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Research Article

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ABSTRACT

***Phyllanthus amarus* is a species used to treat many ailments. The hypotensive effect reported by the pharmacopoeia has been demonstrated for the aqueous extract. This effect is reduced in the presence of atropine. The aim of this work is to evaluate two fractions of *P. amarus* aqueous extract (EAPA), the aqueous fraction (FAPA) and ethanolic fraction (FEPA), on blood pressure. Phytochemical screening shows that EAPA and FEPA differs by the absence of saponins, while EAPA and FAPA differs by the absence of alkaloids. The aqueous extract and fractions both lower blood pressure in a dose-dependent. The ED50 obtained were 25.77 ± 2.76 mg/kg b.w. (EAPA), 22.26 ± 2.59 mg/kg b.w. (FAPA) and 13.41 ± 1.63 mg/kg b.w. (FEPA). Hypotension induced by FEPA is also reduced in the presence of atropine. FEPA does not alter the hypertension induced by adrenaline. Ethanolic fraction of *P. amarus* has a hypotensive effect that is relatively large. These effects result mainly from its cholinergic action of chemical compounds present in this fraction.**

Keywords: *Phyllanthus amarus*, Hypotension, Aqueous fraction, Ethanolic fraction.

INTRODUCTION

Hypertension is a chronic pathology responsible for many cardiovascular and renal complications. It is a real public health problem in developing countries (N'Guetta et al., 2006; Koffi, 2007). In Côte d'Ivoire, the prevalence of hypertension is between 8 and 10 %. The high cost of treatment brings southern populations to use the pharmacopoeia. Thus, many plant species are used to treat this pathology, among them *Phyllanthus amarus*. The whole plant is used as a decoction. She is also involved in the treatment of other conditions such as asthma, liver disease and urogenital infections (Foo and Wong, 1992; Foo, 1995). The hypotensive effect of *P. amarus* reported by the pharmacopoeia was highlighted by Srividya and Periwal (1995). The aqueous extract of the whole plant lowers blood pressure in hypertensive subjects. In addition, Amaechina and Omogbai (2007) also showed that intravenous administration of aqueous extract reduced the one hand systolic, diastolic and strength of contractions of the myocardium. These effects are functions of the dose administered.

The aim of this study is to reveal firstly, the chemical composition of the aqueous extract and two fractions and secondly, to compare their effects on blood pressure.

MATERIALS AND METHODS

Experimental animals

Rabbits (*Oryctolagus cuniculus*), of both sexes weighing between 2 kg and 2.5 kg were obtained from the Animal House of Laboratory of Nutrition and Pharmacology of UFR-Biosciences at Felix Houphouet-Boigny University in Abidjan (Côte d'Ivoire). The rabbits were housed at a constant room temperature with a light/dark cycle of 12/12 hours. The animals were fed and given water *ad libitum.* All animals were fasted for hours, but still allowed free access to drinking water, before the commencement of experiments.

Preparation of *Phyllanthus amarus* extracts

The aqueous extract of *P. amarus* and both fractions were made from fresh whole plants harvested near the Félix Houphouët-Boigny University (Abidjan). They were identified by an expert in Botany (Professor Ake-Assi Laurent, Centre National Floristic, Félix Houphouët-Boigny University).The samples are kept for this species (herbarium No. 3, 141 and 248). Whole plants were harvested and washed brought to boiling in distilled water for 5 to 10 min at 500 g for 1 liter. The decoction was filtered and then lyophilized to obtain a powder of aqueous extract of *P. amarus* (EAPA). Three grams of lyophilisate were dissolved in 250 ml of a solution of 70 % ethanol using a separating funnel for 12 hours. The aqueous phase and the ethanol phase were successively collected and dried using a rotary evaporator (Buchi). The powders obtained represent the aqueous fraction of *P. amarus* (FAPA) and ethanolic fraction of *P. amarus* (FEPA). These extracts were stored at 5 °C. The concentrations to be tested were prepared extemporaneously by dilution in a saline solution (NaCl, 9 ‰).

Phytochemicals compounds research

The phytochemical screening was performed on the total aqueous extract (EAPA) and the two fractions (FAPA and FEPA) of the aqueous extract. A research of different phytochemicals present in the extract was made possible by specific reagents. Sterols and polyphenols have been demonstrated through Lieberman reagent, while the tannins were revealed using the reagent Stiasny. The presence of quinones has been demonstrated using the reagent Borntraeger and saponins through physical testing of the foam. The presence of alkaloids in the extracts was demonstrated through the reagent and Dragendorff Bouchardat.

Recording of blood pressure

The methods were previously described (Konan et al., 2006). The rabbit was anaesthetized with ethyl urethane (40 %) at a dose of 1 g/kg b.w. Then, the animal was put in dorsal *decubitus* on a grid placed in a vat for dissection. The dissection of the thigh and the neck were made in order to expose the saphenous vein and the carotid. Thereafter we carried out the intubation of the vessel and artery. Intubation permitted possible placement of blood carotid and the mercury contained in the tube of U of the pressure gauge of Ludwig in close contact. This mercury was surmounted by a float connected by a wire to a stylet inscriptor. Thus, the variations of the carotid blood pressure which were transmitted to the mercury collected by the float and registered by the stylet inscriptor on paper. Doses to be tested (4.48 to 71.74 mg/kg b.w.) were administrated to the rabbit via a saphenous vein.

Chemicals used

Atropine (ATR), a muscarinic cholinoceptor antagonist and Adrenaline (ADR), an α and β adrenergic receptors were purchased from (Aguettant, France). All drugs were dissolved and/or diluted in saline solution on each day of our experiments.

Ethics

Experimental procedures and protocols used in this study were approved by Ethical Committee of Health Sciences, University Félix Houphouet Boigny. These guidelines were in accordance with the internationally accepted principles for laboratory use and care (National Research Council, 1996; Mosihuzzaman and Choudhary, 2008).

Statistical Analysis

Data obtained from η separate experiments were expressed as means (± standard errors of the means, SEM). Statistical analysis and graphics were carried out using the software GraphPad Instat and GraphPad Prism 5 (San Diego, California, USA), respectively. Statistical analysis of the results was determined by using the unpaired Student's *t*-test. $p < 0.05$ was considered as indicative of significance.

RESULTS

Chemical composition of extracts of *P. amarus*

Sorting phytochemical aqueous extract, ethanolic fraction and the aqueous fraction revealed the presence of different phytochemicals compounds. Alkaloids, flavonoids, polyphenols, saponins, terpenes and sterols were present in the aqueous extract. Both fractions (FEPA and FAPA) differ from the aqueous extract by the absence

of alkaloids in the aqueous fraction and the absence of saponins in the ethanolic fraction. The alkaloids were mainly present in the ethanolic fraction while saponins were mainly present in the aqueous fraction (Table I).

Table I: Chemicals compounds of extracts of *Phyllanthus amarus.*
+ : positive test; **-** : negative test

Chemicals compounds	Aqueous extract (EAPA)	Aqueous fraction (FAPA)	ethanolic fraction (FEPA)
Alcaloïds	+	-	+
Flavonoïdes	+	+	+
Polyphenols	+	+	+
Quinones	-	-	-
Tanins catéchic	-	-	-
Tanins gallic	-	-	-
Saponosids	+	+	-
Terpenes + stérols	+	+	+

Effects of extracts on blood pressure

Dose effect of extracts and determination of ED_{50}

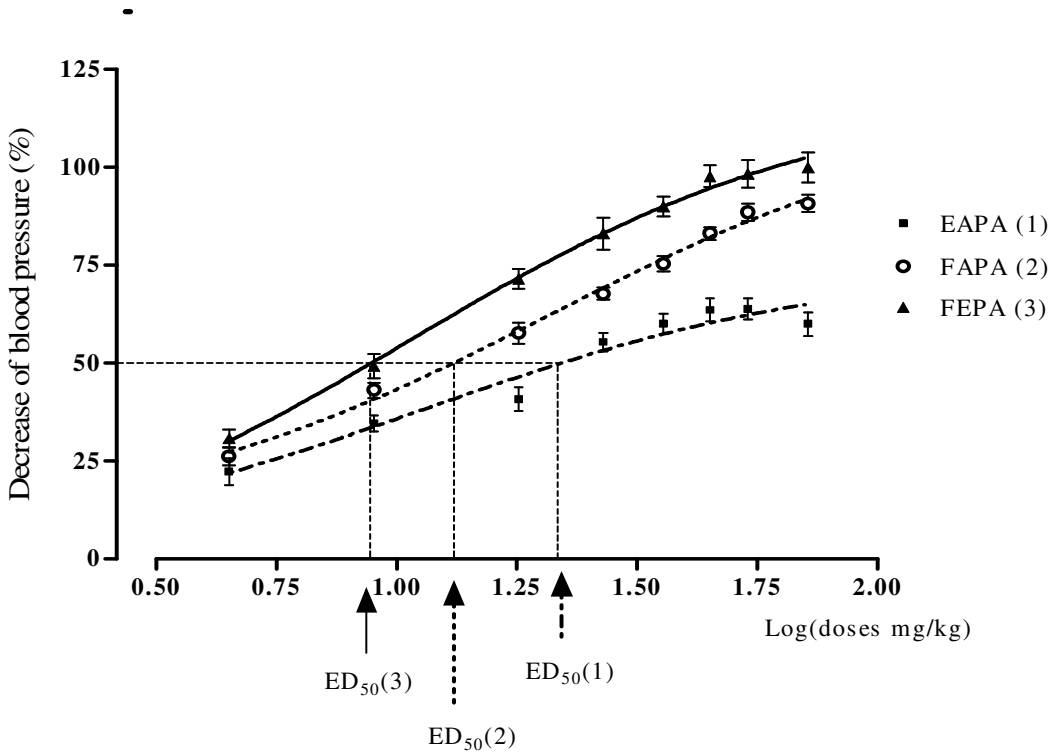


Figure 1: **Determination of the ED_{50} of different extracts of *P. amarus* on blood pressure in rabbits**. The hypotension induced by three (3) extracts increased with dose. Efficaces doses 50% (ED_{50}) were obtained for 13.41 ± 1.63 mg / kg b.w. of FEPA (ethanolic fraction of *P. amarus*), 22.26 ± 2.59 mg / kg b.w. of FAPA (aqueous fraction of *P. amarus*) and 25.77 ± 2.76 mg / kg b.w. of EAPA (aqueous extract of *P. amarus*).

Intravenous administration of increasing doses of each extract (EAPA, FAPA and FEPA) induced hypotension in a dose-dependent manner (Figure 1). The aqueous extract of *P. amarus* (EAPA) allowed the determination of an effective dose 50% (ED_{50}) which amounted to 25.77 ± 2.76 mg/kg b.w. Intravenous injection of increasing doses

of the aqueous fraction *P. amarus* (FAPA) provides an ED_{50} of 22.26 ± 2.59 mg/kg b.w. Increasing doses of the ethanolic fraction (FEPA) afford an ED_{50} which amounted to 13.41 ± 1.63 mg/kg b.w.

Effect of ethanolic fraction (FEPA) with presence of atropine

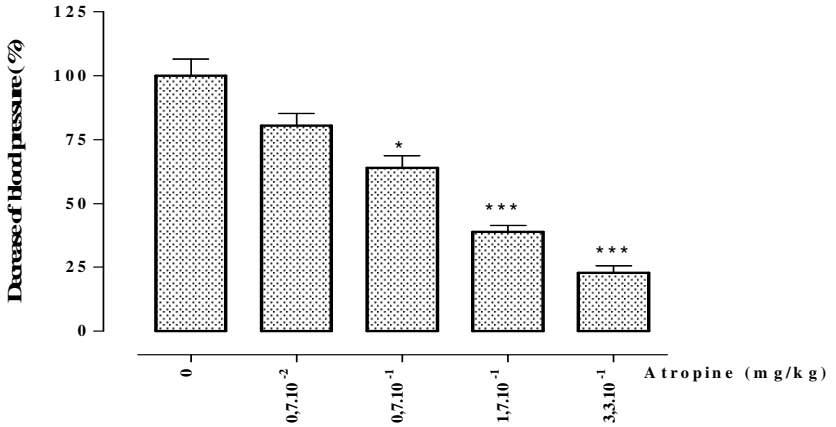


Figure 2: **Effect of FEPA on blood pressure of the rabbit with different doses of atropine.** The decrease of blood pressure is progressively reduced by increasing atropine. FEPA (15 mg / kg b.w.): ethanolic fraction of *Phyllanthus amarus*, * $p < 0.05$, *** $p < 0.001$.

A dose of 15 mg/kg b.w., FEPA induced a decrease in blood pressure of 11.5 ± 0.5 mmHg. This decrease in blood pressure was hypotension recorded maximum (100 %). Hypotension induced by FEPA was progressively reduced by increasing doses of atropine (Figure 2). In the presence of atropine at 0.7×10^{-2} mg/kg b.w., hypotension induced by FEPA (15 mg / kg b.w.) was 9.97 ± 0.33 mmHg. It corresponds to a decrease in blood pressure of 80.53 ± 4.78 %. This extract caused a fuck blood pressure of 7.67 mmHg ± 0.34 (63.88 ± 4.48 %) in the presence of atropine (7×10^{-2} mg / kg b.w.). A dose 1.7 10^{-1} mg/kg b.w. of atropine, hypotension recorded amounted to 4.97 ± 0.23 mmHg (38.88 ± 0.23 %). The highest hypotension was obtained in the presence of 3.3×10^{-1} mg / kg of atropine. It was 2.67 ± 0.33 mmHg and represents a hypotension of 21.07 ± 2.23 %.

FEPA effect on high blood pressure induced by adrenaline

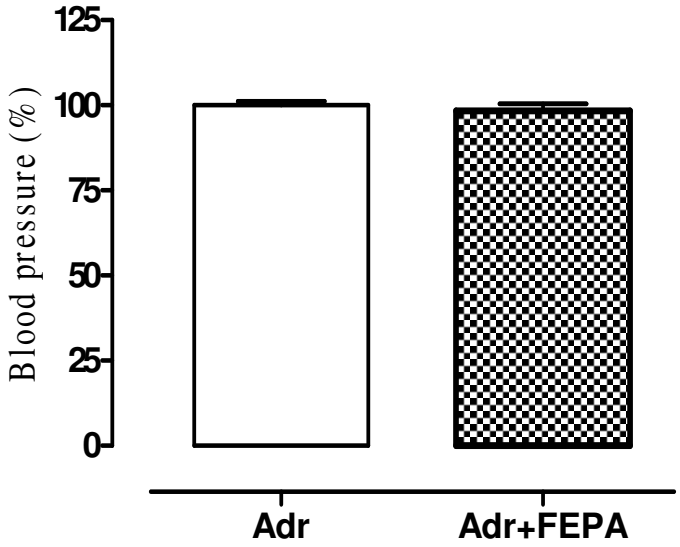


Figure 3: **Effect of ethanolic fraction of *Phyllanthus amarus* (FEPA) on hypertension induced by adrenaline (Adr)**. FEPA does not alter the hypertension induced by adrenaline.

The adrenaline (4.92×10^{-3} mg / kg b.w.) increased the rabbit blood pressure of 30.00 ± 1.30 mmHg (100%). In the presence of FEPA (15 mg / kg), hypertension induced by this dose of adrenaline was 28.72 ± 1.32 mmHg. It represents a high hypertensive effect of 93.33 ± 3.48% (Figure 3). This reduction in hypertension induced by adrenaline in the presence of FEPA was not significant ($p < 0.05$).

DISCUSSION

The qualitative phytochemical analysis showed that the three extracts have slightly different chemical compositions. Alkaloids were present in the aqueous extract (EAPA) and the ethanolic fraction (FEPA). While the saponins were present in the aqueous extract (EAPA) and the aqueous fraction (FAPA). The distribution of these chemicals in both fractions resulting from the polarity of the solvents used (N'Guessan et al., 2009a; N'Guessan et al., 2009b). Thus, saponins were generally present in the aqueous extracts, while alkaloids, polyphenols and flavonoids concentrate better in ethanolic extracts (Sameera et al., 2010; Cavalcante et al., 2011). The aqueous extract of *P. amarus* (EAPA) and the two fractions (FAPA and FEPA) induced hypotension, which increased with dose. However, the ethanolic fraction had the largest hypotensive effect with an ED_{50} relatively lower than the other two extracts. The ED_{50} of FEPA was somewhat different from that obtained with the aqueous extract of *Sesamun radiatum* which also exerts a hypotensive effect depending on the dose (Konan et al., 2006). The highest hypotensive effect of FEPA could result from the nature of chemical compounds. Indeed, Mitamura et al. (2001) showed that mesaconitine, alkaloid extracted from *Aconitum japonicum* induced vasorelaxation pressure. Similar effects were also reported for the fraction of the total alkaloids of *Solanum paludosum* (Monteiro et al., 2012). Hypotension induced by ethanol fraction of *P. amarus* (FEPA) was progressively reduced by increasing doses of atropine. This hypotensive effect could result from the activation of muscarinic receptors by chemical compounds present in this extract. These results corroborate those obtained by Amaechina and Omogbai (2007). In addition, the hypotension induced by aqueous extract of *Gossypium barbadense* was reduced in the presence of atropine (Hasrat et al., 2004). Indeed, the cholinergic mechanism plays an important role in the compensation of hypertension, both peripheral and central (Imai et al., 1989; Lepori et al., 2001). The adrenaline-induced hypertension was not reduced by the ethanolic fraction of *P. amarus* (FEPA). This fraction of the total extract would not add renergic effects on blood pressure.

CONCLUSION

The total extract of *P. amarus* and the two fractions had slightly different phytochemical compositions. However, the ethanolic fraction (FEPA) had the largest hypotensive effect. This effect was a result mainly of the action cholinimimétic chemical compounds present in the extract.

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AUTHORS' CONTRIBUTIONS

All authors contributed equally in the study. They made substantial contributions to the design of the study, the collection of the data as well as the preparation and analysis of the data. They also drafted the manuscript and gave final approval for its submission to the journal for consideration of publication.

DECLARATION OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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