Leptadenia hastata* is widely found everywhere in bush. The availability of *Holarrhena floribunda* is less than *Leptadenia hastata*, due to the pressure of over harvesting and utilisations of the plant. So nowadays, the research institutes, NGOs and universities may cultivate *Holarrhena floribunda* for their purposes. *Cassia sieberiana*'s case is more crucial, as it's use requires the roots of the plant. The possibility of this plant

**Table 1:** Indigenous uses of remedies prepared with *H. floribunda*, *L. hastata*, and *C. sieberiana* (Kerharo and Adam 1974; Neuwinger, 1996; Adjanohoun et al., 1989; Tamboura et al., 1998).

<i>Cassia sieberiana</i>		<i>Leptadenia hastata</i>		<i>Holarrhena floribunda</i>	
Disease treated	Part of the plant used	Disease treated	Part of the plant used	Disease treated	Part of the plant used
Sterility, dysmenorrhea, amenorrhea, venereal disease, sex-impotence	Roots bark	Milk drying, Sex-impotence	Maceration of leaves	Sterility, vaginitis, abortion , hypertension	Maceration or infusion of leaves
Guinea fowl diseases, weakness of newborn	Juice from chewed roots	Trypanosomosis	Whole plant decoction	Blennorrhoea, gonorrhoea Sterility	Decoction of roots
Worms, Diarrhoea, diuretic, stomach pains, fever, bilharzias, leprosy, dropsy	Decoction or maceration of roots or leaves	Acute rhinopharyngitis wounds, injuries	Latex	Abortion, diabetes, dysentery, infectious diseases, local anaesthetic	Maceration or decoction of roots and stems

**Table 2**: Synonyms, phenological description and ecological areas of *Cassia sieberiana*, *Leptadenia hastata*, and *Holarrhena floribunda*

(Kerharo and Adam, 1974; Adjanohoun et al.,1989; Neuwinger, 1996) and data from our own survey in Burkina Faso.

Name of the plant	<i>Cassia sieberiana</i> (DC)	<i>Leptadenia hastata</i> (Pers.) decne	<i>Holarrhena floribunda</i> (G.Don) Dur et Schinz.
Synonyms	<i>Cassia kotschyana</i> Oliv.	<i>Leptadenia lancifolia</i> <i>Cynanchum hastatum</i> <i>Cynanchum lancifolium</i>	<i>Holarrhena africana</i> <i>Rondeletia floribunda</i> <i>Holarrhena wulfsbuzi</i>
Botanical family	Caesalpiniaceae	Asclepadiaceae	Apocynaceae
Vernacular names	Fulani: Sisanghi Moore: kumbrisaka Diola: kaset, buseit Wolof: Sindan, sindâ Lobi: Olonkwo,tekar Bissa: Jambayiga	Fulani: sabato, kasubi Moore: lolongo Diola: busumba amata Wolof: tarhat, darhat	Fulanil: tarki; taraki Moore: Kinkirs yoagba Wolof: séulu; salali
Remarkable phenotypic characteristics	Shrub or small tree, 7-15m high, with large pendulous raceme of bright yellow flowers; persistent long cylindrical and narrow fruit; leaves:20-30 cm long, 4 to 9 pairs of opposite leaflets	Voluble herb with several creeping latex stems; glabrescent leaves; glomerulus and racemus flowers; follicles fruits.	Small to medium size tree (10-15m or 3-4m high ) depending on ecological zones ; oval to lanceolated leaves; Corymbiform axillaries flowers; linear follicles.
Ecology	- Common in savannah woodlands (Sudan and Sudano-guinean countries) wet forest, fringing forest, sandy and lateritic soils.	- Tropical dry lands of Africa; - grows preferably on sandy and dunes or gravelled areas.	Very common in undergrowth close to forest gallery and woodland savannah, or in dry savannah; grows on sandy humid or lateritic soils.

going into extinction is high because the amount of extracted material is quite huge! Consequently, there is an urgent necessity to build a sustainable scheme of management for preservation and multiplication of this species in suitable locations.

### Acute toxicity

#### *Cassia sieberiana*

The orientation test indicated that LD<sub>50</sub> values varied between 10 and 50 mg/kg. Trial conducted on 6 groups of 6 mice each came out with an LD<sub>50</sub> for *Cassia sieberiana* aqueous extracts of 24.40 mg/kg. Calculations using the software gave the lethal values in Table 3. The signs of intoxication are lateral constriction, lack of appetite and asthenia.

**Table 3:** Lethal doses (LD) (mg/kg) after 72 hours and intoxication syndromes of *Leptadenia hastata*, *Cassia sieberiana*, and *Holarrhena floribunda* aqueous extracts in mice.

<i>Leptadenia hastata</i> (Pers.) decne	<i>Cassia sieberiana</i> (DC)	<i>Holarrhena floribunda</i> (G.Don) Dur et Schinz.
LD <sub>.1</sub> = 1294.214	LD <sub>.1</sub> = 8.454	LD <sub>.1</sub> = 121.433
LD <sub>5</sub> = 1389.149	LD <sub>5</sub> = 11.539	LD <sub>5</sub> = 183.318
LD <sub>50</sub> = 1512.879	LD <sub>50</sub> = 24.40	LD <sub>50</sub> = 495.410
LD <sub>95</sub> = 1768.370	LD <sub>95</sub> = 51.88	LD <sub>95</sub> = 1338.828
LD <sub>99</sub> = 1825.867	LD <sub>99</sub> = 70.73	LD <sub>99</sub> = 2021.124

#### *Leptadenia hastata*

The orientation test showed that LD<sub>50</sub> values varied between 1000 and 2000 mg/kg. The trial conducted on 6 groups of 6 mice each gave an LD<sub>50</sub> value of 1512.879 mg/kg for *Leptadenia hastata* aqueous extract. Other critical lethal values obtained by software calculation are provided in Table 3. Signs of intoxication observed are polypnoea and asthenia.

#### *Holarrhena floribunda*

The orientation test showed that LD<sub>50</sub> values varied between 400 and 1000 mg/kg. Experiment using 6 groups of 6 mice each, showed an LD<sub>50</sub> value of 495.410 mg/kg for *Holarrhena floribunda* aqueous extract. Using appropriate software,

calculations led to figures of other critical lethal values mentioned in Table 3. Total eyes closing, lack of appetite and asthenia were observed as signs of intoxication.

## Discussion

Results on traditional uses of local plants got from our field survey are consistent with data obtained by previous authors (Kerarho and Adam, 1974; Bouquet and Debray, 1974; Vasileva, 1969; Basir and Odebiyi, 1974; Levy et al., 1990 ; Duez et al., 1987). Different parts of the plants are used, sometimes solely, some other associated with other plant or material. The local healers treat generally the major signs observed instead of addressing a specific aetiologic agent. The commonest diseases cited were in the digestive area: diarrhoea, gastric pains, dysentery, worms, constipation; in the reproductive area, we noted sterility, sex-impotence, abortion, amenorrhoea, dysmenorrhoea, non delivery, urinary diseases. Other important diseases are diabetes, ascites, fever, rhinopharyngitis, acne and sleeping disease (trypanosomosis).

The LD<sub>50</sub> figures obtained were 24 mg/kg for *Cassia sieberiana*, 1513 mg/kg for *Leptadenia hastata* and 495 mg/kg for *Holarrhena floribunda*. The ratios of LD<sub>5</sub>/LD<sub>50</sub> and LD<sub>50</sub>/LD<sub>95</sub> are quite equal for each plant studied, confirming the validity of our LD<sub>50</sub> test-values (Somé et al., 1995-1996). The last quotient, i.e. LD<sub>5</sub>/LD<sub>95</sub> shows that we got 0.13, 0.22, 0.78 for *Holarrhena floribunda*, *Cassia sieberiana* and *Leptadenia hastata* respectively. It means that *Leptadenia hastata* is very safe for use, as compared to the two others. Based solely on the above LD<sub>50</sub> figures and according to Hodge and Sterner (1943) and Done (1980), it can be concluded that *Cassia sieberiana* is very toxic, compared to *Holarrhena floribunda* (less toxic or noxious) and *Leptadenia hastata* (not toxic). This classification agrees with Allain (2000) who ranks all plants whose LD<sub>50</sub> figures are less than 25 mg/kg in “very toxic” group, between 25mg/kg and 200 mg/kg in “toxic” group, and from 200 mg/kg to 2000mg/kg in “noxious” group. The LD quotients gives the range of safe use of the product i.e., the wave between the therapeutic point and the toxic point. According to Allain (2000), if the LD quotient value is close to 1.0, the plant's is regarded as safer to use than the one with very small (0.22) or closer to zero (0.13). In our experiment, *Leptadenia hastata* which has a high LD quotient is safer to use, whereas the two other plants have small values. These small values means that the wave is short between therapeutic and toxic values. *Cassia sieberiana* has a very small LD<sub>50</sub> (24 mg/kg) and LD quotient too, therefore, great caution is required should it be used. Its use should be discouraged. In the case of *Holarrhena floribunda*, we can explain the situation as did Ouédraogo et al. (2003) and Ouattara/Danté et al., (2003) respectively for *Calotropis procera* and *Fagara xantylloides* : The plant (*H. floribunda*) has a very small LD quotient, but its LD<sub>50</sub> is moderately high (495mg/kg), although the toxic and the therapeutic values are close to each other, the day-to-day quantities used by healers are very far less from these values; then, there may be no risk if properly used. Secondly, the herders and healers are very conscious of the toxicity problems. The doses of dried powder utilized for treatment are quite safe regarding our LD<sub>50</sub> values obtained in this study.

The low toxicity of *Holarrhena floribunda* was reported by Schmit (1950) who used water extracts of barks from trees originating from Senegal, Guinea and Togo, on paramecium and daphnia. Paris (1938) also showed on guinea pigs, the slight toxicity of extracts of *Holarrhena floribunda* roots. Our results agrees with those of Millogo/Koné (1992) who reported on white mice the low toxicity of different amounts of aqueous extracts of leaves of *Holarrhena floribunda*. But, our figure of LD<sub>50</sub> was higher (double), compared to that one obtained by Akah and Nwambie (1993), i.e., 200 mg/kg of alcoholic root extracts.

*Leptadenia hastata* is commonly used as food by many African populations (Hutchinson and Dalziel, 1937). For instance, it's leaves are often chewed by shepherds against polydipsia and mouth dryness (Kerharo and Adams, 1974; Olivier-Boyer, 1986). The main reason for that is their high content of water, minerals and vitamins (phosphorous, glucose, cellulose, vitamins B1, B2, B3, C, and amino-acids) (Nacoulma/Ouédraogo, 1996).

Neuwinger (1996) reported that in many west African regions, from Senegal/Gambia/Guinea to Nigeria, *Cassia sieberiana* is very well known as a very active poison, widely used for hunting and fishing or as arrow poison. These findings are consistent with it's common knowledge in Sudan; there, it is well known that *Cassia sieberiana* will never be eaten by any animal, even when they are in danger of acute starvation. Adjanohoun et al. (1989) reported that the growth substrate of the plant could influence its toxicity, like those growing on termitary which should be more toxic. The age of the plant and the vegetal part used could also explain the variability and high amount of toxicity of *Cassia* gender members (Suliman *et al.*, 1982; El Sayed et al., 1983), although many species are used as food or medicine laxatives, without any toxicity (Salunkhe *et al.* 1982). Our results on the toxicity of *Cassia sieberiana* agrees with those of Dhar et al. (1968) and Mugeru (1970) in goats and sheep respectively. They found that *C. sieberiana* is very toxic.

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