Available online at http://www.ifgdg.org

Int. J. Biol. Chem. Sci. 13(1): 49-62, February 2019

ISSN 1997-342X (Online), ISSN 1991-8631 (Print)

International Journal of Biological and Chemical Sciences

Original Paper http://ajol.info/index.php/ijbcs http://indexmedicus.afro.who.int

Toxicity, cytotoxicity and biological activities of seeds of *Carapa procera* (DC), a native oil tree

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ACKNOWLEDGMENTS

The present work was done with the support from DANIDA, Danish International Development Agency through QUALITREE project. This work was also supported by IAEA through the BKF5011-5014 project.

ABSTRACT

Carapa procera is a native tree which seeds are intensively used for oil extraction. So this study deals with the identification of secondary metabolites, the toxicity and cytotoxicity of *C. procera* hydroalcohol extracts and their estrogenic and androgenic activities. The chemical constituents of *C. procera* extracts were determined by Thin Layer Chromatography (TLC) methods. The toxicity and cytotoxicity were respectively evaluated by the OECD Guideline 425 "Up and Down procedure" (UDP) and the brine shrimp lethality test. Estrogenic and androgenic activities were evaluated by uterotrophic and Hershberger tests and also by in vitro tests, with the yeast assay and MVLN luceferase assay. The MeOH/H_2O extracts of *C. procera* seeds revealed the presence of steroidal glycosides, triterpene, flavonoids and polyphenols. The DCM fraction revealed the presence of triterpene esters & steroid and carotenoids. The MeOH/H_2O has, by in vivo tests, slight toxic effects with mice and shows a lethal effect against the crustacean larvae by in vitro test. The extracts of *C. procera* seeds also show, by in vivo and in vitro tests an estrogenic and anti-androgenic activities. The results show with the presence of flavonoids, polyphenols and glycosides steroids estrogenic and anti-androgenic activities. The seeds of *C. procera* contain some phytochemicals, which can disrupt or reinforce endocrine function.

Keywords: *Carapa procera*, native oil tree, seeds, toxicity, estrogenic, antiandrogenic.

INTRODUCTION

In Burkina Faso, many native tree species are used for their potential composition in oil. For rural communities, these native oils increase and diversify their livelihoods. Women traditionally extract oil from native tree from seeds and this oil is an opportunity for diversification and income generation (Ouédraogo et al., 2013; Tiétiambou et al., 2016).

Carapa procera (*C. procera*) is an oleaginous plant belongs to Meliaceae family which is very appreciated by traditional healers to treat various diseases. The oil from *C. procera* seeds is known to be used as food, cosmetics, veterinary medicine, insecticide and repellent properties (Guillemot, 2004).

DOI: https://dx.doi.org/10.4314/ijbcs.v13i1.5

Regarding the importance of oil from *C. procera* seeds, there is a need to improve extraction method which is still unfortunately traditional. The rural community extraction is not able to purify the oil and several toxic substances have been reported, whereas it is known that some oleaginous plants contain hormone-like substances likely to possess an effect on animal reproduction function. Many phytochemical studies on the seeds of C. procera revealed the presence of flavonoids, phytosterols, triterpenes and tocopherols (Tindo et al., 2012; Goubgou, 2013). Some of these hormone-like substances can bind to the hormone receptor and interfere with endogenous hormone of human and animal reproduction function. Several works suggest the beneficial effects of phytoestrogen like protection against breast and prostate cancer. On the other hand, these compounds can also act as endocrine disrupters which could affect the endocrine system and may cause development and reproductive disturbances. For these reasons, there is a need to characterize the hormonal potency of natural compounds with the ability to bind to the estrogen (Diel et al., 2002).

Despite the highly sought and used of *C. procera* seeds for oil production very few studies have been interesting in the toxicity and the biological effects of these seeds extracts on animal reproductive function.

Due to the use of *C. procera* seeds for oil, the present study investigated the screening of phytochemicals in this seeds, the cytotoxicity, the acute toxicity and the effect of the seeds extracts on male and female reproductive tract by in vivo and in vitro tests.

MATERIALS AND METHODS

Plant material

C. procera seeds were collected in 2015 between 6 and 10 am in West part of Burkina Faso. The seeds were identified by the Herbarium of Ouaga I Pr. Joseph KI-ZERBO University where a voucher sample was preserved for reference under number 6203.

Animal material

The NMRI mice, 27 days old were obtained from the animal house of Ouaga I Pr. Joseph KI-ZERBO University. The room temperature was maintained at (22 ± 3) °C with the 12 h light/12 h 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 tests included in the current work were performed according to the protocols already approved by the Department of Animal Physiology of Ouaga I Pr. Joseph KI-ZERBO University and met the international standards of animals study (Zimmermann, 1983).

Chemical products

Testosterone propionate (Purity 97%) and the 17-béta-Estradiol (Purity 97%) were purchased from the Sigma Chemical Co. (St. Louis, MO, USA).The DMSO 1% was served as dilution liquid for the preparation of different doses. All substances were shipped and stored in glass containers at room temperature. All solvents were analytical grade.

Extraction and isolation

The dried and committed seeds of C. procera (100 g) were successively extracted by maceration with dichloromethane (8 L) and methanol (10 L) at room temperature during 48 h. The extracts were concentrated to dryness under reduced pressure at 40 °C to yield dichloromethane (DCM) crude (3.5 g) and a methanol (MeOH) residue (100 g). Both the extracts were dried and stored at 4 °C until used.

DCM crude (1.5 g) was fractionated by column chromatography over silica gel (40-63 µm, Merck), eluted gradient (from 5/5 to 0/10, v/v), with / ethyl acetate gradient (from 5/5 to 0/10, v/v) and afforded thirteen fractions (A to M). Fraction B was purified by column chromatography over silica gel (40-63 µm, Merck), eluting with n- hexane/ethyl acetate gradient (10/0 to 7/3, v/v) and yielded the compound 1 (25 mg) and three

other fractions. Fraction D was subjected to solid phase extraction (SPE) over silica gel (40-63 μm, Merck) to yield compound 2 (6 mg) by using n-hexane/ethyl acetate (0/10 to with n-hexane/CH_2Cl_2 $CH_2Cl_2$7/3, v/v) as gradient.

The MeOH residue (36 g) was suspended in water (600 mL) during 20 hours, and partitioned with CH_2Cl_2 (3x200 mL) and fraction (0.10 g) and an ethyl acetate fraction (1.03 g). Ethyl acetate fraction was fractionated by column chromatography over Sephadex LH-20 (Pharmacia) as stationary phase and MeOH as eluent and afforded eight fractions (A.1 to A.8). Fraction A.3 was re-chromatographied on silica gel (40-63 μm, Merck) with n-hexane/ethyl acetate (6/4 to 0/10, v/v) as gradient and ethyl acetate (3x200 mL) to yield a CH_2Cl_2 yielded the compound 3 (27 mg). On the other hand, the screening of the chemical constituents was carried out with the extracts of the *C. procera* seeds, using chemical reagents and thin layer chromatography (TLC) methods according to the methodology suggested by Wagner and Bladt (2009).

Acute toxicity test

An intraperitoneal study for determining LD_{50} was performed according to the OECD Guideline 425 "Up and Down procedure" (UDP) (Bruce, 1985; OECD, 2001). In this method, animals were dosed once at a time. If the animal survived, the dose for the next animal was increased and if the animal died, the dose for the next animal was decreased. Six groups of 6 mice (control and test group), each containing an equal number of both male and female, were formed. The first group (control group) received distilled water. Groups 2–6 were intraperitonealy treated with hydro-alcoholic seeds extracts of *C. procera* at the doses of 250, 500, 750, 1000 and 3000 mg/kg respectively. In each case the product volume administered by intraperitoneal was 1 ml/100g body weight. Following administration, the animals were observed for mortality or any sign of abnormality periodically during 1, 24, 48 and 72h. The lethal dose (LD_{50}) was estimated according to the method described by Litchfield and Wilcoxon (1949).

Subchronic toxicity test

Two groups of 10 mice, each containing an equal number of both male and female, received intraperitonealy during 28 consecutive days respectively distilled water (control groupe I) and 82 mg/kg (Group II). Mice were weighed every day before extracts administration and after a follow-up of 2 hours takes place to note signs of toxicity caused by the extract. Each mouse was marked with a unique identification number and behavior was observed daily during the trial period. One day after the last administration, all the mice were autopsied and the organs (lungs, heart, spleen, liver and kidney) were removed and weighed to note the necrotic signs.

Brine shrimp lethality assay

The bioactivity of the extracts was monitored by the brine shrimp lethality test (Meyer et al., 1982). Samples were dissolved in DMSO and diluted with artificial sea salt water so that final concentration of DMSO did not exceed 0.05%. Fifty microliters of sea salt water were placed in all the wells of the 96-well microtiter plate. Fifty microliters (1, 10, 100, 1000 µg/ml) seeds extracts were made in triplicate. Control wells with DMSO were included in each experiment. Hundred microliters of suspension of nauplii containing about 10 larvae was added into each well and incubated for 24 h. The plates were then examined under a microscope (12.5×). 100 µl of methanol was added in each well and after 15 min the number of dead nauplii in each well counted. Lethality concentration fifties (LC50 values) for each assay were calculated by taking average of three experiments using a Finney Probit analysis program (McLaughlin et al., 1991).

Uterotrophic and Hershberger tests

Immature female mice, 24-27 days old, weighted 20 ± 2 g were used for uterotrophic and Herhsberger tests. For each test, animals were divided into four groups, consisting of five mice each. The first group of each test served as a control and received distilled water. The second group of each test received 10µg/kg BW of 17-β-estradiol and Testosterone Propionate per day. The third and fourth group of each test received respectively 50 and 100 mg/kg BW of *C. procera* hydro-alcoholic seeds extracts. The different treatments were administered intraperitonealyduring three (3) consecutive days for Uterotrophic test and 10 consecutive days for Hershberger test. Twenty-four (24) hours after the last treatment, animal were weighed, sacrificed and autopsied. For Uterotrophic test, the uterus and ovaries were removed, separated from fat adhesions and weighed. The increase in uterine weight was taken as a measure of Uterotrophic activity. For Hershberger, testes, epididymis, seminal vesicles, prostate and levator ani muscle were removed, separated from fat adhesions and weighed. The increase of weight of accessory gland was taken as a measure of Hershberger test. Twenty-four (24) hours after the last treatment, animals were weighed, sacrificed and autopsied.

Estrogen and androgen yeast assay

The estrogen-inducible screening assay in the yeast strain *S. cerevisiae* was used as previously described (Routledge and Sumpter, 1997). Cells are stably transfected with the DNA sequence of the hERα. The system also contains expression plasmids composed of two estrogen-responsive elements (ERE) regulating the expression of the reporter Lac-Z gene that encodes the enzyme β-galactosidase (Sohoni and Sumpter, 1998).

Yeasts were handled as previously described (Tham et al., 1998). Stock solutions and the test compounds were prepared in DMSO and added to clear 96-well polystyrene plates (TPP, Switzerland), to a maximum concentration of 1% DMSO. Plates were seeded with 200 ml per well of the assay medium; plates were then sealed and incubated at 32 °C for 2–3 days. Substrate conversion (colour development) was measured at 565 and 690 nm using a plate reader. The readings at 690 nm were used to correct for the increase in turbidity due to growth of the yeast. Samples were tested in quadruplicates, and a standard curve for E2 or DHT (10^{-12}–10^{-8} M) was included in each assay. The concentration of the test oils was ranged from10^{-8} to 5.10^{-6}.

Luciferase-reporter-gene assay

The MCF-7 human breast cancer cell line is a well-established in vitro model characterized by its estrogen responsiveness. It has been frequently used to test the effects of Estrogen mimics on the pathway(s) linking estrogen receptor binding to gene expression and cell proliferation. This investigation tested the effects of *C. procera* seeds oils on this cell line to evaluate their estrogenic activity. MVLN cell line is based on human MCF-7 cells containing an estrogen regulated luciferase reporter gene driven by an ERE of the vitellogenin A2 gene fused to the thymidine-kinase-promoter (Gagne et al., 1992; Pons et al., 1992). Therefore, the specific transcription activity of a test chemical is directly related to the activity of luciferase measured. MVLN cells were maintained as previously described (Pons et al., 1992). On the day of induction, the medium was changed against fresh 1% DCC–FCS medium and cells were treated with the test compounds (10^{-10}–10^{-5}M), or 17β-estradiol (positive control), and vehicle (DMSO). After 24 h, cells were harvested and treated for luciferase assay. The luciferase was extracted as described (Pons et al., 1992). To compare data, the protein content of each extract was measured using the bicinchoninic acid (BCA) protein assay, with bovine serum albumin (BSA) as standard protein. Luciferase activity was calculated in relative light units (RLU) per mg of protein.

Statistical analysis

The results are expressed as mean ± Standard Deviation. ANOVA I followed by a

post hoc multiple comparison was performed. Dunnett's test (SPSS version 20) was used to compare increasing doses of the test compounds with the respective control. P-values <0.05, were considered significant. The PHARMS/PC was used for the determination of the LD50.

RESULTS

Chemical composition

The phytochemical screening on *C. procera* seeds revealed the presence of steroidal glycosides, triterpene, flavonoids, tannins, leucoanthocyanins and alkaloid in the MeOH/H_2O fraction. In the dichloromethane (DCM) extract triterpene esters & steroid, carotenoids and fatty acid with high molecular weight were detected (Table 1). All biological activities were carried out with hydro-alcoholic extracts.

Acute and subchronic toxicity

After 72 h observation, the LD50 and the report LD50/LD1, LD99/LD50 values were respectively 815, 2.90, and 2.90. The security index DL99/DL1 value was 8.41 (Table 2).

For subacute toxicity, all mice survived till the end of 28 consecutive days' treatment. The signs of abnormalities and behavior observed were ruffled hair, sneezes, somnolence, loss of mobility and anorexia. In the two groups, the feces had not significantly changed. *C. procera* hydroalcohol seeds extracts showed 4.96% of body weight decrease compare with the control group. Compare with control group, the weight of lungs, heart, liver, kidney and spleen had not significantly ($p>0.05$) changed.

Brine shrimp lethality assay

C. procera seeds hydroalcohol extracts, press and hexane oils were found to be potent against Brine Schrimp with LC50 value 219, 333, 641 µg/ml respectively (Table 3).

Estrogenic activity

On the average daily weight gain of female mice, the different doses of *C. procera* hydro-alcohol seeds extracts did not show a significant ($p>0.05$) effect compare with control groups DMSO (1%) and (Figure 1).

The doses of 50 and 100 mg/kg of *C. procera* hydro-alcohol seed extracts were exhibited a significant ($p<0.05$) increase on the uterus weight of mice respectively at 58 and 74% compare with the control group (Figure 2).

Androgenic activities

The different treatments did not have a significant ($P>0.05$) effect on the male average daily weight gain compare to control groups (Figure 3). Therefore, on seminal vesicle, prostate and Levator Ani and Bulbocanernous Muscle (LABC), 100 mg/kg of *C. procera* seeds extract induced a significant ($p<0.05$) decrease of these gland compare to control groups (Figure 5).

Estrogen yeast assay and androgenic yeast assay

The estrogenic and androgenicity activities of C. procera seeds extracts were examined by growing the yeast strain overnight in the presence of vehicle, 17-β-estradiol or the different doses of *C. procera* seeds extracts and measuring β-galactosidase activity. Estradiol and DHT (Dihydrotesterone) were the most effective compounds tested inducing maximal β-galactosidase activity. *C. procera* seeds extracts did not exhibit estrogenic or androgenic activities (Figure 6).

Luciferase-reporter-gene assay

Luciferase induction was analysed after 24 h incubation of MVLN cells with 0.001, 0.01, 0.1 µg/ml of *C. procera* seeds oil extracts. E2 at 10^{-8} M was used as a positive control and DMSO as a negative control. The different doses of *C. procera* seeds oil extracts show a significant ($p>0.05$) dose dependent luciferase induction (Figure 7).

Table 1: Chemicals composition of *C. procera* extracts.

CHEMICAL GROUPS	EXTRACTIONS		
	DCM	Meth/H_2O	
		Hydrolyzed extract (aglucones)	***Unhydrolyzed extract (glycosides)***
Triterpene esters & steroid	++ (r-br): triterpenes and phytosterols	ND	ND
Flavonoid aglycone	ND	ND	ND
Alkaloid bases	+ (Dragendorff)	ND	ND
Coumarins	ND	ND	ND
Carotenoids	++	ND	ND
Fatty acids of high molecular weight	++	ND	ND
Steroidal glycosides & triterpene	ND	++(r-br) : Phytosterols	ND
Flavonoids	ND	++ (red) : flavonols	ND
Anthraquinone	ND	ND	ND
Leucoanthocyanins	ND	++	ND
Tannins	ND	ND	++ (blue) : gallic
saponosides	ND	ND	+ (suds height >1,5cm)
alkaloid salts	ND	ND..	+ (Dragendorff)

ND: Not detected; +: average; ++: abundant

Table 2: Lethal doses (LD) (mg/kg) after 72 H of *C. procera* extracts on mice.

GROUPES	DOSES	MORTALITY (%)	LD (Lethal Doses)		LDx/LDy	
I	Distilled water	0	**LD1**	280	**LD5/LD50**	0.46
II	250	0	**LD5**	383		
III	500	17	**LD50**	815	**LD50/LD95**	0.46
IV	750	50	**LD95**	1731		
V	1000	67	**LD99**	2364	**LD5/LD95**	0.16
VI	3000	100				

Table 3: Effects of *C. procera* seed extracts and oils on Brine shrimp lethality.

Plants	Material	Percent (%) death at 24H				LC50 (µg/ml)
		1 µg/ml	10 µg/ml	100 µg/ml	1000 µg/ml	
C. procera	Seed	0	1	5	70	219
	Press oil	0	0	8	63	333
	Hexane oil	0	0	0	48	641

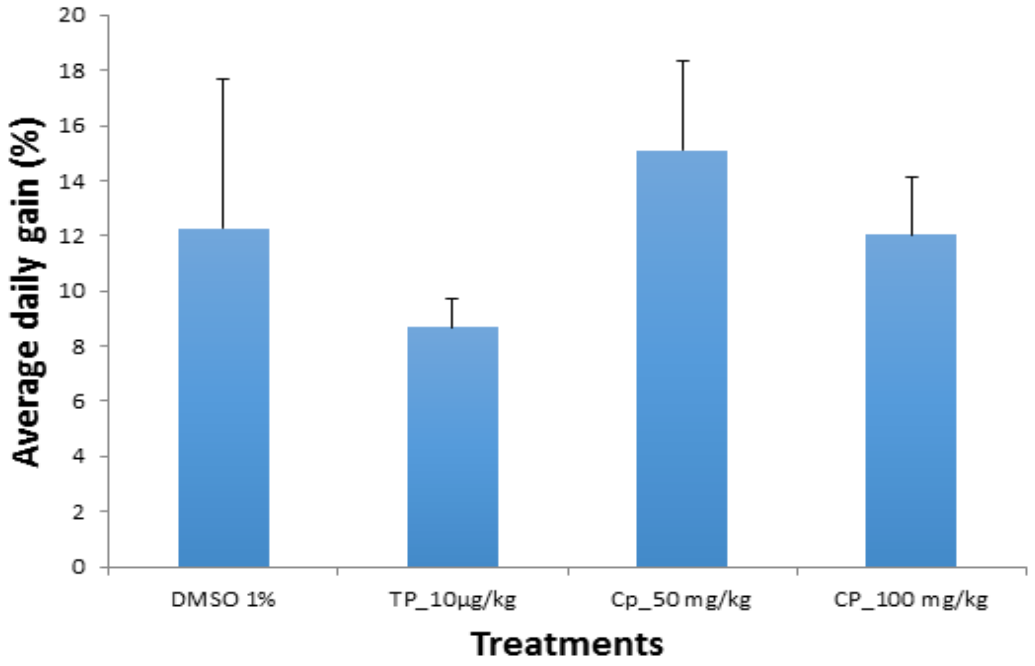

Figure 1: Effect of treatments on average daily gain of female mice. Each histogram represents the mean ± SEM of the values for 5 animals.

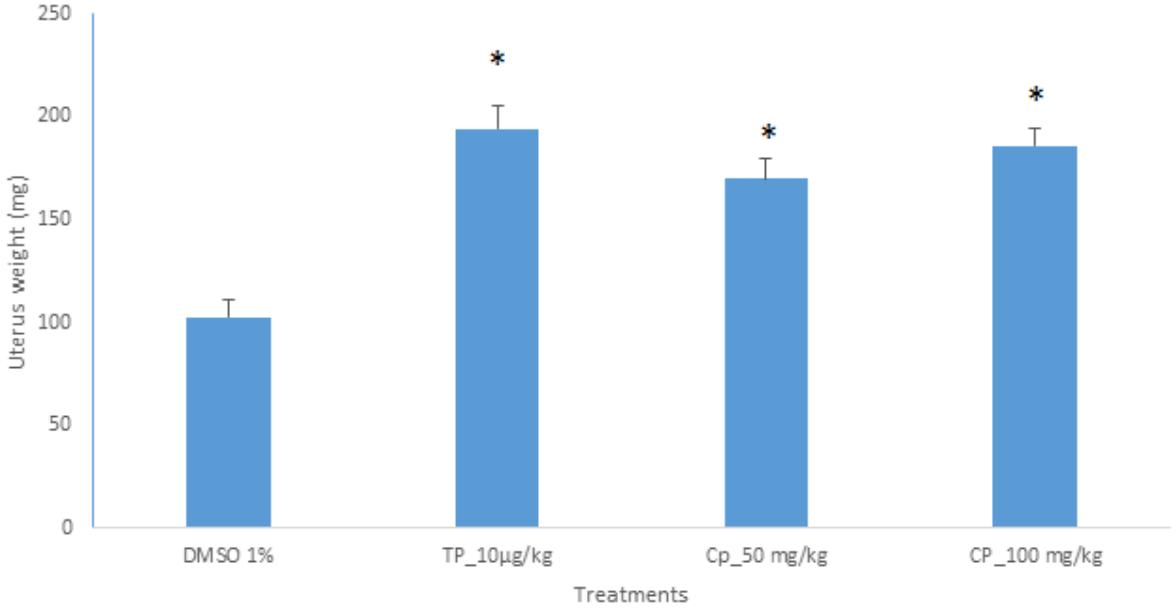

Figure 2: Effect of treatments on uterus weight. *p<0,05 (Values significantly different from those of the control group (ANOVA and Dunnett's test); each histogram represents the mean ± SEM of the values for 5 animals.

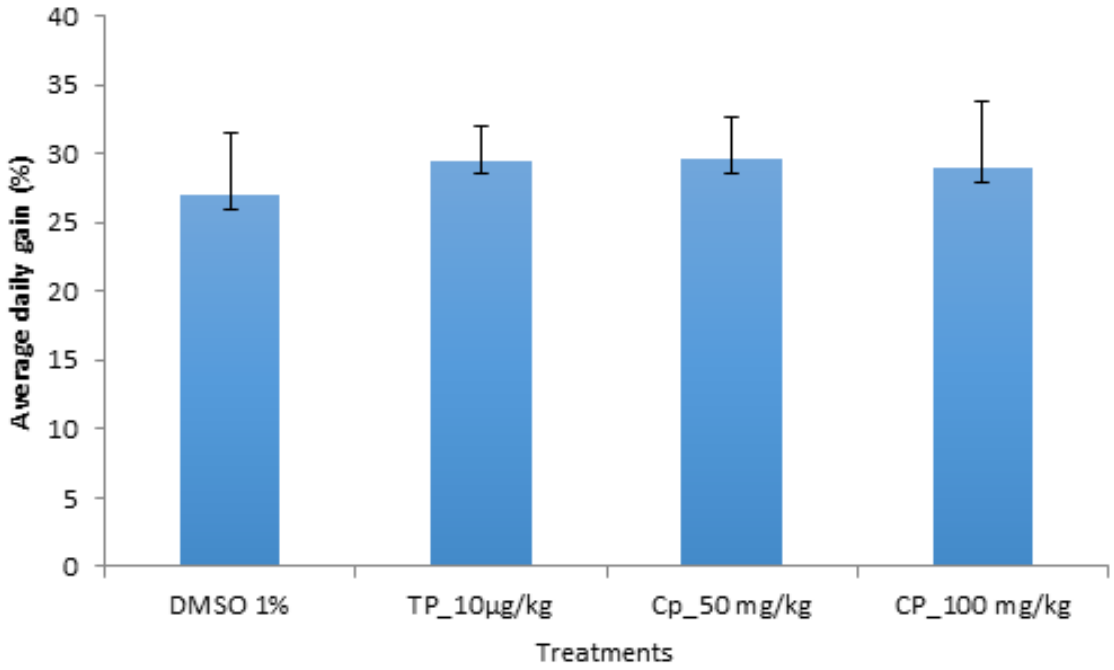

Figure 3: Effect of treatments on the average daily weight gain of female mice. Each histogram represents the mean ± SEM of the values for 5 animals.

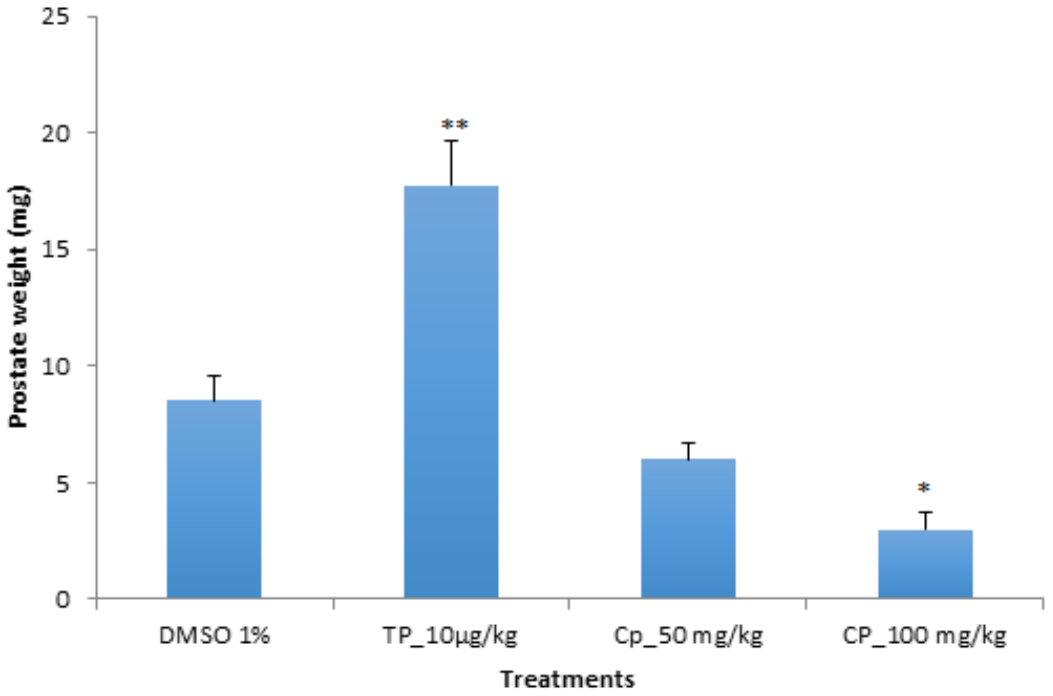

Figure 4: Effect of treatments on the evolution of prostate weight. *p<0.05, **p<0.01 (Values significantly different from those of the control group (ANOVA and Dunnett's test); each histogram represents the mean ± SEM of the values for 5 animals).

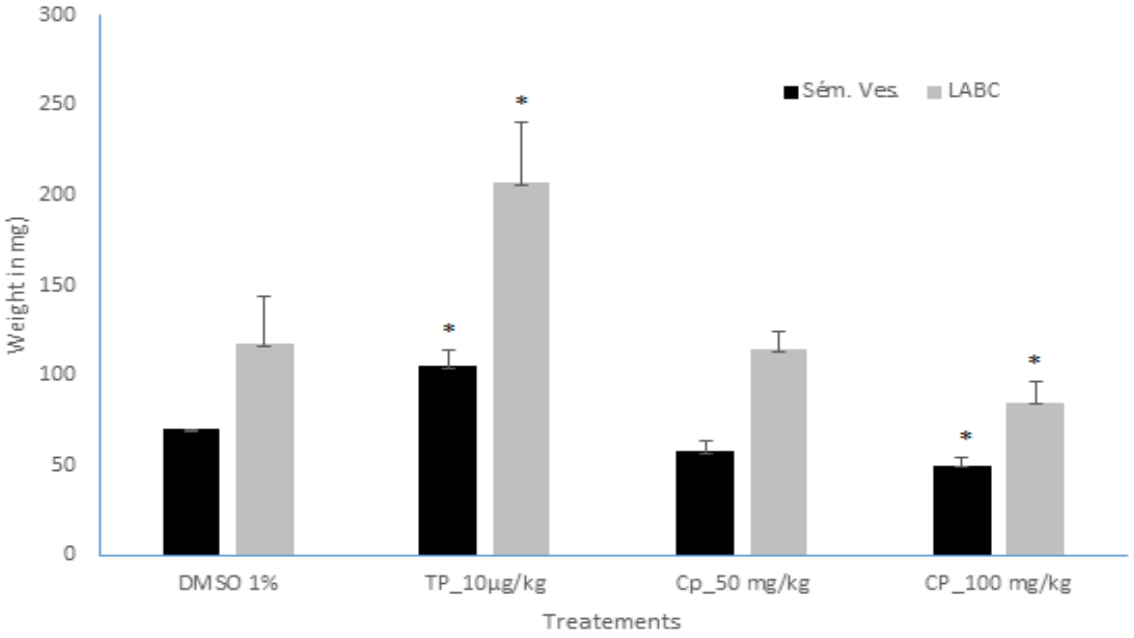

Figure 5: Effect of treatments on seminal vesicles and LABC weight. *p<0,05 (Values significantly different from those of the control group (ANOVA and Dunnett's test); each histogram represents the mean ± SEM of the values for 5 animals).

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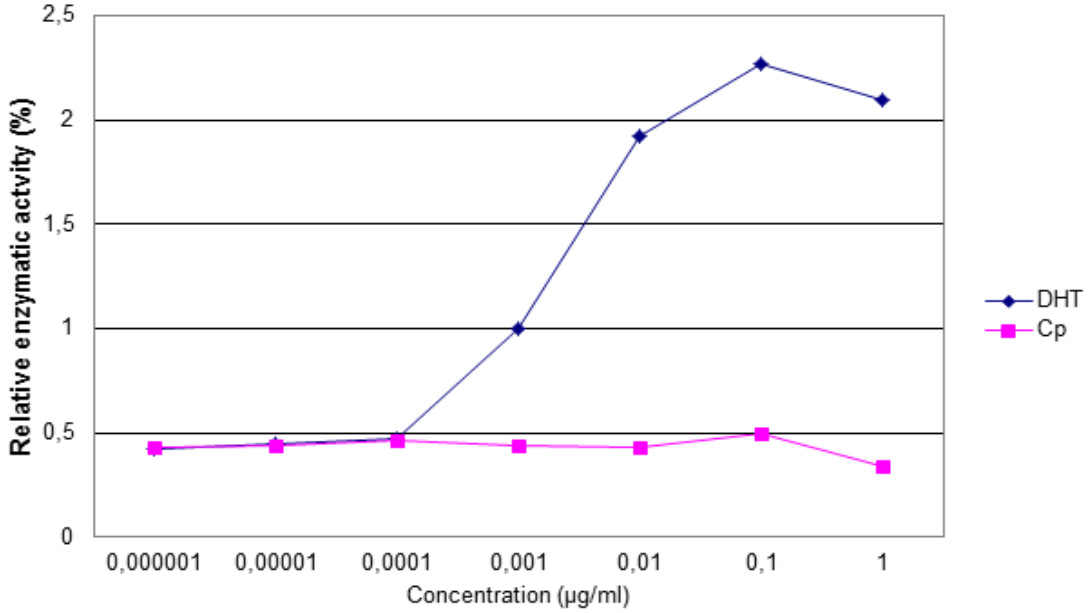

Figure 6: Androgen inducible yeast receptor assay. Androgen potential of Cp seed extracts were tested in concentration dependent manner. The recombinant yeasts were incubated at 32 °C for 48 h with test compounds. Data represent the mean± SEM of Three independent experiments (Cp: *Carapa procera*; DHT: Dihydrotesterone).

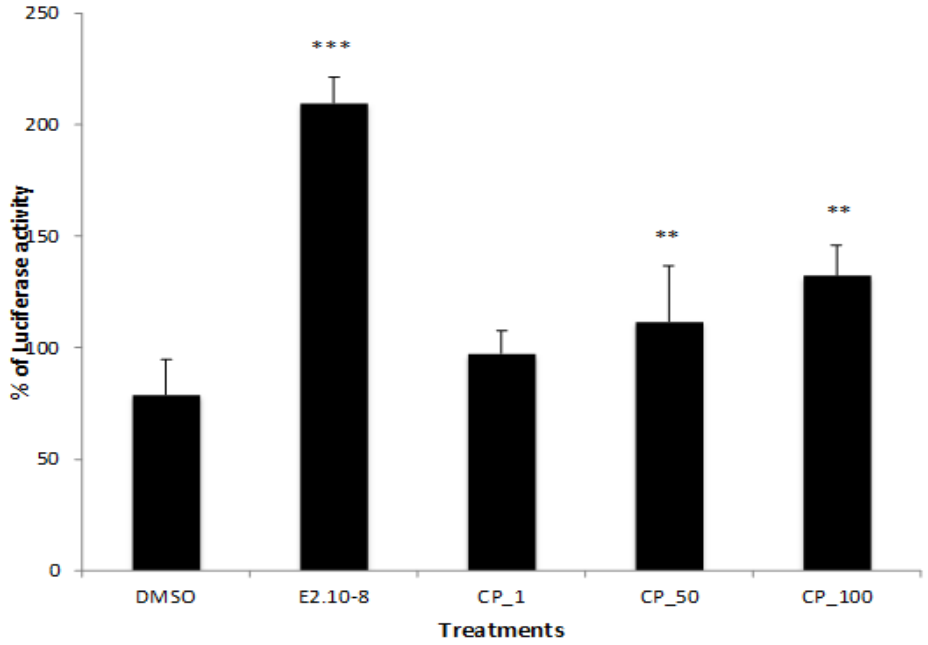

Figure 7: Effect of Cp seed oils extracts on luciferase induction. MVLN cells were exposed to substances for 24 h at 1, 10 and 100 mg/kg (0,001, 0,01; 0,1 µg/ml). E2 (10^{-8} M) was used as standard agonist. All the substances were dissolved in DMSO. Each concentration was tested in duplicate. DMSO (0.1%) was used as control. **p<0,01, ***p<0,001, significantly different from the control group (ANOVA I followed by a post hoc multiple comparison).

DISCUSSION

The seeds of *C. procera* are widely used by women in rural communities for oil extraction. This oil, which is highly appreciated for its nutritional, cosmetic and medical qualities, allows women to increase their incomes and thus reduce their poverty (Weber et al., 2009). Although several studies have focused on *C. procera* oil, very little data exist on the biological activities evaluation of *C. procera* seeds extracts.

The phytochemicals screening of *C. procera* seeds have showed the presence of carotenoids, triterpens esters, steroid and alkaloid in DCM extracts. In hydro-alcohol extracts the screening shows the presence of steroidal glycosides and triterpens, flavonoids, leucoanthocyaninis, polyphenols and saponosides. The presence of flavonoids and triterpens explain why these extracts are used for biological activities evaluation.

The presence in *C. procera* seeds of flavonoids as flavonols are considered as beneficial phytoestrogens for the resolution of deficiencies of estrogen in humans and animals physiology (Martinez, 2012) and triterpenic and steroidic glycosides such as phytosterols are considered asphyto-androgen for testosterone synthesis.

The LD50 of *C. procera* hydro- alcoholic extract is classified in the scale of Hodge and Sterner (1943) as slightly toxic and in class II described as moderately dangerous according to the scale of OMS. These results are similar with those of Lompo et al. (1995) which indicated a low toxicity in mice of the extract of Kaya senegalensis, a plant from the same family (Meliaceae) as *C. procera*.

The equal ration of LD5/LD50 and LD50/LD95 confirm the validily of our LD50 test values (Somé et al., 1996). The quotient DL_5/DL_{95} indicates a value of 0.16 more inferior to 1. It means that *C. procera* hydroalcohol extract is safe of use and have a large marge of maneuverability (Hodge and Sterner, 1943). *C. procera* seeds for therapeutic use should be made with caution for its low toxicity. The extract probably contains toxic substances that could cross the intestinal barrier and pass in the blood and cause damage to vital organs.

This is the case of tannins which can easily infiltrate the blood and body tissues when they are consumed in excess. Intraperitoneal administration of *C. Procera* hydro-alcoholic extracts even if it has a low toxicity can provoke failures of vital organs and a decrease of body.

The brine shrimp mortality assay is widely accepted as a convenient probe for potential pharmacological activity (Meyer et al., 1982). Toxic constituents of plant extracts showing lethal effects against the crustacean larvae may elicit interesting effects at lower, non-toxic doses (McLaughlin, 1991; 1993). According to Meyer et al. (1982) extracts derived from natural products, which have LC50 ≤ 1000 mg/mL are known to possess toxic effects. Hence the seeds of *C. procera* were more toxic than press and hexane oils. In the most cases, toxicity is associated with pharmacological properties, it was concluded that *C. procera* seeds can have best bioactivity (Wanyoike et al., 2004). This result is similar to those obtain by *in vitro* with acute toxicity test.

C. procera's hydro-alcoholic seeds extracts as well as 17-βestradiol caused a significant increase of the weight of uterus but did not have significant effect on the weight of animals. These results have confirmed some works (Cruz et al., 2017) which have indicated that the administration of estradiol can allow, during four months, the weight loss of female before they find their normal weight. The increase of the weight of uterus, an estrogen dependent gland, suggests that the extracts of *C. procera* contain various secondary metabolites, which can have estrogenic activity.

In fact, the phytochemical screening MeOH/H_2O extracts of *C. procera* showed that it contain flavonoids, flavonol, steroidal glycosides and polyphenols which can explain this estrogenic activity (Hodek et al., 2002; Bayala, 2005). It is well known that some chemical features in the structure of flavonoids are required to obtain an estrogenic response (Choi et al., 2008) and the hydroxyl group at position 4′ promotes the estrogenic activity and the estrogenic potency (Zand et al., 2000; Zingue et al., 2016). The increase of the uterus weight can be explained by the effect of flavonoids on estrogen receptor and induced a proliferation of cells of endometrium as indicated by Kouakou et al. (2008) and Benneteau-Pelissero (2010). The discovery of ERβ, the second ER, by Kuiper et al. (1996) was the most significance for the understanding of estrogen action. Some compounds of plant origin have been demonstrated to bind with higher affinity to this type of receptor than to ERα (yeast assay ERalpha) (Kuiper et al., 1998). The other important, which can explain estrogen action was the discovery of co-activators (Onate et al., 1995) and co-repressors (Lavinsky et al., 1998), which are involved in the initiation and regulation of gene transcription by the ER. Such co-activators are believed to determine the agonistic and antagonistic properties of compounds and to be responsible for the tissue specific action of selective estrogen receptor modulators (SERMs).

For Hershberger test, the hydro- alcoholic extracts of *C. procera* seeds are showed a significant decrease of the weight of prostate, seminal vesicle and *levator ani* muscle. All these glands are androgen dependent organs. These results reveal that the extracts of *C. procera* can contain some

chemical components which are possessing anti-androgenic activities. These results could explain the estrogenic effects of *C. procera* extracts because flavonoids are also known to produce anti-androgenic activity and affect male fertility (Bhargava, 1989). The antiandrogenic activity of *C. procera* extract is probably due to the estrogenic activity of flavonoids. A potent anti-androgen can create an estrogenic activity (Sohoni and Sumpter, 1998) and inhibit alpha reductase (Liang and Liao, 1992; Blanchard and Robaire, 1997).

Although the Uterotrophic and Hershberger assays in rodents are a good tool to assess estrogenic/antiestrogenic and androgenic/ antiandrogenic potency of natural products, these methods are not suitable for the screening of large suspected estrogenic/androgenic chemicals (Kornera et al., 1999).

The yeast assay did not show an estrogenic and androgenic effects when compare to control group. Therefore, the luciferase activity of MVLN cells incubated with various concentrations of *C. procera* extracts exhibit as 17-β-estradiol a significance increase of luciferase induction. These results confirm the estrogenic activity obtain with the uterotrophic assay. The luciferase activity induced by *C. procera* seeds extracts can be due to an estrogen-like receptor compound that bound and activated the receptor (Le Bail et al., 1998).

The fact that *C. procera* extracts show an estrogenic activity in uterotrophic assay and antiandrogenic activity with Hershberger assay can be explained by the complexity of sex hormones actions. Sex hormones like, estrogen and androgen generally act by binding at receptor sites and a synthetic chemical can disrupt this way by number of mechanisms. A chemical may mimic the action of the natural ligand by being able to act on this receptor site, or it may interfere with the receptor in some other way, and be able to block the action of the hormone (Iguchi, 1992). For example o,p-DDT, which was shown originally to be a weak estrogen, also possesses anti-androgenic activity (Kelce et al., 1995).

Some chemicals can also perturb the normal functioning of the sex hormones by inhibiting the enzymes responsible for steroid hormone biosynthesis and/or inducing enzymes responsible for steroid metabolism (Majdic et al., 1996).

Conclusion

C. procera seeds extracts has for *in vivo* tests slighty toxic effects and show a lethal effects against the crustacean larvae by *in vitro* tests. The seeds extracts of *C. procera* has also an estrogenic and anti-androgenic properties due its high amount of flavonoids, glycosides steroids and triterpernoids. Phyhormones still need evaluating for their safety on human systems, beneficial and harmeful doses. This estrogenic effect has been confirmed by the luciferase activity of MVLN cells incubated with various concentrations of *C. procera*. The uses of *C. procera* seeds for oils extraction need to pay more attention to the extraction system to prevent endocrine disrupting substances from passing through the oil. However, further studies have to be developed to achieve a better understanding regarding the interactions between the ligand-receptor binding of these compounds and ERs.

COMPETING INTERESTS

The authors declare that they have no competing interest.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all the authors. The collection and the identification of the seeds of *C. procera* were done by BB, SB and HHT. VM, OY participated in the extraction and phytochemical screening. *In vivo* and *in vitro* tests were made by BB, SB and VM. The obtained results were statistically analyzed by BB and SB. The supervision of the works was made by HHT and BB. All the authors read and approved the final manuscript.

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