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Full Length Research Paper

Phytochemical composition, acute toxicity and phytohormonal activity of hydroalcoholic extract of *Pentadesma butyracea* (Clusiaceae Sabine (1824)) seeds

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***Pentadesma butyracea* is a rainforest species of Clusiaceae family with multi-values for human health care according to previous ethnobotanical survey. In spite of this traditional use of *P. butyracea*, there is a lack of scientific knowledge of its biological activities. Therefore, the aim of this study was to identify the major phytochemical compounds of *P. butyracea* hydroalcoholic seeds extract and to assess phyto-hormonal activities. Phyto-chemical screening of dichloromethane and hydroalcoholic seeds extracts were achieved. Subsequently, acute toxicity study was performed on mice to assess extracts safety use. Phyto-hormonal activities of hydroalcoholic extract of seeds were evaluated by uterotrophic and Hershberger's bioassays. Phyto-chemical screening of seeds of *P. butyracea* showed the presence of flavonoids, tannins, phytosterols, polyphenols, leucoanthocyanes and fatty acids. Acute toxicity investigation showed no mortality of mice at the dose of 2000 mg/kg. Hydro-alcoholic extract of seeds significantly increased ($p<0.05$) the weight of uterus of immature female mice while prostate and seminal vesicles weight of immature male mice were significantly ($p<0.05$) reduced. In conclusion, the hydroalcoholic extract of seeds of *P. butyracea* is practically nontoxic and contains chemical groups which induced estrogenic and anti-androgenic activities. The seeds extract of *P. butyracea* have great potential which could be useful for management of menopausal symptoms disorders and hormone-sensitive diseases.**

Key words: *Pentadesma butyracea* extract, phyto-chemical, acute toxicity, phyto-hormonal activities.

INTRODUCTION

Natural products including medicinal plants have a great potential for human and animal well-being. Many pharmaceutical drugs are derived from medicinal plants (Rates, 2001) and until now, people from developing countries rely mostly on medicinal plants for their primary health care. Furthermore, medicinal plants and diets supplement can have a great benefit for treatment or prevention of chronic diseases such as cancers and cardiovascular diseases (Liu, 2003). Therefore, with the increase of chronic diseases cases in developing countries and especially in Burkina Faso, more interest should be given to the possible contribution of medicinal

plants for health care of low income people. Although this natural source of medication is cheap and efficient, there is a lack of scientific knowledge with regard to safety and active dose for several medicinal plants, which limits their rational use. *Pentadesma butyracea* is one of them.

P. butyracea is a dense forest species belonging to Clusiaceae family. It can be found in Western African countries such as Burkina Faso, Benin, Cameroon, Ghana, Côte d'Ivoire (Sinsin et al., 2003; Ouedraogo et al., 2013; Noudogbessi et al., 2013a). It is used by traditional healers to improve lactogenic activity of mammal, to manage breast pain and to treat pregnancy disorders such as abortion (Sinsin et al., 2003; Avocevou-Ayisso et al., 2011). Previous investigations (Batista et al., 2009; Tala et al., 2013; Noudogbessi et al., 2013b) showed that *P. butyracea* has anti-tumor and anti-plasmodial activities.

Nevertheless, few scientific investigations were performed to assess its biological activities on reproduction systems. Therefore, this study was conducted for two main objectives: to identify the major chemical compounds of *P. butyracea*, and to assess its acute toxicity and phyto-hormonal effects on mice reproduction tracts.

MATERIALS AND METHODS

Plant material

Seeds of *P. butyracea* were collected in June, 2013 from Banfora town located some 440 km from the capital Ouagadougou, in western part of Burkina Faso. These seeds were identified and authenticated by the Herbarium of University Ouaga I Pr. Joseph KI-ZERBO where a voucher specimen was deposited with a reference number (ID number: 16973, sample number: 6847). Seeds were air-dried in ventilated room, shielded from dust and sun.

Extraction and phytochemical screening

The dried seeds of *P. butyracea* were powdered with an electrical apparatus. One hundred gram of dried powdered was extracted in continuous extraction apparatus (soxhlet) until exhaustion successively with 500 ml of n-hexane, 500 ml of dichloromethane (DCM), 500 ml of acetate of ethyl and hydro-alcoholic solution 80% (v/v). The solvent of each extract was completely removed by evaporation (40 to 50°C) in a rotavapor. All the extracts were freeze-dried and stored at 4°C. The yield of the extraction was 39.64, 0.24, 3.8 and 12.63 % for n-hexane, DCM, acetate of ethyl and hydro-alcoholic solution, respectively. Various colorimetric tests were used on DCM and hydro-alcoholic seed extracts to identify the major groups of secondary metabolites according to Ciulei (1982). The following phytochemical compounds were checked, phytosterols and triterpens, flavonoids aglycones, anthracinosides aglycones, alkaloids, coumarins, cardenolides, fatty acids with high molecules weight, flavonoids, leuco-anthocyans and tannins.

Chemicals

The 17β-estradiol (Purity 97%) was purchased from the Sigma Chemical Co. (St. Louis, MO, USA) and pantestone (testosterone undecanoate) was obtained from local pharmacy. The dimethylsulfoxid [DMSO 1% (v/v)] was used as dilution liquid for preparation of the various doses. All substances were shipped and stored in glass containers at room temperature. All solvents were of analytical grade.

Biological studies

Animal model and ethic consideration

Naval Medical and Research Institute (NMRI) mice of 5 to 7 weeks-old 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 12 h light/12 h dark cycle and humidity at 50 ± 10%. The animals are fed with industrial pellets with 29% protein and have free access to drinking water. All the tests 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 animals' study (Zimmermann, 1983).

Acute toxicity

Acute toxicity was assessed as described previously (Organisation for Economic Co-operation and Development (OECD), 2001) with slight modification. Seven weeks-old mice of both sex weighting 24.53 ± 5.2 g were used. Twenty-four hours before test commenced, mice were fasted and 4 h before that time, drinking water was removed. Nine mice were randomly divided into 3 groups of 3 animals. One group received 1000 mg/kg body weight (BW); the 2nd; 2000 mg/kg BW and the 3rd one was treated with 0.24 ml of DMSO at 1% and constitutes the control group. All the mice were observed systematically for 1, 24, 48, 72 h after administration, with intoxication syndromes and mortality recorded.

Uterotrophic and Hershberger bioassay

Uterotrophic assay was achieved as described previously (OECD, 2007) with slight modification. Thirty immature female mice, 5 weeks-old and weighing 15 ± 2.11 g, were randomly divided into 5 groups of 6 mice each. The first group received 0.15 ml of DMSO at 1% and was considered as blank control. The second group received 10 µg/kg of 17β-estradiol and was considered as positive control. The three other groups received 50, 100 and 200 mg/kg of *P. butyracea*, respectively. All treatments were done intraperitoneally during 3 consecutive days.

For Hershberger assay, previous guideline for OECD (2009) was used with slight modification. Thirty-five immature male mice of 6 weeks old and weighed 20 ± 2.8 g were randomly divided into 5 groups of 7 mice. The first group was treated with 0.2 ml of DMSO at 1% and considered as blank control. The second group was

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Table 1. Phytochemical screening of DCM and hydroalcoholic extract of *P. butyracea* seeds.

Chemical group	Extract	
	DCM	Hydro-alcoholic
Phytosterols and triterpens	++	++
flavonoid aglycones	-	-
anthracinoside aglycones	-	-
Alkaloids	-	-
Coumarins	-	-
Cardenolides	+	-
Fatty acids with high molecule weight	++	++
Flavonoids	-	+
Leuco-anthocyans	-	++
Tannins	-	++
Saponosides	-	-
Alkaloid salt	-	-
Reducing compounds	-	++

++: abundant; +: average; -: no-detected.

treated with pantestone and was considered as positive control. The other three groups were treated, respectively with 50, 100 and 200 mg/kg of *P. butyracea*. All the treatment was done through intraperitoneal route administration for 10 consecutive days. Twenty-four hours after the last administration, mice were weighed and autopsied. Hormono-dependent sex glands were removed and separated from adherent tissues and weighed. Uteri, ovary, vagina and adrenal glands were removed and weighed for uterotrophic bioassay. For Hershberger bioassay, seminal vesicles, prostate, testicle, epididymis and elevator anis and bulbo-carvenous (LABC) were removed and weighed.

Statistical analysis

Data were presented as mean ± standard error of mean (SEM) and analyzed by using Graph Pad Prism version 5.03. One-way analysis of variance (ANOVA) followed by Dunnett's comparison test were used to assess differences between groups. A value of $p < 0.05$ was considered as statistically significant.

RESULTS

Phytochemical screening

Phyto-chemical screening of DCM and hydro-alcoholic extracts of *P. butyracea* seeds showed the presence of secondary metabolites such as tannins, phytosterols, triterpens, cardenolides and leuco-anthocyanes. Flavonoids were found only in hydro-alcoholic extract. Coumarins, alkaloids, anthracenosides and saponosides were not detected (Table 1).

Acute toxicity

After 72 h, no mortality was recorded at 1000 and 2000 mg/kg. Nevertheless, sleepiness behavior was recorded.

Uterotrophic bioassay

Animal body weight

Three consecutive days of treatment with hydro-alcoholic extract of *P. butyracea* seeds did not change significantly ($p > 0.05$) the animal body weight of groups treated with 50, 100 and 200 mg/kg BW when compared to control. Nevertheless, there were slight impairments of the body weight of animals given the extract (Figure 1).

Organ weight

The doses of 50, 100 and 200 mg/kg BW caused significant increase ($p < 0.05$) of the weight of uteri, ovaries and vagina in comparison to positive control (Figures 3 and 4). The weight of adrenal glands did not change significantly ($p > 0.05$) when compared to blank control (Figure 3).

Hershberger bioassay

Animal body weight

After ten consecutive days of treatment, the doses 50, 100 and 200 mg/kg BW of hydroalcoholic extract of *P. butyracea* seeds did not cause significant ($p>0.05$) change in immature mice body weight when compared to control groups (Figure 2). Nevertheless, the body weight of these animals was slightly impaired.

Organs weight

The doses 50, 100 and 200 mg/kg BW did not induce

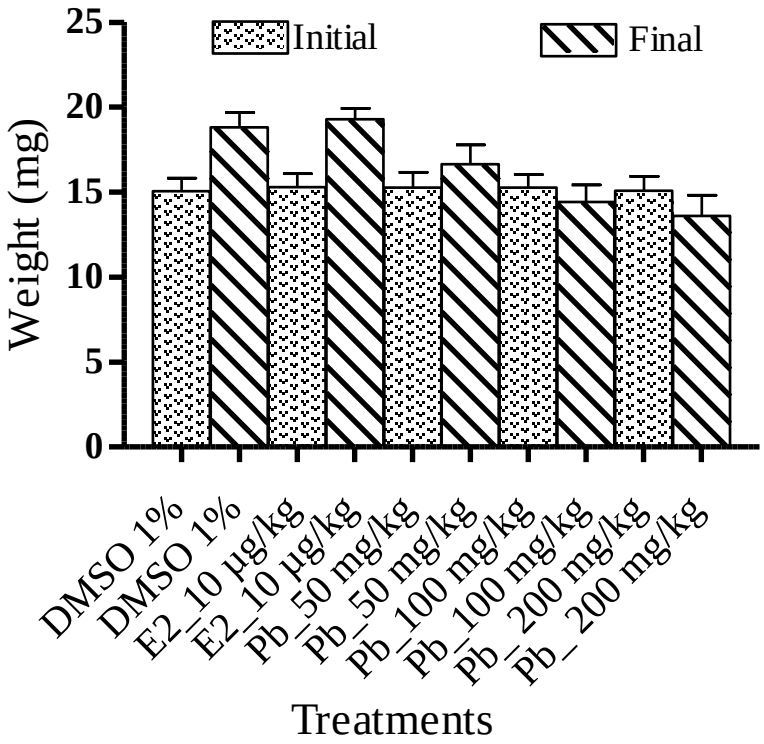

Figure 1. Immature female mice (NMRI) body weight before and after 3 consecutive days of treatment with hydro-alcoholic extract of *P. butyracea* seeds (DMSO: dimethylsulfoxyd, E2: 17-β-estradiol, Pb: *Pentadesma butyracea*).

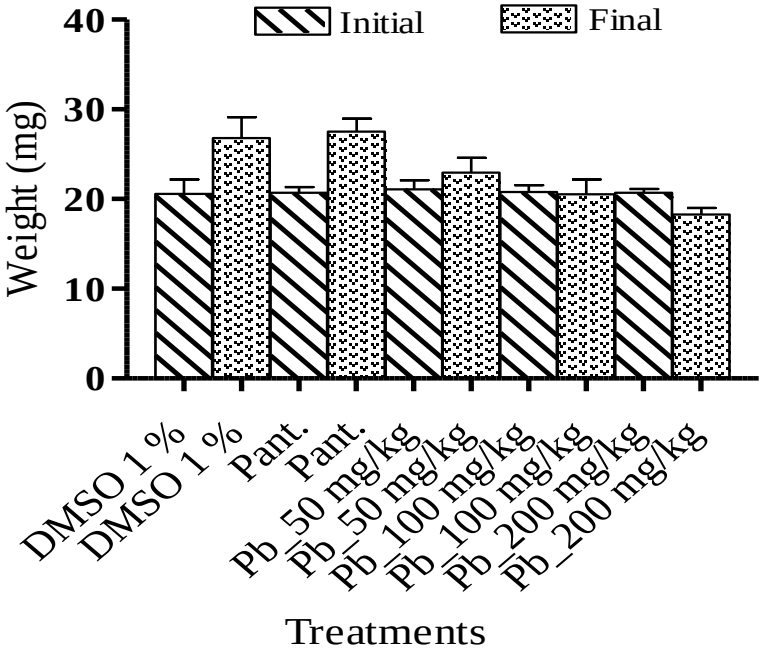

Figure 2. Immature male mice (NMRI) body weight before and after 10 consecutive days of treatment with hydro-alcoholic extract of *P. butyracea* seeds (DMSO: dimethylsulfoxyd, Pant.: Pantestone, Pb: *Pentadesma butyracea*).

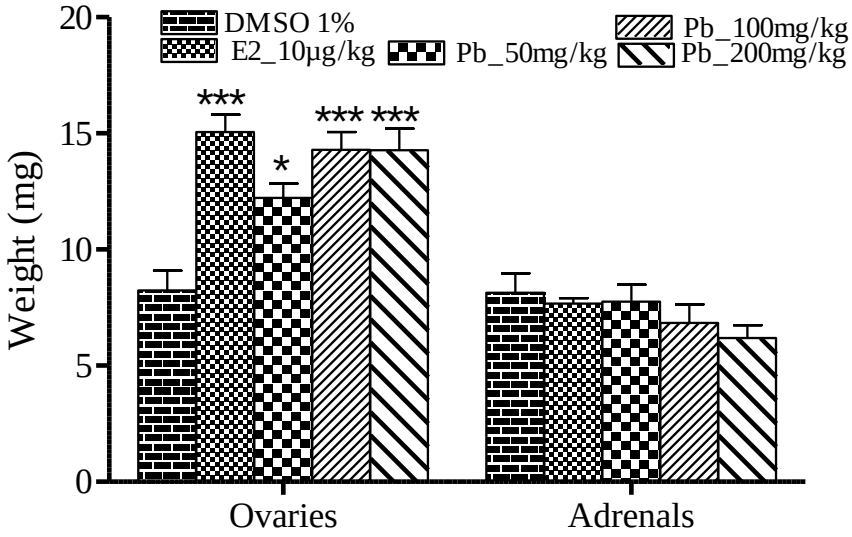

Figure 3. Ovaries and adrenals weight of immature female mice (NMRI) treated with hydro-alcoholic extract of *P. butyracea* seeds after 3 consecutive days of treatment (DMSO, E2: 17-β-estradiol, Pb: *Pentadesma butyracea*, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$).

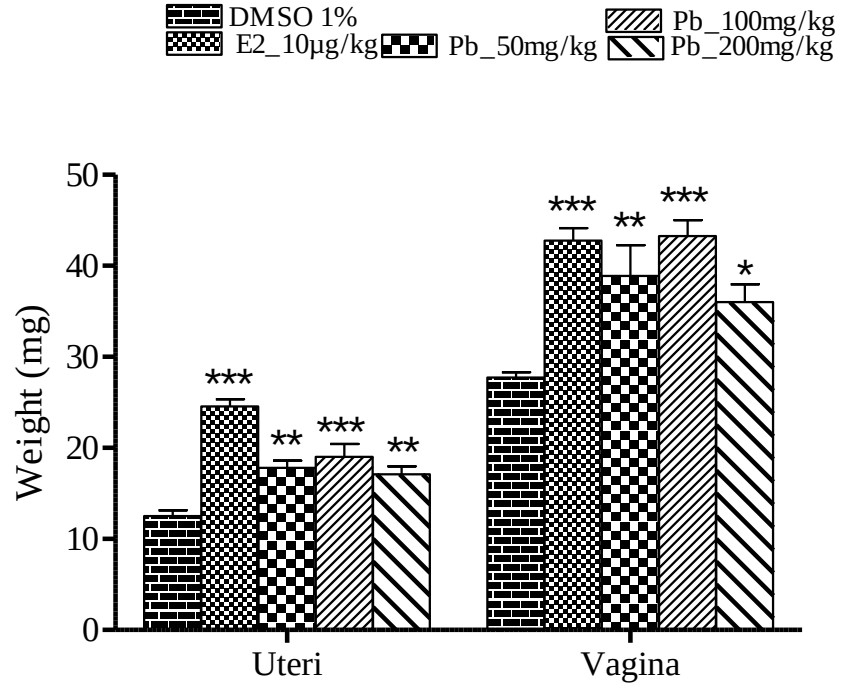

Figure 4. Uteri and vagina weight of immature female mice (NMRI) treated with hydro-alcoholic extract of *P. butyracea* seeds after 3 consecutive days of treatment (DMSO, E2: 17-β-estradiol, Pb: *Pentadesma butyracea*, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$).

significant ($p>0.05$) change of epididymis and testicles weight in comparison to blank control (Figure 5). In comparison to positive control, the dose of 100 mg/kg BW of *P. butyracea* induced significant reduction ($p<0.05$)

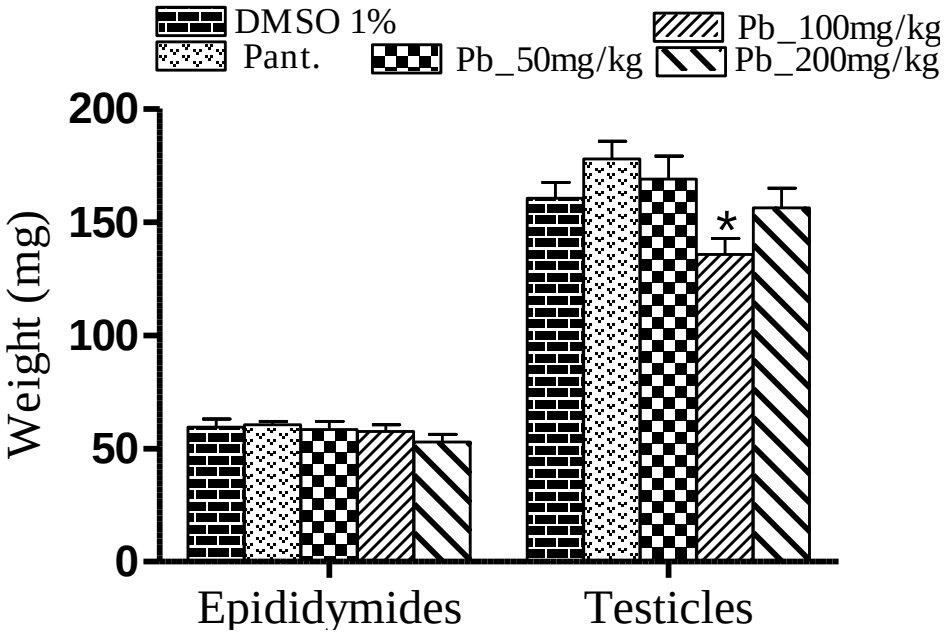

Figure 5. Epididymides and testicles weight from immature mice (NMRI) treated with hydro-alcoholic extract of *P. butyracea* seeds for 3 consecutive days (DMSO, Pant.: Pantestone, Pb: *Pentadesma butyracea*, *: $p < 0.05$).

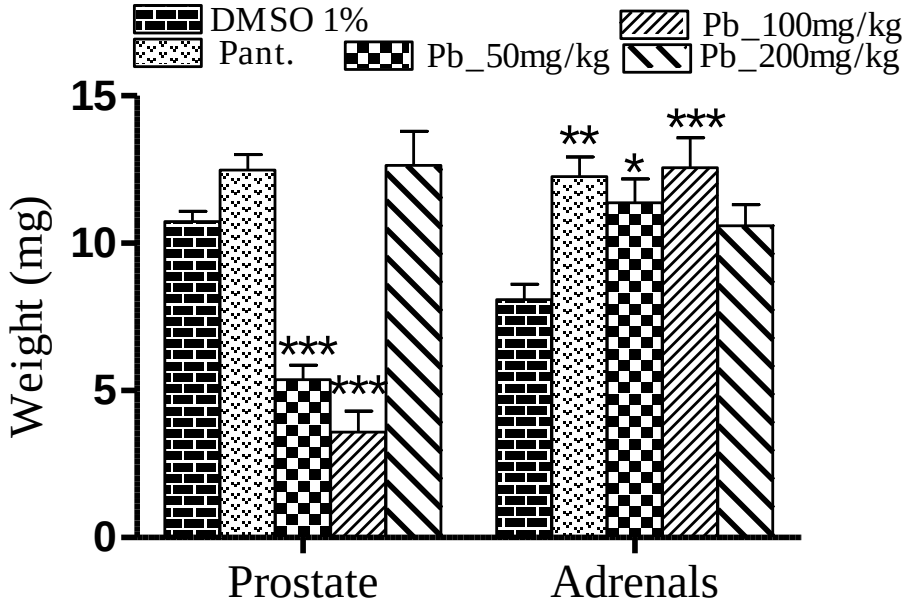

Figure 6. Prostate and adrenal glands weight from immature male mice (NMRI) treated with hydro-alcoholic extract of *P. butyracea* seeds for 10 consecutive days (DMSO, Pant.: Pantestone, Pb: *Pentadesma butyracea*, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$).

of testicles weight (Figure 5). Prostate weight was reduced significantly ($p<0.05$) in comparison to control values whereas adrenals weight was significantly ($p<0.05$) increased with the dose of 50 and 100 mg/kg BW (Figures 6 and 7). All the doses caused significant ($p<0.05$) decrease of seminal vesicles weight in comparison to blank control (Figure 7). LABC weight was reduced significantly ($p<0.05$) with dose of 100 mg/kg BW

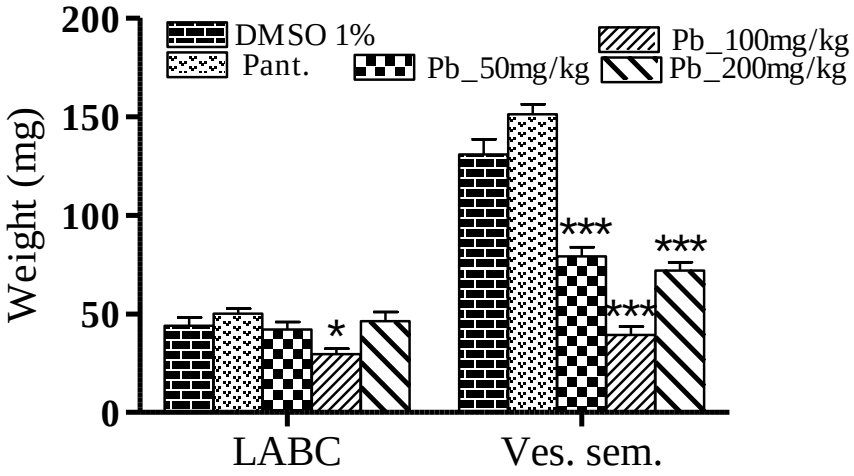

Figure 7. LABC and seminal vesicles weight from immature male mice (NMRI) treated with hydro-alcoholic extract of *P. butyracea* seeds for 10 consecutive days (DMSO, LABC: levator anis and bulbocarvenous, Ves. Sem.: Seminal vesicles, Pant.: Pantestone, Pb: *Pentadesma butyracea*, *: $p < 0.05$, **$p < 0.01$, ***: $p < 0.001$).

when compared to control values (Figure 5).

DISCUSSION

Phytochemical screening showed that *P. butyracea* seeds contain secondary metabolites including phytosterols, triterpens, tannins, flavonoids and leuco-anthocyanes. Previously, phytochemical investigations on bark, roots and leaves showed the presence of similar phyto-chemical compounds in this plant (Tchobo et al., 2007, 2013; Noudogbessi et al., 2013a). However, some compounds such as coumarins and saponosides found by Noudogbessi et al. (2013a) were not detected in *P. butyracea* hydroalcoholic seeds extracts. This difference could be explained either by solvent used for the extraction or by the organ or growing locality soil of plant used for the extraction (Lachman et al., 2008; Kajdzanoska et al., 2011; Noudogbessi et al., 2013a).

Several investigations suggest that phytochemical compounds have health benefits including positive impact on cancer (prostate and breast), cardiovascular diseases prevention, and menopausal symptoms management (Bacciottini et al., 2007; Lui, 2003). Among phytochemicals from plants, flavonoids are the most mentioned as phytoestrogen and therefore, well-known as the compound which could be involved in several sex hormonal activities (Koffi et al., 2009; Aguiar and Barbosa, 2014). In our two extracts, flavonoids were found only in hydro-alcoholic extract, which has been selected for toxicological and *in vivo* assay.

P. butyracea seeds and butter are used in foodstuffs and traditional medicine (Sinsin et al., 2003; Avocevou-Ayisso et al., 2011; Ouedraogo et al., 2013). For the first-time, acute toxicity of hydro-alcoholic extract of *P. butyracea* seeds on mice was investigated. No mortality was recorded and lethal dose (LD_{50}) of hydro-alcoholic seeds extract could be more than 2000 mg/kg according to OCDE 423 (2001). The extract can then be classified as practically non-toxic. Therefore its phytohormonal activities were investigated.

Reproductive target glands function is under the control of hypothalamo-pituitary-axis through steroidal hormones regulation. Therefore, reproductive organs, especially uterus are very sensitive to estrogen which is essentially produced by ovaries of mature mammals. Ovariectomized mice or immature mice have low level of estrogen. These mice need external source of estrogen for the growing or functionality of uterus and other accessory glands. This is the principle underlaying the use of uterotrophic bioassay to screen substances with estrogenic/anti-estrogenic activities (Padilla-Banks et al., 2001). Uterotrophic bioassay of hydro-alcoholic extract of *P. butyracea* seeds on immature female mice showed that the weight of estrogeno-dependent organs (uteri, vagina and ovaries) increased quite significantly. The hypothalomo-pituitary-gonadic axis of these animals was not yet functional and subsequently, the change in sex glands weight is probably due to the extract effect. These findings are consistent with Bayala et al. (2006) and Siangcham et al. (2010) who showed that estrogenic compounds induce an increase of sex glands weight, especially uterus. Indeed, estrogenic compounds cause this weight increase through mitotic activity and retention of substance in target cells, especially in uterus cell (Bayala et al., 2006; Lienou et al., 2012; Essien and

Effiong, 2014).

Hershberger bioassay showed that the extract caused a decrease of prostate, seminal vesicles and LABC weight. The extract could mediate anti-androgenic activity. It has been reported that the anti-androgenic compounds caused reduction of androgeno-dependent glands weight (Piyachaturawat et al., 1999; Bayala et al., 2011, 2012). Anti-androgenic activity can be exhibited by two essential ways (Rashed et al., 2014). Firstly, competitive action can be mediated between androgens and anti-androgens for binding androgen receptors. Secondly, anti-androgens could inhibit 5-α reductase activity, an enzyme which controls the conversion of testosterone to dihydro-testosterone, the more potent androgen acting substance on prostate. In both instances, androgeno-dependent sex glands functionality could be reduced and lead to a weight reduction.

P. butyracea is useful for management of hormone-dependent diseases and menopausal symptoms. Its use as foodstuffs could have benefic effects for prevention of hormone-dependent diseases. It has been reported that compounds which are able to interact with hormonal function can have great potential to relieve hormono-dependent related diseases (Saunier et al., 2011; Rashed et al., 2014). Many results claimed that plant extract with estrogenic activities can be used for management of menopausal symptoms (Geller and Studee, 2006; Xu et al., 2014). Furthermore, treatment with potential to decrease androgen hormone action is a good candidate for prostate disorders management (Grant and Ramasamy, 2012).

Conclusion

Hydroalcoholic extract of *P. butyracea* seeds is rich in secondary metabolites including phytosterols, triterpens, flavonoids, tannins, leuco-anthocyanes. Until 2000 mg/kg of BW, hydroalcoholic extract of *P. butyracea* seeds did not cause mortality of adult mice therefore, the extract is quite safe. Biological studies revealed that the extract has estrogenic and anti-androgenic activities on immature NMRI-spell out female and male mice, respectively. Therefore, *P. butyracea* is a good candidate for management of hormono-dependent diseases such as breast cancer, prostate cancer and menopausal symptoms. Nevertheless, subsequent investigations are needed.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

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