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Ethnobotanical Survey and Biological Activities of Two Lactogenic Plants in the Cascades Region of Burkina Faso

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Abstract: An ethnobotanical survey for a better knowledge of lactogenic plants has been conducted in Cascades region of Burkina Faso. Biological activities of the two most used plants were evaluated. The survey concerned 25 species classified into 22 genera and 19 families. The most represented families were Moraceae, Apocynaceae, Fabaceae-Caesalpinioideae and Malvaceae. The most used species were *Euphorbia hirta, Calotropis procera* and *Ficus sycomorus* with respectively 16.98%, 13.21% and 13.21% followed by *Ficus platyphylla* (9.43%) and *Carica papaya*, *Holarrhena floribunda*, *Scoparia dulcis*, *Sorghum bicolor*, each with 3.77% of frequency use. Plants organs mostly used for medicinal preparation were leaves, whole plant for herbaceous species, fruits and seeds. The main mode of medicinal preparation was decoction with 60.42% of frequency use. The most route of administration was oral (68.75%). Biological activities evaluation concerned *Euphorbia hirta* and *Calotropis procera* which were the most used plants. The acute toxicity of aqueous extracts of *Calotropis procera* (AECP) and *Euphorbia hirta* (AEEH) showed a LD_{50} of 2063 and 603 mg/kg body weight (b.w), respectively. These LD_{50} values permitted to classify these plants as slightly toxic herbal drugs. The uterotrophic test showed that the AECP and AEEH exhibited estrogenic activity. In conclusion, important medicinal plants in the Cascades region of Burkina Faso were used to stimulate or ameliorate lactation. However further studies must be conducted to confirm the traditional use of these plants.

Keywords: Ethnobotanical Survey, Toxicity, Estrogenic Activity

1. Introduction

Ethnobotanical investigation constitutes an important factor to know and appreciate the medicinal potentials of the vegetal biodiversity in Burkina Faso [1]. Medicinal plants were used by people for their health care [2]. In Burkina Faso, most people (70%) in particular, in rural areas use the traditional medicine and medicinal plants for the treatment of various pathologies and diseases [3] [4] [5]. Lactogenic plants are the most of the medicinal plants that were used by traditional healers [6]. Those lactogenic herbs are among the most widely used especially in developing countries where infant feeding is based on milk as substitutes [7].

In the present study an ethnobotanical survey was realized to check off the plants traditionally used in the Cascade region against lactation pathologies such as lactation insufficiency. The two most plants *Euphorbia hirta* (*E. hirta*) and *Calotropis procera* (*C. procera*) were used for some biological activities. For their innocuity and their safe of use the acute toxicity was evaluated. The effect of these extracts on the female reproductive tract was evaluated by uterotrophic assay. All animal experiments in this study, were carried out in accordance with University of Ouaga I Pr. Joseph KI-ZERBO, Ethical Committee Acts.

2. Materials and Methods

2.1. Study Area

Cascades region is located at some 440 km from the capital city of Ouagadougou in the south-western part of Burkina Faso. The surface area of this region is 18407 km² (6.7 % of the national area) [8]. The region has two provinces Comoé and Léraba and is limited in the East by the South-west region, in the North by Hauts-Bassins region, in the West by Mali republic and in the South by Ivory Coast republic (Figure 1). The region has a south-sudanian climate with a precipitation from 900 to 1000 mm [9]. Its population was estimated at 531 808 persons [8]. The main economic activity practiced by populations is agriculture. The choice of the Cascades region was based firstly on the best knowledge of the different localities. Secondly on the good mastery of the local languages where the more spoken is Dioula. Finally, on the basis that no survey has been conducted on lactogenic plants.

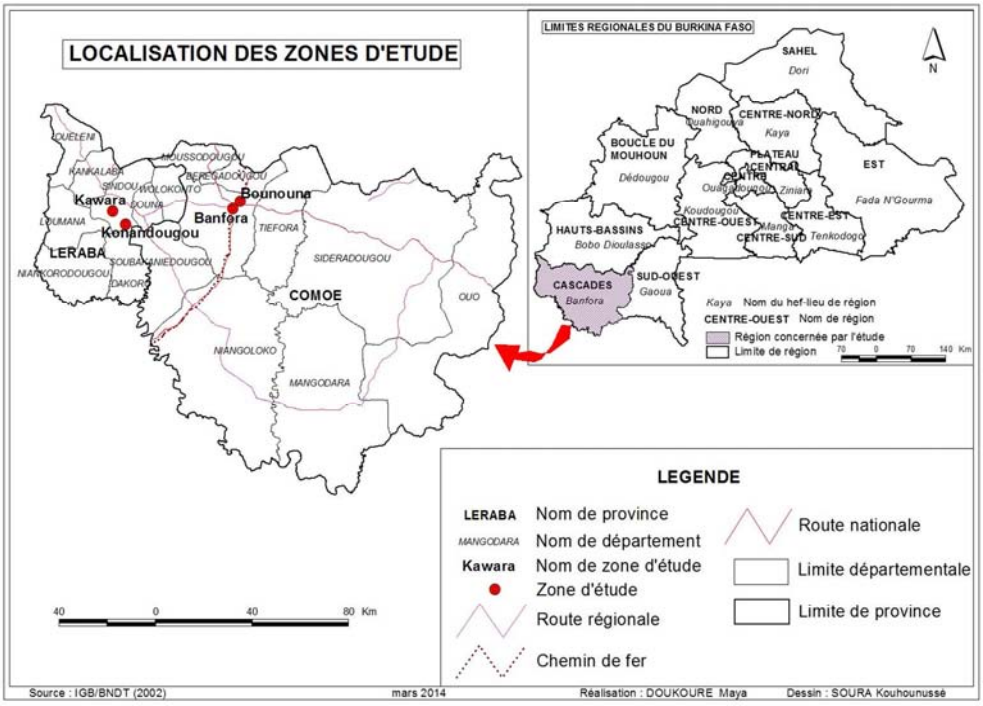


Figure 1. *Localization of the study area.*

2.2. Data Collection

Ethnobotanical survey was achieved in Cascades region from December 30, 2013 to January 10, 2014 for data collection. This survey was based on semi-structured interviews with 36 people including traditional healers and mothers who have a knowledge on the lactogenic plants. Plant species, vernacular names, organs of plants, mode of preparation and mode of administration were recorded during the interviews. Therefore, specimens were collected in Bounouna forest and were identified by the Herbarium of University Ouaga I Pr. Joseph KI-ZERBO where the voucher samples were deposited under numbers 6845 and 6846 for *C. procera* and *E. hirta*, respectively.

2.3. Collection of Plant Materials

Whole plants of *E. hirta* and the branch leaves of *C. procera* were collected in Banfora province of Comoé (Cascades region) in morning at 6 to 11 AM in May 2014. The two plants sample were washed with tap water, dried under ventilation in the shade. The dried sample of the two plants were pulverized using a mechanical grinder. The powder obtained was used for extraction.

2.4. Preparation of Plant Extracts

One hundred fifty (150) g of dried materials of each plant were macerated in 1500 ml of solvent: distilled water and a mixture methanol/water (80/20 v/v) during 24h and 48h, respectively at room temperature (25 and 30°C). The mixture of aqueous extract was then filtered through tissue wool and the filtrate was centrifuged at 2000 rpm/min. Hydro-alcoholic extract was filtered through whatman paper. The filtrate and the supernatant obtained were then concentrated by rotary evaporator under reduced pressure at temperature (40-50°C) and dried within sweating-room at temperature of 40°C. The two dry extracts were packaged in bottle and stored in the refrigerator. The yield of dry aqueous and methanol/water extracts were respectively 14.9% and 9.7% for *E. hirta*, 20.5 % and 10.8 % for *C. procera.*

2.5. Animal Model

Male and female immature and adult NMRI (Naval Medical Research institute) mice were obtained from the animal house of University Ouaga I Pr Joseph KI-ZERBO. The room temperature was maintained at 22±3 °C with the

12h light/12h dark cycle and humidity at 50 ± 10%. The animals were fed with industrial pellets with 29% protein and have free access to drinking water. All the tests in this work were performed according to the protocols already approved by the Department of Animal Physiology of University Ouaga I Pr Joseph KI-ZERBO and met the international standards of animal study [10].

2.6. Acute Toxicity Test in Mice

For the toxicity evaluation, we used the method of [11] adapted by [12]. The toxicity study was achieved in two tests, a preliminary and a definitive test. For the preliminary test, eight (08) groups of three (03) mice each have been used for aqueous extracts of *C. procera* (AECP) and another eight (8) group of three mice each for aqueous extracts of *E. hirta* (AEEH). The different groups of AECP and AEEH were treated with 50, 100, 200, 400, 800, 1000, 2000 and 3000 mg/kg b.w respectively.

The aim of the preliminary test was to determine the dose which causes 0% and 100% of mortality in mice. The definitive test consists to evaluate the Lethal Dose (LD).

Then six (06) groups of six (06) mice each have been used for *C. procera* and seven (07) groups of six (06) mice each for *E. hirta*. For AECP, the 6 groups of animals were intraperitonealy treated with 1000, 1500, 1750, 2000, 2500 and 3000 mg/kg respectively and for AEEH, animals for the 7 groups were intraperitonealy treated with 100, 200, 400, 600, 800, 1000 and 2000 mg/kg respectively.

The different doses were extemporaneously prepared with distilled water. Animals were dosed once at a time. After the treatments, mice were observed 1h, 24h, 48h and 72h. Intoxication syndromes and the number of dead mice were recorded 1h, 24h, 48h and 72h after the administration.

2.7. Uterotrophic Test of Aqueous Extracts of C. Procera and E. Hirta

Immature female mice, approximately seven week days old and weighing 20.07 ± 0.23g were used. Experimental mice were randomly divided into six (06) groups of six (06) mice each. Mice of group 1 were treated with DMSO (1%) and served as blank control, and those of group 2 were treated with 17-β-estradiol at the dose of 10µg/kg b.w. and served as reference control. The mice of group 3 and 4 received AEEH, respectively at the dose of 50 mg/kg and 100 mg/kg b.w. Those of group 5 and 6 received AECP at the dose of 50 mg/kg and 100 mg/kg b.w, respectively. The treatments were achieved for 3 consecutive days. Twenty-four (24) hours after the last treatment, the mice were weighed, sacrificed by cervical dislocation and weight of ovaries, uterus, vagina and adrenal gland were recorded.

2.8. Data Analysis

The results are expressed as mean ± Standard error of mean (SEM). ANOVA I followed by Dunnett's Multiple Comparison Test was performed. The Excel version 2013 and Graph Pad Prism version 5.03 were used respectively for data recording and graphs drawing. P-values < 0.05, were considered significant. The Win LD_{50} software, version 2.0 was used to determine the lethal doses.

3. Results

3.1. Ethnobotanical Survey

Ethnobotanical survey showed 25 species as potential lactogenic plants. These species belong to 19 families and 22 genera. Moraceae, Apocynaceae, Fabaceae-Caesalpinioideae and Malvaceae were the most dominant families of lactogenic plants (Table 1).

Table 1. *Plant species and use frequency.*

Species	Family	Frequency use (%)
Euphorbiahirta	Euphorbiaceae	16.98
Calotropisprocera	Asclepiadaceae	13.21
Ficus sycomorus	Moraceae	13.21
Ficus platyphylla	Moraceae	9.43
Holarrhenafloribunda	Apocynaceae	3.77
Caricapapaya	Caricaceae	3.77
Sorghum bicolor	Poaceae	3.77
Scopariadulcis	Scrophulariaceae	3.77
Annonasenegalensis	Annonaceae	1.89
Saba senegalensis	Apocynaceae	1.89
Leptadeniahastata	Apocynaceae	1.89
Guierasenegalensis	Combretaceae	1.89
Ipomoeaasarifolia	Convolvulaceae	1.89
Diospyrosmespiliformis	Ebenaceae	1.89
Cassia obtusifolia	Fabaceae- Caesalpinioideae	1.89
Cassia sieberiana	Fabaceae- Caesalpinioideae	1.89
Flemingiafaginea	Fabaceae- Faboideae	1.89
Parkiabiglobosa	Fabaceae-Mimosoideae	1.89
Agelanthusdodoneifolius	Loranthaceae	1.89
Gossypiumherbaceum	Malvaceae	1.89
Hibiscus sabdariffa	Malvaceae	1.89
Ficus ingens	Moraceae	1.89
Gardeniaternifolia	Rubiaceae	1.89
Vitellariaparadoxa	Sapotaceae	1.89
Ocimumamericanum	Lamiaceae	1.89

The most potential lactogenic plants species used by traditional healers are *Euphorbia hirta* (16.98%) followed by *Calotropis procera* (13.21%), *Ficus sycomorus* (13.21%), *Ficus platyphylla* (9.43%) and *Carica papaya*, *Holarrhena floribunda*, *Scoparia dulcis*, *Sorghum bicolo*r each with 3.77% (Table 1).

Whole plant or different organs of plant including leaves, fruits or seeds, stems, bark, root and flowers are used by the traditional healers. Organs of plants mostly used in the preparation of medicinal recipes are leaves (32.69 %), whole plant (26.92%), fruits or seeds (15.38%), branch leaves (9.62%), bark (7.69%), stem (3.85%), root and flower with 1.92% each (Figure 2).

We noted three (03) main modes of preparation using medicinal plants: decoction, trituration and maceration. Decoction was the most method of preparation of recipes (60.42%) followed by trituration (33.33%) and maceration (6.25%) (Figure 3).

Oral administration and cutaneous application (washing and massage) were the route of administration recommended with 68.75 % and 31.25 %, respectively (Figure 4).

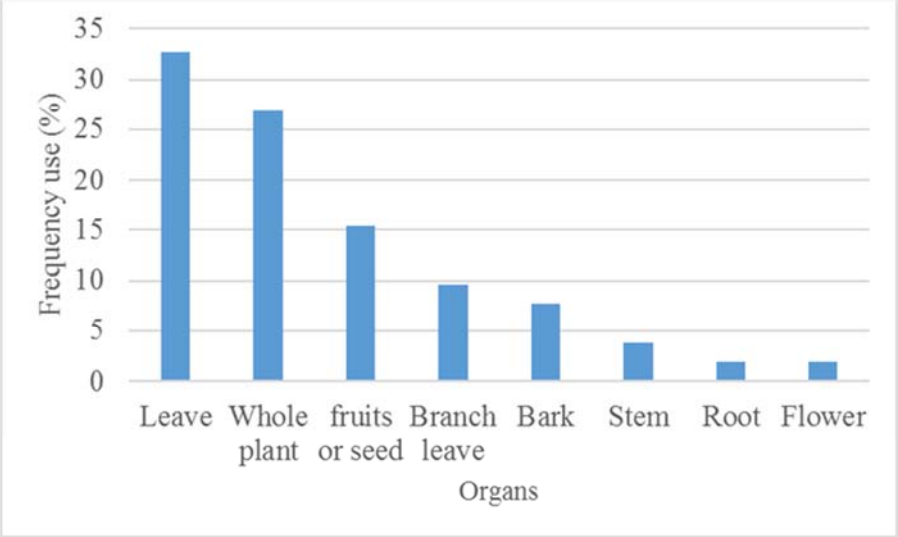


Figure 2. *Use frequency of plant organs.*

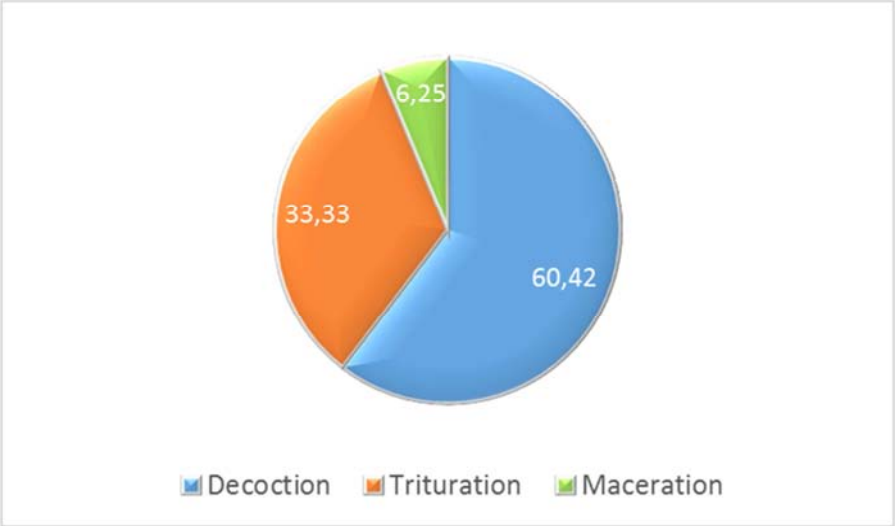


Figure 3. *Frequency of different modes of preparation.*

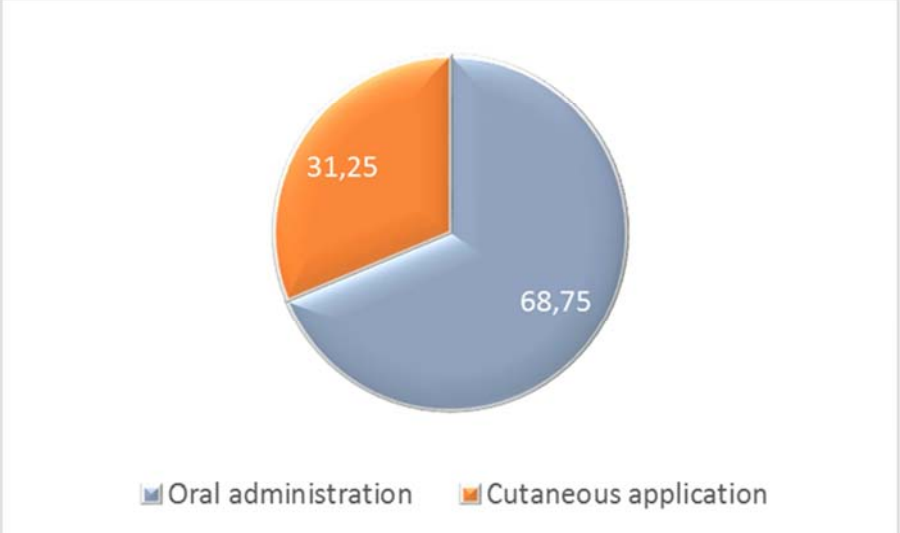


Figure 4. *Frequency of different modes of administration.*

3.2 Acute Toxicity Study of the Plants Extracts

The preliminary test of *C. procera* indicated that LD values varied between 1000 and 3000 mg/kg. For *E. hirta,* the preliminary test showed that 2000 mg/kg causes 100% of mortality. The definitive test of the acute toxicity showed that LD_{50} of *C. procera* was 2063 mg/kg and 603 mg/kg for *E. hirta*. The intoxication signs of the two extracts were abdominal constrictions, lack of appetite and immobility. The ratio LD_5/LD_{50} and LD_{50}/LD_{95} were 0.66 and 0.66; 0.277 and 0.277 for *C. procera* and *E. Hirta* respectively. The ratio LD_5/LD_{95} was 0.44 and 0.076 for *C. procera* and *E. hirta* respectively (Table 2).

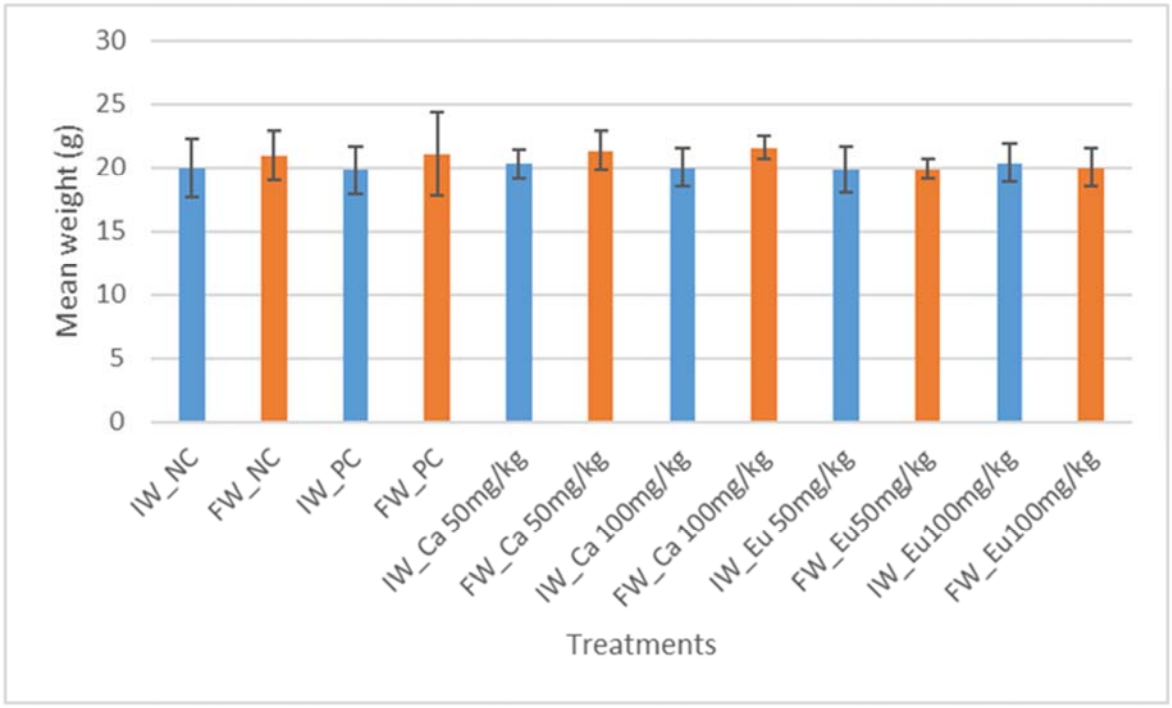
Table 2. *Lethal doses (LD) (mg/kg) and proportion of (LD) after 72 h of C. procera and E. hirta*

Treatments	LD	Values	Ratios of LD
	DL_1	1154	DL_5/DL_{95}= 0,44
	DL_5	1368	
Calotropis procera	DL_{10}	1498	DL_5/DL_{50}= 0,66
	DL_{50}	2063	
	DL_{90}	2841	
	DL_{95}	3111	DL_{50}/DL_{95}= 0,66
	DL_1	98	DL_5/DL_{95}= 0,076
	DL_5	167	
Euphorbia hirta	DL_{10}	222	DL_5/DL_{50}= 0,277
	DL_{50}	603	
	DL_{90}	1636	
	DL_{95}	2172	DL_{50}/DL_{95}= 0,277

3.3. Uterotrophic Test

AECP and AEEH at the doses of 50 and 100 mg/kg b.w. showed no significant difference ($P > 0.05$) on mice body weight compared to control groups (Figure 5). On the weight of adrenal glands, AECP and AEEH at the doses of 50 and 100 mg/kg showed any significant ($P > 0.05$) difference compared to control groups. On ovaries, the doses of 50 and 100 mg/kg of AEEH show similarly to 17-β-estradiol, a significant increase ($p<0.05$) of the weight of this organ compared to control group. Ovaries weight increased a dose-dependent manner when compared of those to control group (Figure 6).

Both doses 50 and 100 mg/kg of AECP and AEEH, similarly to 17-β-estradiol dose, induced a significant ($p<0.05$) increase of the weight of uterus. Indeed, the AECP increase the weight of uterus in a dose-dependent manner. However, the low dose (50mg/kg) of AEEH induced a significant ($p<0.001$) increase of the weight of uterus than the high dose (100 mg/kg). Among the different treatments, only the dose 100mg/kg of AECP caused a significant ($p<0.001$) increase of vagina weight (Figure 7).



IW: Initial Weight; FW: Final Weight; NC: Negative Control; PC: Positive Control; Ca: Calotropis procera; Eu: Euphorbia hirta

Figure 5. *Body weight of mice before and after treatment with the aqueous extracts of Calotropis procera and Euphorbia hirta.*

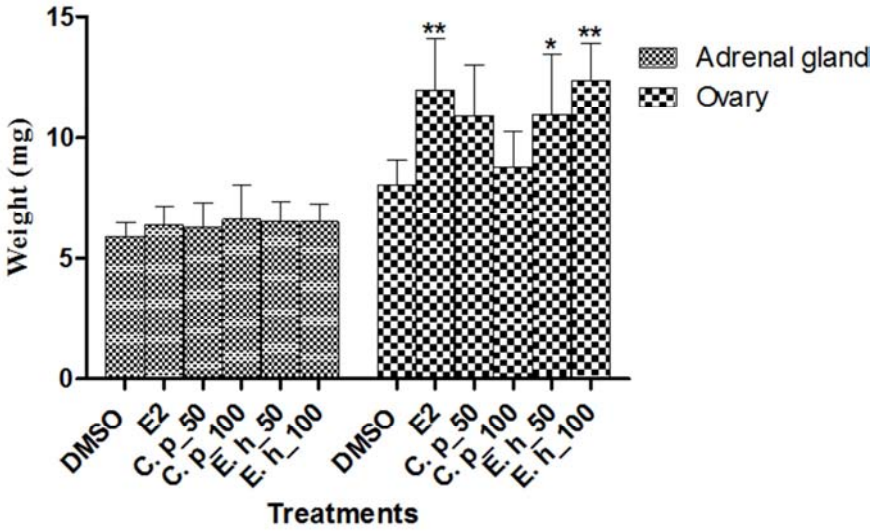


Figure 6. *Effect of AECP and AEEH on ovary and weight (C. p: Calotropis procera, E. h: Euphorbia hirta, E2: 17β-estradiol, DMSO: dimethylsulfoxide) *P<0.05 **P<0.01.*

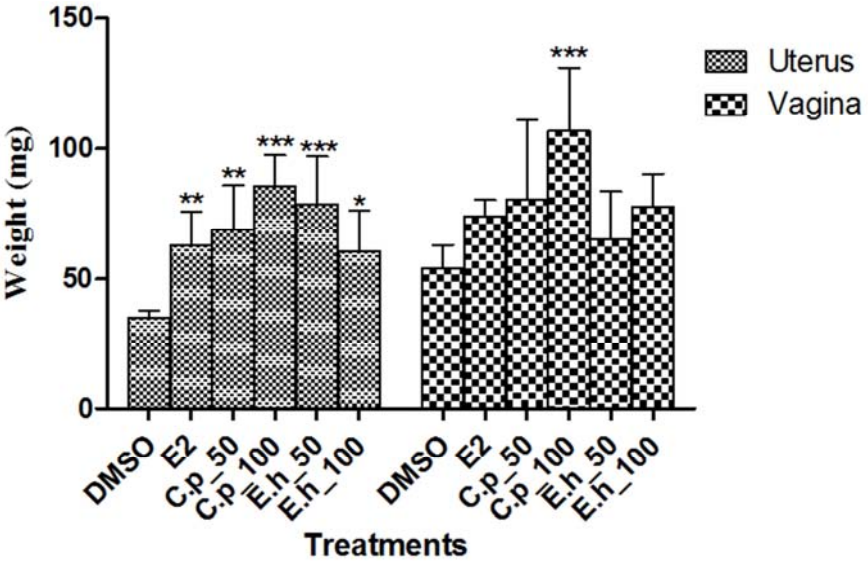


Figure 7. *Effect of aqueous extracts of Calotropis procera and Euphorbia hirta on uterus and weight(*P<0.05 **P<0.01 ***P<0.001).*

4. Discussion

Ethnobotanical survey conducted in Cascades region has permitted to identify 25 species used as lactation herbs. The most used species were *E. hirta* followed by *C. procera* and *Ficus sycomorus*. *E. hirta* was the most dominant species; this result is similar to Sawadogo [13] and Nacoulma [3], who have noted that this species are used in the lactation by the traditional healers. Moreover, another results [14] have showed that Euphorbiaceae family belongs to the most family of medicinal species plants which are used for the treatments of lactation disorders and menstrual disorders in waterside soil of cynegetic area of Pendjari (Benin).

Some authors already showed that *C. procera* and *Ficus sycomorus* possess lactogenic property and they noted that *C. procera* is very effective [3] [15]. Organs of plants mostly used for medicinal preparation were leaves. These results are in agreement with those of other authors who also indicated that the leaves are the organ of the plant mostly solicited in the preparation of medicinal recipes [14] [16] [17].

Among the different methods of preparation, the decoction was the main mode of medicinal preparation. Our results are similar to those of other researchers [18] [19] [17] who showed that the decoction is the most method used by the traditional healers for their medicinal preparation. Our results also indicated that the oral drinking was the most route of administration. This result is similar to other authors [18] [16] [17].

Many investigations on *C. procera* and *E. hirta* have reported that the two plants have various pharmacological properties. In this study, we evaluated the acute toxicity and estrogenic activities.

Concerning the acute toxicity, our results showed that the lethal doses (LD_{50}) were 2063 and 603 mg/kg of b.w. for *C.*

procera and *E. hirta*, respectively. According to the scale of Hodge and Sterner (1943) in N'Guessan and co-workers [20], *C. procera* would be classified as practically non-toxic and *E. hirta* as slightly toxic. The ratios of LD_5/LD_{50} and LD_{50}/LD_{95} are quite equal for each plant, confirming the validity of our LD_{50} test-values. The quotient LD_5/LD_{95} was 0.44 and 0.076 for *C. procera* and *E. hirta*, respectively. It means that *C. procera* is safe for use compared to *E. hirta.*

According to Mossa and co-workers [21], the acute toxicity study of the ethanolic extract of aerial parts of *C. procera*, administrated orally showed no mortality or no behavioral changes in mice up to a dose of 3000mg/kg b.w. This difference can be explained by the type of extract, the mode of extract preparation (decoction) and the route of administration. Moreover, Ouédraogo and co-workers [22] have found that aqueous and hydro-alcoholic extract of the roots of *C. procera* at dose of 2000 mg/kg administrated orally to NMRI mice did not cause any mortality 72h after the treatment. They have also noted any behavioral changes in mice 14 days after the treatment. The difference with our results may be explained by the route of administration and the organ of plant used which can influence the effect of toxicity.

It was also reported that the hydro-alcoholic extract of *E. hirta* administrated orally by intragastric at dose of 2000 mg/kg to albino mice did not cause any mortality [23]. The difference with our results may be explained by the route of administration and the type of extract which can also influence the effect of toxicity.

The ERα is widely expressed in mammary epithelium and play an important role in the development of mammary gland [24]. It is therefore important to evaluate the estrogenic effect of *C. procera* and *E. hirta* extracts. The uterotrophic assay of aqueous extracts of *C. procera* and *E. hirta* in immature mice showed for both plants an increase of the fresh weight of the uterus. These results suggested that AECP and AEEH possess an estrogenic activity. Concerning AECP, our results are different to those of Circosta and co-workers [25], who found that the aqueous and ethanolic extracts of *C. procera* roots do not possess estrogenic activity. This difference may be explained by the organ of plant used, the mode of extract preparation (decoction) and mainly the different components which exist in our extract. The AEEH at low dose display a strong estrogenic activity that did not exhibit at higher dose. Similarly, to others researchers who have found that some compounds exhibit a strong estrogenic activity at low doses [26] [27] [28]. Our result may be explained by the mechanisms action of some phytoestrogens. Indeed, Makela and co-workers [29] are reported that some compounds which have estrogen properties, at low doses can act in a similar ER mediated mechanism as endogenous estrogen. Moreover, this result may be also explained by the saturation property of estrogen receptors [30].

It is well-known that estrogen and progesterone are involved in the mechanism of regulation of lactation. Indeed, it has been showed that the injection of 17-β-estradiol (0.1mg/kg b.w.) and progesterone (0.5mg/kg b.w.) per day during one-week initiate lactation in cow [31]. In addition, it had been establish that estrogen causes mammary duct growth and the combination of estrogen and progesterone was required for lobulo alveolar development of the mammary gland [32].

5. Conclusion

This study allows to identify 25 species classified into 22 genera and 19 families which are used as lactogenic plants by traditional healers. The most used species were *E. hirta* followed by *C. procera* and *Ficus sycomorus*. Organs of plants mostly used for medicinal preparation were leaves. The main mode of medicinal preparation was decoction and the most way of administration was oral. The LD_{50} values permitted to classify these plants as slightly toxic herbal drugs. The uterotrophic activity of aqueous extracts of *C. procera* and *E. hirta* in immature mice showed that these extracts have an estrogenic activity. These studies show the large number of plants used for the improvement of milk production in the Cascades region of Burkina Faso. However further studies must be conducted in laboratory animals to confirm the traditional use of these plants as lactogenic plants.

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