ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 11, Issue, 06, pp.4254-4259, June, 2019

DOI: https://doi.org/10.24941/ijcr.35556.06.2019

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

POLYPHENOL, LYCOPENE CONTENTS AND ANTI-HYPERTENSIVE ACTIVITY OF AQUEOUS EXTRACT OF *LYCOPERSICON ESCULENTUM* Mill FRUITSIN RABBITS

[1]Syndoux Dembélé, *[2]Koffi N'dri Emmanuel, [3]Goze Nomane Bernard and [1]Anin Atchibri Anin Louise

[1]Laboratory of Nutrition and Food Safety(LNFS), Research Training-Unit of Sciences and Food Technologies, University Nangui Abrogoua, 02 BP 801 Abidjan 02, Cote d'Ivoire
[2]Advanced teacher's training college of Abidjan (ENS), Department of Sciences and Technologies, 08 BP Abidjan 08, Cote d'Ivoire
[3]Laboratory of Physiology, Pharmacology and Pharmacopeia, Research Training-Unit of Sciences Nature, University Nangui Abrogoua, 02 BP 801 Abidjan 02, Cote d'Ivoire

ARTICLE INFO

Article History:
Received 24th March, 2019
Received in revised form
20th April, 2019
Accepted 26th May, 2019
Published online 30th June, 2019

Key Words:

Tomato, Polyphenol,
Lycopene, Antihypertensive

**Corresponding author:*
Koffi N'dri Emmanuel

ABSTRACT

Objective*:** The aim of this study was to quantify some compounds of the aqueous extracts of two tomato varieties grown in Cote d'Ivoire and assess their anti-hypertensive activity on arterial blood pressure in rabbit. ***Material: Two ripe tomato varieties were collected from different tomato fields in Yamoussoukro district *(Cote d'*Ivoire*)* during season of June to July 2018. These samples were dried and crushed. The powder obtained was used for different extract preparations. ***Duration*** : This study was carried out from june 2018 to february 2019. The determination of polyphénol and lycopene contents was carried out at the National Laboratory of Public Health. Then, antihypertensive activity was evaluated at Animal Physiology Laboratory of Felix Houphouët Boigny University. ***Methodology***: Lycopene and polyphenol contents were determined by high performance liquid chromatography (HPLC) method. The antihypertensive activity was performed on rabbits by manometric method using Ludwig's manometer. **Results** : Lycopene content of tomato aqueous extracts was ranging from 0.01 to 89.9 mg/100g of extract. The most abundants polyphenolic compounds identified were caffeic acid (1.37 to 2.14 mg/100 g) and quercetin (1.10 to 2.19 mg/100 g) of extract. Other phenolic compounds like, catechin, flavanone, cinnamate, coumarine and tanninol were identified. Efficacy dose (ED_{50}) of aqueous extracts varies between 26.25 mg/kg bw for the macerated of *Local cotelette* variety and 31.62 mg/kg bw for the macerated of *Cobra F1* variety. ***Conclusion*** : Tomato aqueous extracts reduced rabbit arterial blood pressure. This decrease will be due to compounds with antihypertensive activity contain in extracts such as lycopene and quercetin. As a result, tomato in take would be beneficial for hypertensives.

***Citation: Syndoux Dembélé, Koffi N'dri Emmanuel, Goze Nomane Bernard and Anin Atchibri Anin Louise,* 2019.** "Polyphenol, lycopene contents and anti-hypertensive activity of aqueous extract of *Lycopersicon Esculentum* Mill fruitsin rabbits", *International Journal of Current Research*, 11, (06), 4254-4259.

INTRODUCTION

Hypertension is among the major risk factors for cardiovascular diseases (Murray, 1997). It is defined as an abnormal and permanent elevation of arterial blood pressure. People is hypertensive when the systolic (SAP) and diastolic (DAP) arterial blood pressures is higher than 140 mmHg and 90 mmHg respectively (Benkhedda, 1956). According to epidemiological data, the number of hypertensives will increase to 1.5 billion (OMS, 2010) and 150 million by 2025 in the world and in sub-Saharan Africarespectively (Fourcade, 2007). In Côte d'Ivoire, the prevalence of this pathology is 33.4 % (MSLS, 2015). Hypertension appears in the list of incurable diseases according to WHO (OMS, 1998), its treatment remains a difficult task despite the availability of several types of drugs that lower and regulate arterial blood pressure by different mechanisms (Waeber, 1999). Considering the risk of high arterial blood pressure that populations are exposed as well as the high cost of the treatments of this affection by allopathic medicine, studies are undertaken to find effective and less costly remedies. There is accordingly that two varieties of tomato grown in Cote d'Ivoire, one hybrid (*Cobra 26 F1*) and the other traditional (*Local cotelette*) commonly called African tomato would be a promising alternative in the treatment of hypertension. The aim of this study was to ascertain compounds of the aqueous extracts of two tomato varieties (*Cobra 26 F1* and *Local cotelette*) using high performance liquid chromatography technique and assess their pharmacological effects on arterial blood pressure in rabbits.

MATERIAL AND METHODS

Plant material: Two varieties of tomato namely *Cobra 26 F1*(hybrid variety) and *Local cotelette* (traditional variety) were used. They were respectively harvested from three different fields in N'gattakro and Zatta, two localities in Yamoussoukro district (Cote d'Ivoire) from June to July 2018.

Animals: 12 rabbits of *Oryctolagus cuniculus* (Leporideae) species were used to assess hypertensive activity of tomatoes extracts. They come from breeding farms in Abobo (Abidjan, Cote d'Ivoire). These animals were fed with pellets (Ivograin, Cote d'Ivoire) and watered. They were acclimatized for a week at the laboratory of Animal Physiology of Research Training-Unit of Biosciences of Felix Houphouet Boigny University of Abidjan-Cocody (Cote d'Ivoire) in order to regulate and harmonize their physiological states before experiments. For experiments, only rabbits weighted between 1.9 and 2.1 kg were used.

Methods

Preparation of the tomato aqueous extracts: Fresh tomatoes were crushed and dried at 60°C. for 48 hours. They were ground in a porcelain mortar. The powder obtained was used for the extract preparations using maceration and decoction process.

Maceration process: 100 g of tomato powder were dissolved in 1L of distilled water. This powder remained soaked in water at ambient temperature (30 ± 2 °C under stiring for 24 hours. After, the mixture was centrifuged at 1000 rpm for 10 min. The supernatant was collected and dried at 60° C during 48 hours.

Decoction process: It consisted in heating at reflux for 10 min a mixture of 100 g of tomato powder in 1 L of distilled water. After cooling, this mixture was centrifuged at 1000 rpm for 10 min. The supernatant was recovered and dried at 60 ° C for 48 hours.

HPLC analysis: This analysis was carried out with a Precision instruments HPLC, Model 201 (Kyoto, Japan) equipped with a C18 column (ODS 2: 250 x 2 mm, Waters, USA) and an electrochemical detector, connected to an integrator (Waters 746, Saint-Quentin in Yvelines, France). Operating conditions for various compounds identification and quantification were established by Yap *et al.* (1997).

Determination of lycopene content: 20 µL of extract were analyzed by HPLC on a C18 column (ODS 2: 250 × 2 mm, Waters, USA). The pump flow rate was kept constant at 2 $mL.min^{-1}$. Elution was carried out with 425 mL of acetonitrile-methanol mixture (350 mL-75 mL, V/V), 50 mL of aqueous solution of ammonium acetate (4%, m/V) and 50 mL of dichloromethane. Elution was carried out in an isocratic mode for 20 minutes. Analyzes were performed in triplicate. Lycopene chromatograms were recorded at λ_{max} = 292 nm. The lycopene content was expressed as mg/100 g trans-lycopene equivalent.

Determination of polyphenol content: 20 µL of extract were analyzed by HPLC on a C18 column (ODS 2: 250 × 2 mm, Waters, USA). The pomp flow rate was kept constant at 0.6 $mL.min^{-1}$. Elution was carried out with acetonitrile/water/acetic acid mixture (210/237/3, V/V/V) and it was carried out in an isocratic mode for 15 minutes. Polyphenols wavelength detection was fixed at λ_{max} = 280 nm (Figure 2). The different analyzes were done in duplicate. For polyphenol quantifications, standard extracts of polyphenol (catechin, coumarin, tannin, quercetin, caffeic acid, sodium cinnamate and flavanone) were co-injected with tomato studied extracts. Standards having the same peaks as tomato extracts were quantified in mg/100 g equivalent of the corresponding standard.

In vivo antihypertensive activity of tomatoes aqueous extracts: Antihypertensive activity of tomatoes extracts was assessed recording to the method described by Etou *et al.,* (2019) using a LUDWIG manometer. Rabbits were anesthetized using 40 % solution of ethyl urethane administered intraperitoneally at the dose of 1 g/kg bw. The hairs of the neck and thigh were shorn to expose the saphenous vein and the carotid artery. These vessels were respectively intubated using a syringe containing the Mac Ewen heparinized solution. The carotid artery was intubated with a catheter connected to a U-tube pressure gauge, which collects direct arterial blood pressure in the carotid. This method measures the reference arterial blood pressure in rabbits. Changes in the carotid blood pressure, transmitted to the mercury column of the device, are recorded with a pen that translates the movements of mercury on the smoked paper placed on a cylinder driven at constant speed. The tomato aqueous extracts were extemporaneously dissolved in the Mac Ewen solution and then injected into the rabbit through the saphenous vein previously dissected with a syringe every 15 minutes at different doses (10, 20, 30, 40 and 50 mg / kg bw).

Statistical analysis: Statistical analysis and graphs were achieved using Graph Pad Prism 5.01 (San Diego, California, USA) software. Results are expressed as mean of individual values assigned to standard error (mean ± SEM). The significance of differences between the percentages of decrease compared to the reference arterial blood pressure is obtained by analysis of variance (ANOVA) and multiple comparison test using Tukey–Kramer method. According to this test when $p < 0.05$, and $p < 0.001$ the observed differences are significant, and highly significant, respectively. The dose at which the half effect 50 % of extracts was obtained (Efficacy dose at 50 %, ED_{50}) were calculated with this software.

RESULTS

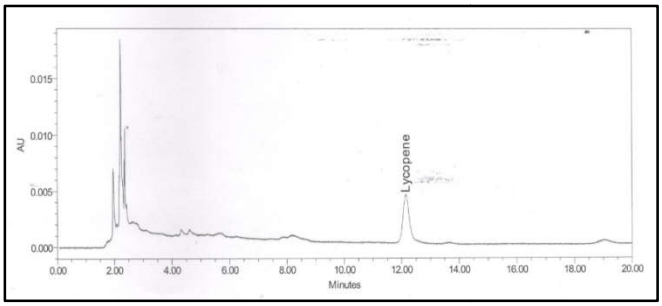
Lycopene content of the tomato aqueous extracts: The lycopene HPLC profile of four aqueous extracts from two tomato varieties grown in Cote d'Ivoire obtained by maceration and decoction (figure 1). Lycopene content of the aqueous extracts obtained by maceration is ranging between 9.4 and 89.94 mg/100 g of extract while the aqueous extracts obtained by decoction ranging between 0.007 and 0.008 mg/100 g of extract. These results show that aqueous extracts obtained by maceration contain more lycopene than those obtained by decoction. Extract from traditional variety (*Local cotelette*) also contains more lycopene than *Cobra F1* variety (Table 1).

Polyphenol contents of the tomato aqueous extracts: Figure 2 presents phenolic HPLC profile of tomatoes aqueous extract. Phenolic compound such as catechin, quercetin, caffeic acid, sodium cinnamate, coumarin and flavanone are present in all the tomato aqueous extracts.

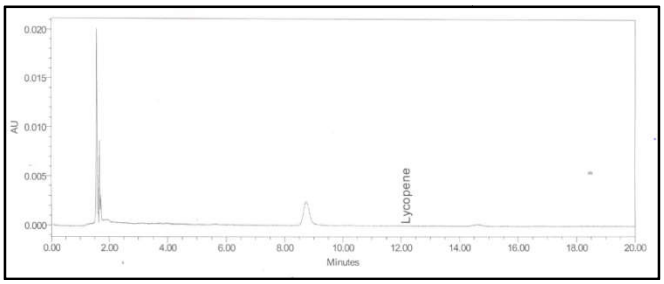
Table 1. Lycopene contents of four tomato aqueous extracts

Extracts	Lycopene (mg/100g)
Cobra F1 (*maceration*)	9.4 ± 0.02^{b}
Cobra F1 (*decoction*)	0.008 ± 0.00^{a}
Locale cotelette(*maceration*)	89.94 ± 0.01^{c}
Locale cotelette(*decoction*)	0.007 ± 0.00^{a}

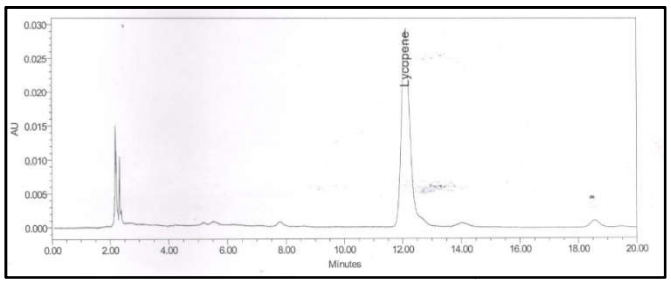
Values are the average of 3 tests ± standard error, Values with the same letter in the same column are not statistically y different at p<0.05.



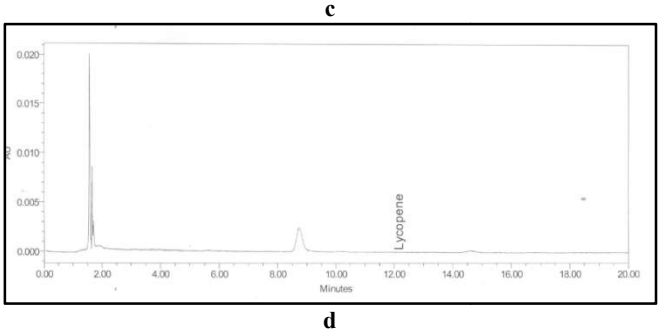
a



b



c



d

a= extract obtained by maceration of Cobra 26 F1, b= extract obtained by decoction of Cobra 26 F1, c= extract obtained by maceration of Local Cotelette, d= extract obtained by decoction of Local Cotelette

Figure 1. Chromatogram of lycopene of aqueous extracts of tomato varieties

However, sodium cinnamate and tanninol were not identified in the aqueous extract of *Cobra F1* obtained by maceration. Moreover, tanninol is absent in the aqueous extract of *Cobra F1* obtained by decoction. Catechin content of the aqueous extracts is ranging between 0.36 and 0.91 mg/100 g of extract. Extracts from the *Cobra F1* variety are richer in catechin than those from *Local cotelette* variety. However, the aqueous extract obtained by maceration of *Cobra F1* variety has a low catechin content compared to that obtained by decoction of this same variety. The quercetin content in these different extracts is at contents ranging between 1.10 and 2.19 mg/100 g of extract. The extracts of the hybrid variety *Cobra F1* contain more quercetin than those of the traditional variety *Local cotelette*. Caffeic acid content is ranging between 1.37 and 2.14 mg/100 g of extract. The extract obtained by decoction of *Local cotelette* variety and that obtained by maceration of the *Cobra F1* variety are the richest in caffeic acid with 2.14 and 2.08 mg/100 g of extract respectively. Sodium cinnamate in the various extracts are at levels between 0.02 and 0.04 mg/100 g of extract. The aqueous extract by maceration of the *Cobra F1* variety does not contain sodium cinnamate. Coumarin is present in all extracts, but at low rates i.e. around 0.02 and 0.03 mg/100g of extract. The flavanonecontent varies between 0.05 and 0.17 mg/100g of extract while the tanninol present only in the extracts of the traditional variety (*Local cotelette*) at contents between 0.02 and 0.03 mg/100 g of extract. Extracts from tomatoes grown in Cote d'Ivoire are rich in polyphenol including quercetin and caffeic acid. However, results obtained indicate that the extraction method and the tomato variety affected significantly phenolic content of the extracts.

Effect of the fruits aqueous extract from *Lycopersicum esculentum* Mill fruit on arterial blood pressure in rabbit: Aqueous extracts obtained by maceration and decoction were evaluated on arterial blood pressure in rabbit at doses ranging between 10 and 50 mg / kg bw (Figure 3). The normal mean arterial blood pressure values of rabbits in this experiment were 109 mmHg (*Local cotelette* decocted), 96 mmHg (Local cotelette macerated), 102 mmHg (Cobra F1 decocted) and 126 mmHg (Cobra F1 macerated) respectively. These values represent the reference arterial blood pressures. The respective injection on different intravenous preparations in increasing doses of *Local cotelette* decocted (LCOT DEC), *Local cotelette* macerated (LCOT MAC), *Cobra F1* decocted (COBRA DEC) and *Cobra F1* macerated (COBRA MAC) ranging between 10 and 50 mg / kg bw. induced in rabbits, dose-dependent and reversible hypotensions. The highly significant percentage decreases *(p<0.001)* in arterial blood pressure ranged from 14 to 40 % (LCOT DEC), 16 to 46 % (LCOT MAC), 10 to 36 % (COBRA DEC), and 10 to 43 % (COBRA MAC) respectively compared to the respective normal values of arterial blood pressure in the interval of considered doses.

The average values obtained after three experiments were used to plot curves shown in figure 3-E' expressing decreases in arterial blood pressure at doses ranging from 10 to 50 mg / kg bw of LCOT DEC, LCOT MAC, COBRA DEC and COBRA MAC. These decreases in arterial blood pressure of rabbits varied from 15.21 ± 2.60 to 42.57 ± 4.90 % (LCOT DEC), 17.44 ± 1.80 to 47 ± 3.80 % (LCOT MAC), 10.87 ± 2.40 to 38.26 ± 4.10 % (COBRA DEC) and 11.32 ± 2,30 to 44.55 ± 4.40 % (COBRA MAC) relative to rabbits reference arterial blood pressures. The values of the efficacy dose at 50 % (ED_{50}) determined from these curves were 27.97 mg/kg bw (LCOT DEC), 26.25 mg/kg bw (LCOT MAC), 30.83 mg/kg bw (COBRA DEC) and 31.62 mg/kg bw (COBRA MAC).

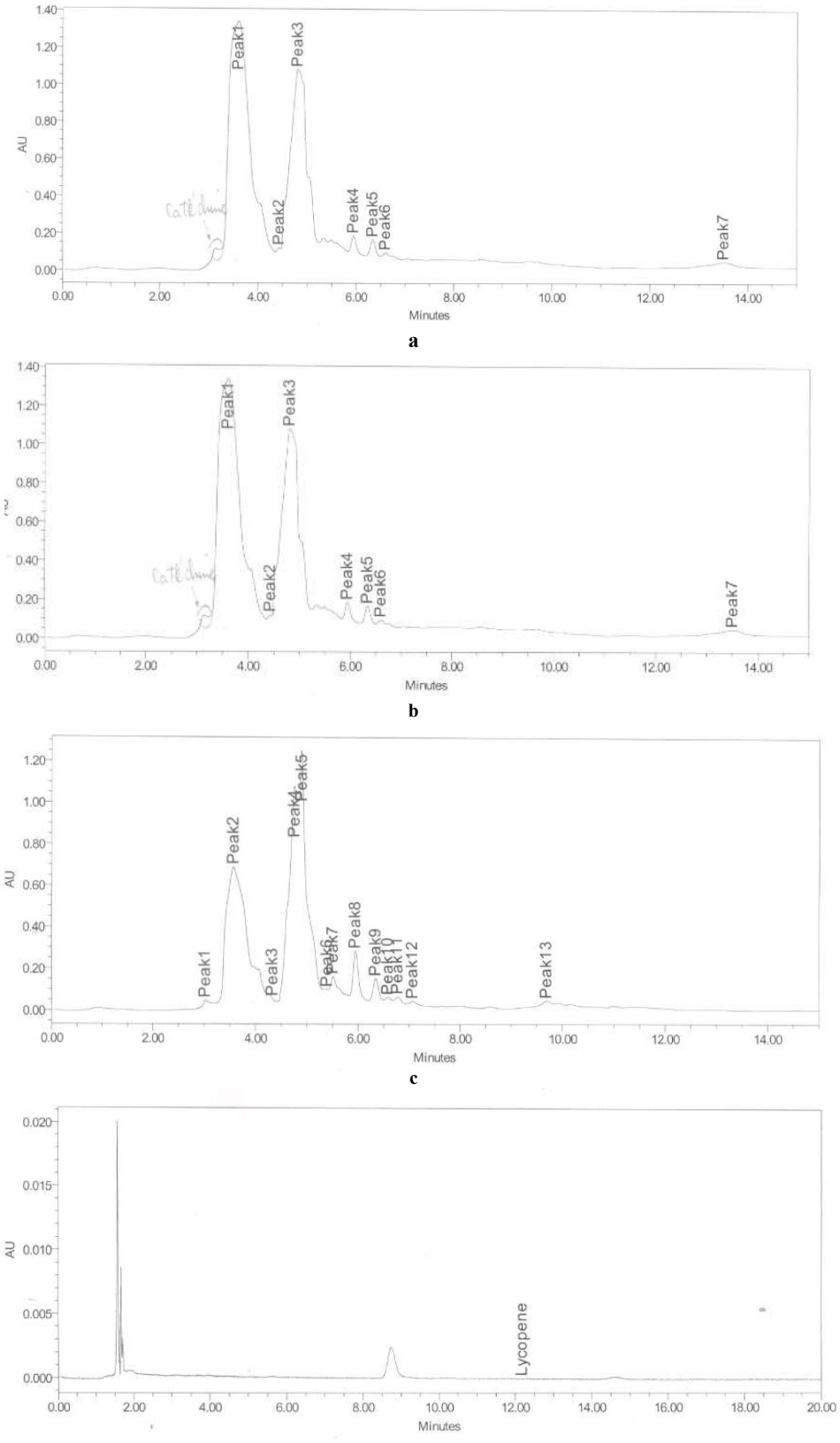
DISCUSSION

The recommended daily dose of lycopene is 10 to 20 mg/kg bw, tomato intake or products based on tomato or lycopene supplementation may help against deficiency lycopene and prevent hypertension (Paran, 2001).

Table 2. Polyphenol contents of four tomato aqueous extracts

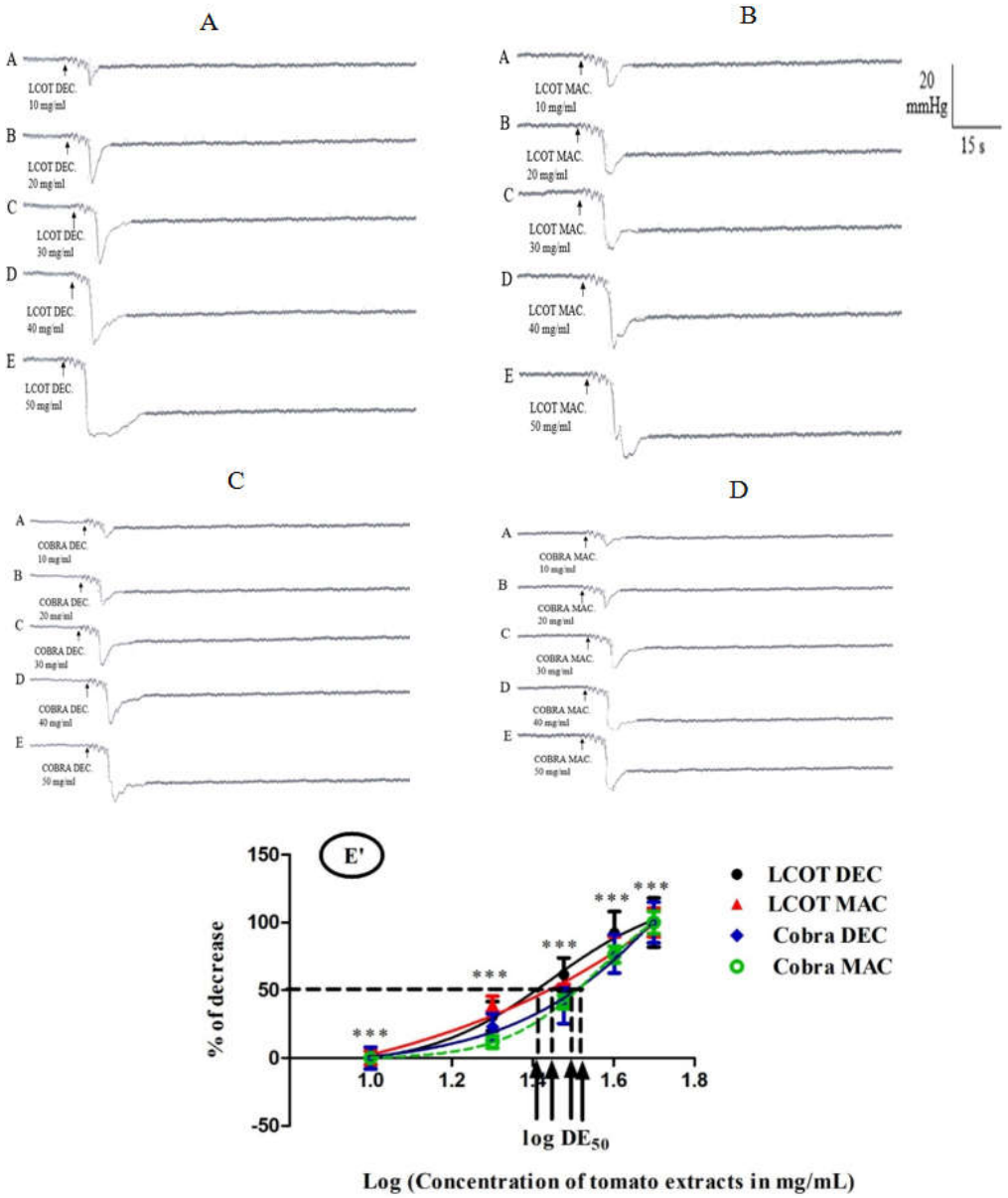
Extracts	Catechin	Quercetin	Caffeic acid	Sodium cinnamate	Coumarin	Flavanone	Tanninol
L. cotelette (AEM.)	0.36±0.05[a]	1.24±0.23[a]	1.56±0.11[a]	0.04±0.00[b]	0.02±0.00[a]	0.17±0.03[c]	0.02±0.01[a]
L. cotelette (AED)	0.37±0.08[a]	1.10±0.19[a]	2.14±0.12[b]	0.02±0.01[a]	0.03±0.01[a]	0.06±0.03[a]	0.03±0.01[a]
Cobra F1 (AEM)	0.67±0.03[b]	2.17±0.18[b]	2.10±0.22[b]	-	0.03±0.01[a]	0.05±0.02[a]	-
Cobra F1 (AED)	0.91±007[c]	2.19±0.20[b]	1.37±0.17[a]	0.04±0.00[b]	0.02±0.01[a]	0.11±0.03[b]	-

Values are the average of 3 tests ± standard error, Values with the same letter in the same column are not statistically y different at p<0.05. AEM= Aqueous extract obtained by maceration, AED= Aqueous extract obtained by decoction. Contents are expressed in mg/100g.



e= extract obtained by maceration of Cobra 26 F1, f= extract obtained by decoction of Cobra 26 F1, g= extract obtained by maceration of Local Cotelette, h= extract obtained by decoction of Local Cotelette

Figure 2. Chromatogram of phenolic profile of the aqueous extracts of tomato varieties



A to E : Control recordings (before arrows) and effects of extracts (after arrows) at 10 mg/ml(A), 20 mg/ml (B), 30 mg/ml (C), 40 mg/ml (D) and 50 mg/ml (E) on rabbit arterial blood pressure.
A', B', C'et D' : Dose–response effects of LCOT DEC, LCOT MAC, Cobra DEC and Cobra MAC on rabbit arterial blood pressure.
E' : Dose-response curves of tomato extracts (n=3). ***p<0.001 : highly significant difference compared to decrease in percentage of reference arterial blood pressure.

Figure 3. Dose-response effect of the fruits aqueous extract of *Lycopersicum esculentum* on rabbit arterial blood pressure

In addition, these extracts contain a quercetin which promotes the lowering of arterial blood pressure in hypertensive subjects (Edwards, 2007). These extracts are a potential source of caffeic acid, polyphenols which has a high antioxidant capacity. Caffeic acid is able to slow down or prevent lipids oxidation (Emma, 2009). It also protects cells against action of free radicals as trolox (water soluble vitamin E) (Chung, 2006). Then, catechin is a very important flavonoid that is responsible for the inhibition of histidine decarboxylase, an enzyme that promotes the conversion of histidine to histamine (Emma, 2009). The latter is at the origin of the increase in organism toxicity. Chen *et al*. (Chen, 2002), showed that catechins protect body from toxic effect of several compounds. According to *Codex Alimentarius* (2006) coumarin is the phenolic which should not be added to foods and beverages. Its acceptable daily intake is 0.1 mg/kg bw according to European Food Safety Authority (EFSA). Extracts that were studies contain a tiny amount that ranges from 0.02 to 0.03 mg/100 g of extract. In order to assess the effects of tomato extracts on hypertension, the study of the pharmacological effects of four aqueous extracts of tomato on arterial blood pressure in rabbits was performed. Results revealed that these extracts induced a dose-dependent and reversible hypotension for doses ranging between 10 and 50 mg/kg bw. These extracts are therefore endowed with a real hypotensive potential. These results are similar to those of many medicinal plant extracts such as *Hibiscus sabdariffa* (Malvaceae) (Mojiminiyi, 2007) and *Bambusa vulgaris* (Poaceae) (N'guessan, 2009). Comparing the efficacy dose at 50 % (ED_{50}) of these four extracts, it appears that LCOT MAC with an ED_{50} equal to 26.25 mg/kg bw is the most effective because having the smallest ED_{50} value. The decrease in arterial blood pressure observed during intravenous injection of these four tomato extracts could be related to the combined action of lycopene and quercetin. Indeed, it is known that in tomato, one of the cholinomimetic substances responsible for the decrease in arterial blood

pressure is lycopene (Engelhard, 2006). As proof, Paran *et al*. (2001) found that in 30 grade 1 hypertensive patients aged between 40 and 65 years who used only treatment based on lycopene extracted from tomato for 8 weeks, a systolic blood pressure decrease of 9 mmHg and a diastolic blood pressure of 7 mmHg was found. In addition, these results also remained acetylcholine (ACh) effects on arterial blood (Emma, 2009). It indicates that there are cholinomimetic substances in these tomato extracts that act like ACh. However, ACh induces hypotension mainly by two ways. Indeed, in auricles, stimulation of M_2 muscarinic receptors activates G-protein composed of three subunits (α, β, γ). The α subunit directly stimulates potassium channels while the βγ complex promotes the degradation of arachidonic acid (Hartzell, 1988). The K^+ ions output induced a hyperpolarization which results in a reduction of calcium entry resulting in a weak release of calcium by sarcoplasmic reticulum. This leads to a decrease in the strength of contractions of heart and subsequently hypotension (Engelhard, 2006 and Noda, 1983). In ventricles, stimulation of M_2 receptors inhibits adenylcyclase by a mechanism that involves an inhibitory G-protein. This results in a decrease in the production of cyclic AMP (cAMP) which in turn causes decrease in protein kinase A (PKA) activity. This decrease in PKA activity inhibits calcium channels, resulting in low calcium release by sarcoplasmic reticulum (Hartzell, 1988). All these reactions lead to hypotension. It would be interesting to use a competitive antagonist cholinergic receptors to ensure the cholinomimetic effect of these aqueous extracts. This will explore the cholinergic pathway involved in this decrease in arterial blood pressure.

Conclusion

This study revealed that the aqueous extracts of two varieties of tomato *local cotelette* and *Cobra F1,* grown in Cote d'Ivoire are rich in polyphenols (quercetin, caffeic acid) and lycopene. These aqueous extracts have anti-hypertensive activity. Among these extracts, *locale cotelette* macerated have the best anti-hypertensive activity. As a result, the intake of this variety would be could be advised in traditional medicine for hypertension treatment.

REFERENCES

Benkhedda, S., Les nouvelles recommandations de l'OMS société internationale de Best, C.H., The first clinical use of insulin. *Diabetes*, 1956(5): pp. 65-67, 2001.

Chen, L., X. Yang, H. Jiao, and B. Zhao, Tea catechin protect against lead-induced cytotoxicity, lipid peroxidation and membrane fluidity in Hep G2 cells. *Toxicology Sciences,* 69(1): pp. 149-156, 2002.

Chung, M., Walker PA, and Hogstrand C, Dietary phenolic antioxidants, caffeic acid and Trolox, protect rainbow trout gill cells from nitric oxideinduced apoptosis. *Aquatic Toxicology,* vol. 80, : pp. 321-328, 2006.

Commission du Codex Alimentarius PROGRAMME MIXTE FAO/OMS SUR LES NORMES ALIMENTAIRES COMITÉ DU CODEX SUR LES ADDITIFS ALIMENTAIRES Trente neuvième session CX/FA 07/39/12, 1-11. 2006.

Coumarin in flavourings and other food ingredients with flavourings properties- Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and materials in contact with food (AFC). doi: 102903 : j.efsa.2008793, 2008.

Edwards, R., T. Lyon, S. Litwin, A. Rabovsky, J. Symons, and T. Jalili, Querctin reduces blood pressure in hypertensive subjects. *J Nutr,* vol. 137, 2007.

Emma, M., A. Marinova, N. Toneva, and Yanishlieva, Comparison of the antioxidative properties of caffeic and chlorogenic acids. *Food Chemistry,* vol. 114: pp. 1498-1502, 2009.

Engelhard, Y., Gazer B, and P. E., Natural antioxidants from tomato extract reduce blood pressure in patients with grade-1 hypertension: a double blind placebo controlled pilot study. *Am. Heart J,* 151(1), 100, 2006.

Etou Ossibi, A.W., R.D.G. Elion Itou, and J. Nzonzi, Effets de l'extrait aqueux de Lippia multiflora Moldenke (Verbenaceae) sur la pression artérielle, la fréquence cardiaque et les ondes de l'électrocardiogramme chez le rat normotendu. *Revue CAMES-Série Pharm. Méd. Trad. Afr,* 17 (1): pp. 1-9, 2014.

Fourcade, L., P. Paule, and B. Mafart, Arterial hypertension in Sub-saharan Afica. Nitric oxide and inhibits angiogenesis. Circulation, 106: pp. 913-919., 2007.

Hartzell, H.C., Regulation of cardiac ion channels by catecholamines Acetylcholine and second messenger systems. *Prag. Biophys. Mol., ,* 52:: pp. 165-247, 1988.

Katzung, B., Pharmacologie Fondamentale et Clinique (7ème édn) Edition Piccin: Padoue, Italie, 150, 2007.

Mojiminiyi, F.B.O., M. Dikko, B.Y. Muhammad, P.D. Ojobor, O.P. Ajagbonna, R.U. Okolo, U.V. Igbokwe, U.E. Mojiminiy, M.A. Fagbemi, S.O. Bello, and T.J. Anga, Antihypertensive effect of an aqueous extract of the calyx of Hibiscus sabdariffa. Fitoterapia, 78: pp. 292-297, 2007.

MSLS, Ministère de la Santé et de la Lutte contre le SIDA. Programme National de développement sanitaire 2015.

Murray, C.J. and A.D. Lopez, Global mortality, disability, and the contribution of risk factors Global Burden of disease study. Lancet, 349: pp. 1436-1442, 1997.

N'guessan, K., G.N. Zirihi, and D.T. Etien, .,. , Hypotensive effect of Aqueous Extract of Bambusa Vulgaris Sheets on the Arterial Pressure of Rabbits. . American Journal of Scientific Reseach,, (Issue 2): pp. pp60-72. , 2009.

Noda, M., H. Takahashi, and T. T., Structural homology of Torpedo californica Acetylcholine receptor subnits nature.302: pp. 528-532, 1983.

OMS, Communiqué OMS/39. Assemblée mondiale de la santé. les maladies non transmissibles sont une priorité mondiale., 1998.

OMS, Rapport sur la santé dans le monde. 2010.

Paran, E. and Y. Engelhard, Effect of tomato's lycopene on blood pressure, serum lipoproteins, plasma homocysteine and oxidative stress markers in grade I hypertensive patients. Am. J. Hypertens., 14, 141A, 2001.

Racke, K. and S. Matthiesen, The airway cholinergic system: physiology and pharmacology. Pulmonary Pharmacol. Ther, 17: pp. 181-198, 2004.

Reimann, H., W. Lorenz, M. Fischer, R. Frolisch, H. Meyer, and A. Schmal, Histamine and acute haemorrhagic lesions in rat gastric mucosa: Prevention of stress ulcer formation by (+)-catechin and inhibitor of specific histidine decarboxylase in vitro. DOI 10. 1007 / BF 01964883, Vol 7, Number 1: pp. 69-73, March 1977.

Waeber, B., M. Burnier, and H.R. Brunner, Le point sur le traitement de l'hypertension artérielle. Schweiz Med Wochensch, 129: pp. 14-16, 1999.

Yap, S., Y. Choo, and C. Ooi, Quantitative analysis of carotenes in the oil from different palm species. Elaeis,. Ong ASH., Goh SH, 3 pp. 369-378, 1997.

****