World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

Effect of fractions from *Carissa edulis (*Forssk.) Vahl *(Apocynaceae*) leaves on hypertension induced in Wistars rats

Marius Adjagba [1,*], Razack Osseni [2], Ferdinand Adounkpe [3], Arnaud Agbanlinsou [1], Latifou Lagnika [4], Gaudens Djego [5], Raphaël Darboux [1], Bonaventure Awede [2] and Anatole Lalèyè [1]

[1] *UFR of Human Biology. Faculty of Health Sciences, University of Abomey-Calavi, Benin.*
[2] *UFR of Human Physiology. Faculty of Health Sciences, University of Abomey-Calavi, Benin.*
[3] *National Narcotics and Toxicology Laboratory, University of Abomey-Calavi, Benin.*
[4] *Laboratory of Biochemistry and Molecular Biology, Faculty of Technical Sciences, University of Abomey-Calavi, Benin.*
[5] *Applied Ecology Laboratory, Faculty of Agronomic Sciences, University of Abomey-Calavi, Benin.*

World Journal of Advanced Research and Reviews, 2022, 15(02), 424–431

Publication history: Received on 29 June 2022; revised on 02 August 2022; accepted on 04 August 2022

Article DOI: https://doi.org/10.30574/wjarr.2022.15.2.0781

Abstract

High blood pressure is an important health problem because of its prevalence and its several complications. The high cost of its management and the side effects of conventional drugs have led patients to use medicinal plants. *Carissa edulis* is one of the plants used in the traditional management of hypertension. The aim of this study was to determine the effects of crude extract and fractions of *Carissa edulis* in L-NAME hypertension rats models. The phytochemical analysis of the extracts was carried out by using the thin layer chromatography method. The pharmacological effect was evaluated in Wistar rats rendered hypertensive by administering the N (ω) -Nitro-L-Arginine-Methyl Ester (L-NAME). The crude extract was administered at 500 mg / kg (b/w) and the fractions at 20 mg / kg (b/w). Blood pressure was measured by a non-invasive method. Four fractions were obtained after fractionation. Many chemical compounds were detected diversely distributed in either in the crude extract or in the fractions. The crude aqueous extract induced a significant decrease in blood pressure from 137, 00 ± 8,01 mmHg to 94,75 ± 2.84 mmHg. In the other hand, only the aqueous fraction exerted the highest effects by reducing the blood pressure from 137,00 ± 8,01 mmHg to 93,5±8,54 mmHg.

The results obtained justify the traditional use of the leaves of *Carissa edulis* in the treatment of high blood pressure.

Keywords: *Carissa edulis*; Medicinal plant; L-NAME; High blood pressure; Ethnobotanical surveys

1. Introduction

High blood pressure, the major risk factor of cardiovascular diseases is an important health problem worldwide because of its prevalence and its multiple complications. The therapeutic management of hypertension is a long term and expensive process especially for developing countries population. The high cost of its management and the side effects of conventional drugs have led patients to use medicinal plants [1].

*Carissa edulis (*Forssk.) Vahl. is a plant which belongs to the family of Apocynaceae and is distributed in tropical Africa (Botswana, Cameroun, Benin) and Asia (Cambodge, Japan and Myanmar) [2]. The root decoction is used against malaria, indigestion, post-partum pains and against chest pains. [3]. The roots' infusion is used against stomachache, Herpès

* Corresponding author: Marius Adjagba
UFR of Human Biology. Faculty of Health Sciences, University of Abomey-Calavi, Benin.

simplex virus infection and cataract when used as eye drop. In Benin, the roots are used as aphrodisiac, against women sterility and hypertension. *Carissa edulis* is also used as a source of dye [4].

Pharmacological studies on *Carissa* species have indicated significant antiplasmodial [5], diuretic (Nedi T et al, 2004)[6], anticonvulsant [2] , antibacterial [3, 7–10], anti-oxidant and anti-tumor [5, 11–13], antiviral [14, 15] , antiemetic [9], anti-hyperlipidemic [10], analgesic, anti-inflammatory, antipyretic activities [16, 17], vasorelaxant [18], cardioprotective [19] , hepatoprotective [16–18], antidiabetic [5] and anti-helminthiasis activities [20].

Considering the use of *Carissa edulis* in folk medicine as antihypertensive, this study aimed to evaluate the antihypertensive activities of the crude extracts and fraction of the plant in order to verify its ethnomedical use.

2. Material and methods

2.1 Vegetal material

Fresh leaves of *Carissa edulis* were harvested at Abomey-Calavi a closed country to Cotonou (South of Benin) and a voucher specimen was authenticated at the National herbal Centre of Benin AA6482/HNB. They were dried under the lee of sun at a temperature between 20-25°C during three weeks. The dried leaves were pulverized and the powder was stored at room temperature until use.

150 g of the dried powder of *Carissa edulis* were extracted at 80°C with 500ml of distilled water during 30 minutes. The decoction was filtered and then poured into an evaporating dish to evaporate the water over a water-bath at a temperature of 80°C. The brown dried extract obtained was stored in refrigerator at 4°C.

In the other hand, 50 g of the aqueous extract were dissolved with 500 ml of distilled water and then introduced in a 2L conical flask. A first separation was carried out with dichloromethane (700ml three times) and successively with ethyl acetate and n-butanol. The organic phases were recovered and evaporated at 30°C on a rotary evaporator. The residual aqueous phase was also evaporated at 70°C on the rotary evaporator under reduced pressure.

2.2 Phytochemical analysis

The phytochemical analysis of the crude extract and the various fractions was carried out using a thin-layer chromatography (TLC) as described by Bladt and Wagner (2001) [13]. 5 mg of each extract was dissolved in 1 ml of appropriate solvent (1: 1 merhanol / water mixture, dichloromethane and ethyl acetate). On silica gel chromatography plates (60F254, Merck), 10µl of each mixture are deposited and the migration was carried out by means of suitable solvent for each desired chemical group. Thus, for the screening of coumarins, flavonoids, tannins, triterpenes and anthocyanins derived, the migration solvent used includes ethyl acetate / formic acid / methanol / acetic acid / water (100: 11: 11: 26). The solvents used for the searching of alkaloids, anthracenes, and glucosides are mixed of ethyl acetate, methanol and water (in proportions 100, 13.5: 10). For lignans, terpenes and sesquiterpenes, saponins, naphthoquinones, the migration solvents mixure wererespectively: chloroform / methanol / water (70: 30: 4); Chloroform / methanol / water (65: 25: 4); Chloroform / acetic acid / methanol / water (64: 32: 12: 8) and toluene / formic acid (99: 1).

2.3 Animal Design

Male wistar rats weighing between 180 - 200g were used housed in cages and maintained in a light controlled environment (12:12h light-dark cycle). They had free access to food and water.

For the crude extract, 15 rats were assigned to three groups of five rats

- Control group: Rats received distilled water from day 1 to day 14 (L-NAME for the 7 first days and which received specific treatment from day 8 to day 14 as follows:
- (N(G)-Nitro-L-Arginine Methyl Ester (L-NAME) group: rats were treated with L-NAME) at 20 mg/Kg of body weight from Day 1 to Day 7 and received distilled water from day 8 to day 14.
- Treated Group: Rats of this group were previously treated with L-NAME followed by the aqueous extract of *Carissa edulis* at 500 mg/kg of body weight from day 8 to day 14.

For the fractions, 35 male rats were randomly divided into 7 groups of five.

Group 1: Rats received distilled water from day 1 to day 14 (control group
Group 2: Rats were treated with L-NAME from Day 1 to Day 7 and received distilled water from day 8 to day 14.
Group 3: Rats of each subgroup were treated with one of the fractions of *Carissa edulis* 30 mg/kg of body weight for 7 days after 7-day-administration of L-NAME.
Group 4: animals in this group were treated with Losartan at 100 mg/kg of body weight from day 8 to day 14 after L-NAME administration from day 1 to day 7.

2.4 Measurement of blood pressure

The arterial pressures of the rats were taken by non-invasive measurement with the CODA device (Kent Scientific Corporation, USA) on D0, D4, D8, D12 and D15.

The CODA device allows the measurement of caudal arterial pressure in rats. This method of measurement consists in the use of a sleeve causing an occlusion of the blood flow at the level of the caudal artery. This sleeve is linked directly to a CODA voltage controller which is connected to a computer. Once the systolic and diastolic blood pressure values were obtained, the system automatically calculates the mean pressure while the cuff deflates completely. This value can also be calculated using the following formula: PAM = PAD + 1/3 (PAS-PAD).

2.5 Data processing and analysis

Arterial pressure values are given on average ± standard error. The data were entered in Excel and analyzed using GraphPad Prism 5 software. The analysis of variance (ANOVA test) was used. The significance threshold P was set at 5% ($P < 0.05$).

3. Results

3.1 Aqueous extraction and liquid-liquid partition

The aqueous extraction carried out from 100 g of the powder of the leaves of ***Carissa edulis*** yielded 18.32g of a dry extract of a slightly dark brown color with a yield of 18.32%. The liquid-liquid partition from 50 g of crude aqueous extract resulted in four (4) fractions: the aqueous fractions (Aqueous F.), dichloromethane (FDM), acetate of ethyl (FAE) and butanol (F. BUTANOL). The masses, extraction yields, colors and aspects of the various fractions are shown in Table 1.

Table 1 Summary of liquid-liquid partition efficiency results

Fractions	Mass of fractions (g)	Extraction yield (%)	Colors and aspects after evaporation
F AQUEUX	22,80	45,60	Light brown and powder
F BUTANOL	9,95	19,90	Orange and powder
F AE	0,81	1,62	Paste and sticky yellow orange
F DM	0,86	1,72	Pasty and sticky dark green
F CYCLO	0,20	0,40	Pasty and sticky dark green
Total	34,62	69,24	

The extraction yields were determined based on the weight of the dry crude aqueous extract. The highest yields were obtained with the aqueous phase followed respectively by the butanol phases, dichloromethane, ethyl acetate. On the basis of very low yield obtained for the cyclohexane phase, we carried out a thin-layer chromatographic analysis of the various fractions in order to define their chromatographic profile with a view to possible regrouping. The reading of the plate revealed that the cyclohexane and dichloromethane fraction had the same profile and therefore consisted of the same families of chemical compounds. We have thus discarded the cyclohexane fraction (very low yield). The results of this partition showed that during the partition a small quantity of components was drained into the organic phases, which justifies the higher yield obtained for aqueous fraction (45, 60%). In the organic phases, the butanol fraction gave the highest yield (19, 90%) while the cyclohexane fraction gave the lowest yield with 0.44%.

3.2 Phytochemical analysis

The results of the phytochemical screening was given in table 2. Eleven (11) chemical compounds were distributed differently in the crude aqueous extract as well as in the different fractions. Lignans and saponosides were present in the crude extract and in all the fractions. Tanins, anthocyanin pigments, glycosylated coumarins, anthocyanin pigments, alkaloids, glycosylated flavonoids and anthracens were absent only in the dichloromethane fraction, but present in the crude extract, aqueous fraction, butanol fraction and in the ethyl acetate fraction. Naphtoquinones were detected only in the dichloromethane fraction. Bitter principles were highlighted in the aqueous fraction, butanol fraction and in the ethyl acetate fraction, while the triterpens in the ethyl acetate and dichloromethane fractions.

Table 2 Phytochemical screening of the crude extract and the different fractions of *Carissa edulis* Vahl

Samples	Crude Extract	Aqueous fraction	Butanol fraction	Ethyl acetate fraction	Dichlromethane Fraction
Tanins	+	+	++	++	-
Triterpenes	-	-	-	+	++
Anthocyanin pigments	+	+	++	++	-
Glycosylated Coumarins	++	++	+	+	-
Lignans	++	++	++	++	+
Saponosides	++	++	++	++	+
Bitter Principles	-	+	++	++	-
Alkaloids	+	+	+	++	-
Glycosylated Flavonoids	++	++	+	+	-
Anthracene Derivatives	+	+	++	++	-
Naphtoquinones	-	-	-	-	+

Absence (-), (+) Low presence, (++) Strong presence

3.3 Effects of crude aqueous extract and fraction on arterial hypertension

Table 3 Comparative mean arterial pressure of the rats subjected to the study of the antihypertensive activity of the crude extract

	D0	D8	D15
Control	89,60 (3,94)	91,40 (11,17)	88,80 (7,22)
L-name	93,33 (7,45)	137,00(13,45)	119,67 (12,33)
L-name/crude extract	93,75 (5,09)	137,00 (8,01) **	94,75 (2,84) **

The data are expressed in mmHg and as mean +/- SEM; The values assigned to the letter A are significantly different from the value of the witness batch; The values assigned to the letter B are significantly different from the value of the L-NAME batch
(*: P-value <0.05)and (**: p-value <0.01)are Significantly different from witness group.

Tables 3 and figure 1 showed the mean arterial pressures of the different groups of retreated with the crude extract of *C. edulis*.

Administration of L-NAME from D0 to D7 resulted in a significant increase in both control and treated group of rats. The MAP increased about 40 mmHg. Consequently, a significant decrease of rats MAP was observed when treated by crude aqueous extract of *C. edulis* from 137,00 ± 8,01 mmHg (J8) to 94, and 75 ± 2.84 mmHg (D15).

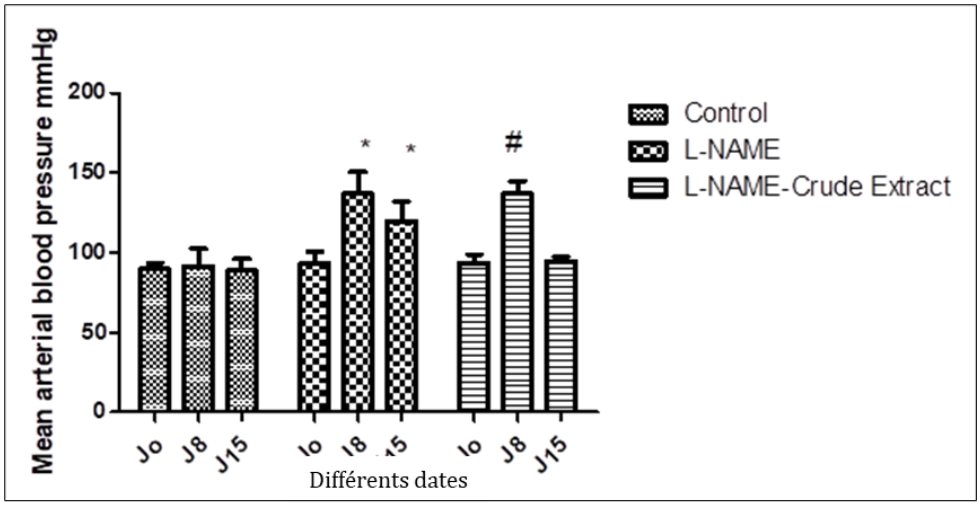
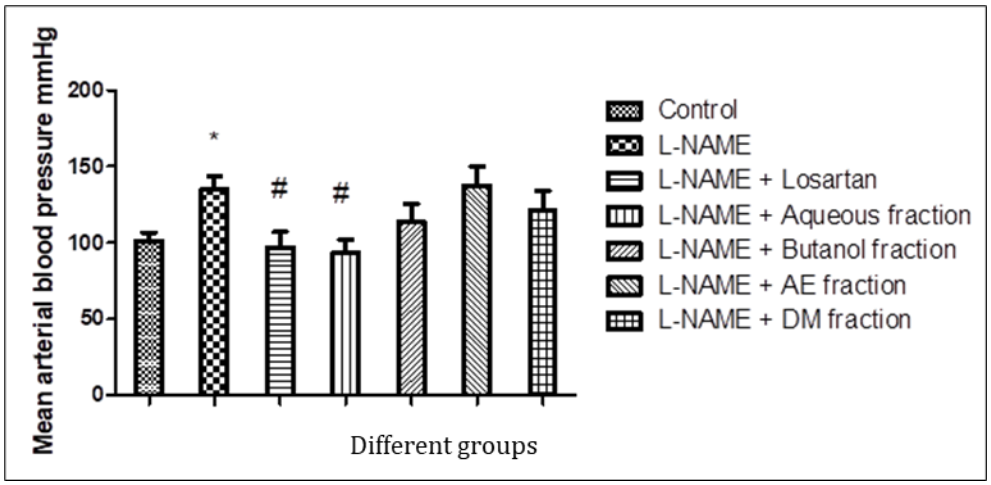


Figure 1 Effect of the crude extract of *Carissa edulis* on the arterial pressure of rats

Table 4 Comparative mean arterial pressure of the rats subjected to the study of the antihypertensive activity of the different fractions

	PAS	**PAD**	**PAM**
CONTROL	132,8±3,69	86,8±6,32	101,6±5,11
L-NAME	167±4,16	119,67±12,33	135±8,89 [b]
L-NAME/Losartan	120±10,07	86,33±10,84	97±10,39 [a]
L-NAME/F AQ	119±12,81	81,75±6,81	93,5±8,54 [a]
L-NAME/F BUT	145,5±11,84	98,5±12,28	113,75±11,93
L-NAME/F AE	162,8±13,64	125,8±11,75	137,8±12,2
L-NAME/F DM	152±13,32	106,2±14,39	121±13,48

F: Fraction; BUT: Butanol; DM: Dichloromethane; AE: Ethyl acetate; The values assigned to the letter a are significantly different from the value of the control batch; The values assigned to the letter b are significantly different from the value of the L-NAME group; *:P-value<0.05 and **: p-value <0.001 Significantly different from control group. PAS: systolic arterial pression; PAD: diastolic arterial pression; PAM: Mean arterial pression



* Significant compared to control group;# Significant compared to L-NAME group

Figure 2 Mean arterial pressure of the different groups of rats

Table 4 and Figure 2 showed the effect of the administration of the various fractions on the arterial pressure of the rats. After administration of L-NAME,rats blood pressure increased significantly and the administration of either Losartan or the different fractions normalized the MAP. The best effect was recorded with the aqueous fraction. Although the butanol fraction induced a significant decrease it was not able to normalize the MAP of the rats. Ethyl acetate and dichloromethane fractions had no effect on the rats MAP.

4. Discussion

The aqueous decoction is the method used by healers to prepare herbal recipes. It is also the most widely used method for the preparation of recipes used in the traditional management of hypertension [21, 22]. This technique has therefore been used to carry out the aqueous extraction of the plant drug.

The liquid-liquid partition is carried out by using increased polarity solvents. Empirically, the aqueous extraction was chosen, followed by the alkaline extracts in conventional therapeutics. This choice is generally advisable because most active pharmacological molecules dissolve there.

The results of this partition showed that during the partition, a small quantity of extracts was drained into the organic phases. This justifies the yield obtained for the aqueous fraction (45.60%). In the organic phases, the butanol fraction gave the highest yield (19, 90%).

The phytochemical screening revealed eleven (11) chemical compounds distributed differently in the crude aqueous extract and the different fractions.

The different fractions obtained and the crude aqueous extract were tested on a model of hypertension induced by L-NAME in Wistar rat. Indeed, an inhibition of nitrogen monoxide (NO) synthesis at the endothelial level is induced by L-NAME and underlied the development of arterial hypertension in the rat. This inhibition of NO leads to vasoconstriction by increasing peripheral vascular resistance and is a relatively stable model of hypertension. According to Biancardi et al (2007) [23], vasoconstriction induced by sympathetic tone, in response to L-NAME administration, plays an important role in the initiation and maintenance of hypertension.

The dose of L-NAME at 20 mg / kg body weight used in our study is comparable to that used by Mali et al (2012), and Bachav et al., (2012) [24, 25]. Arterial pressures obtained after L-NAME administration were significantly higher ($p < 0.05$) than in normal untreated rats, indicating the efficacy of L-NAME at this dose. The aqueous extract of *C. edulis* significantly normalized ($P < 0.05$) the blood pressure at a dose of 500 mg / kg.

In the other hand, only the residual aqueous fraction revealed the most important antihypertensive activity.

Our results were similar with those previously published by Adjagba (2015) , Osseni (2016), Adjagba (2017) [26–28] who in their work demonstrated that at a dose of 500 mg / kg body weight, the crude aqueous extract of *Tridax procumbens*, *Gmelina arborea* and *Crateva adansonii* significantly reduced MAP in the rat. Considering the model of hypertension used during this study, the mechanism of action of the crude aqueous extract and the active fraction derived from the fresh leaves of *Carissa edulis* may be a relaxation of the vascular smooth musculature leading to a decrease in peripheral resistances due to the inhibition of the action of L-NAME.

Al-Youssef and Hassan 2010 [5], have shown that the petroleum ether, ethyl acetate and aqueous extracts of *Carissa edulis* possess a marked potency for lowering the blood pressure in rats at a dose dependent manner, while butanol extract has not shown any significant decrease in arterial pressure at higher dose.

The observed antihypertensive property could be related to the activity of the secondary metabolites detected in the aqueous extract and which were concentrated in the aqueous fraction.

It has been shown that flavonoids induced vasorelaxation and increased nitric oxide (NO) production by endothelial cells [29, 30].

Cardiac glycosides have effects on the heart. These are the active ingredient in many different heart medicines in clinical use and they are the major class of medications used to treat heart failure. The cardiotonic activity and prolonged blood pressure lowering effect of *Carissa edulis* was previously reported [5]. The cardiac activity of water-soluble fraction has been attributed to the presence of the odoroside glucosides including odoroside H and F [5].

Phytochemicals such as the flavonoids, tannin, (polyphenols) and terpenes are kwon to induce antioxidant properties [21, 31, 32] and are thus liable to protect lipids by lipid peroxidation inhibition, blood and other body fluid from damage induced by oxidative stress.

Some alkaloids were known to have muscarinic activity (Liu et al., 2003)[33]. It has also been documented that saponins have some hypotensive activity [34, 35].

Moreover it has been also noted a diuretic effect of extracts of *Carissa edulis* that may also explain the antihypertensive effect [2].

5. Conclusion

The crude aqueous extract induced a significant decrease in blood pressure. In the other hand, only the aqueous fraction exerted the highest effects by reducing the blood pressure. Furthermore, vasodilator properties of the extract were conferred on it by the presence of numerous secondary metabolites and their safety has been proven. This present results support the ethnomedical use of *Carissa edulis* as an antihypertensive agent.

Compliance with ethical standards

Acknowledgments

This study was financially supported by Rectoract of the University of Abomey-Calavi BENIN. The authors thank all participants in this study.

Disclosure of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

Statement of ethical approval

The studies were conducted in accordance with internationally accepted principles for laboratory animal use and care (EEC directive of 1986: 86/609 EEC). There is no ethics committee for animals in BENIN.

References

[1] Agban A, Gbogbo KA, Hoekou YP, et al. Évaluation de l ' activité antifongique des extraits de Cassia alata L . et Candida albicans. Int J Biol Chem Sci 2013; 7: 1041–1047.

[2] Ya'u J, Yaro AH, Abubakar MS, et al. Anticonvulsant activity of Carissa edulis (Vahl) (Apocynaceae) root bark extract. J Ethnopharmacol 2008; 120: 255–258.

[3] Fartyal M, Kumar P. Bioactivity of Crude Extracts of Carissa carandas Linn. Extracted in Polar and Non Polar Solvents. Int J Eng Sci Innov Technol 2014; 3: 186–191.

[4] Olivier M, Zerbo P, Boussim J, et al. Les plantes des galeries forestières à usage traditionnel par les tradipraticiens de santé et les chasseurs Dozo Sénoufo du Burkina Faso. Int J Biol Chem Sci 2013; 6: 2170–2191.

[5] Al-Youssef HM, Hassan WHB. Phytochemical and Pharmacological Aspects of Carissa Edulis Vahl : a Review. Int J Curr Res Chem Pharm Sci 2014; 1: 12–24.

[6] Nedi T, Mekonnen N, Urga K. Diuretic effect of the crude extracts of Carissa edulis in rats. 2004; 95: 57–61.

[7] Apocynaceae CE, Jean K, Tokoudagba MD, et al. Phytochemistry and antibacterial activity of extracts of parkia biglobosa. Int J Sci Acad Res 2021; 02: 3241–3245.

[8] Achenbach H, Waibel R, Addae-Mensah I. Lignans and other constituents from Carissa edulis. Phytochemistry 1983; 22: 749–753.

[9] Ahmed S, Hasan MM, Ahmed SW. Anti-emetic effects of bioactive natural products. 2013; 4: 390–433.

[10] Kaunda JS, Zhang YJ. The Genus Carissa: An Ethnopharmacological, Phytochemical and Pharmacological Review. Nat Products Bioprospect 2017; 7: 181–199.

[11] Semenya SS, Maroyi A. Medicinal plants used by the Bapedi traditional healers to treat diarrhoea in the Limpopo Province, South Africa. J Ethnopharmacol 2012; 144: 395–401.

[12] Muthee JK, Gakuya DW, Mbaria JM, et al. Ethnobotanical study of anthelmintic and other medicinal plants traditionally used in Loitoktok district of Kenya. J Ethnopharmacol 2011; 135: 15–21.

[13] Itankar PR, Lokhande SJ, Verma PR, et al. Antidiabetic potential of unripe Carissa carandas Linn. fruit extract. J Ethnopharmacol 2011; 135: 430–433.

[14] Allahverdiyev AM, Abamor ES, Bagirova M, et al. Investigation of antileishmanial activities of Tio2@Ag nanoparticles on biological properties of L. tropica and L. infantum parasites, in vitro. Exp Parasitol 2013; 135: 55–63.

[15] Bagla VP, Mcgaw LJ, Eloff JN. The antiviral activity of six South African plants traditionally used against infections in ethnoveterinary medicine. Vet Microbiol 2012; 155: 198–206.

[16] Itankar PR, Lokhande SJ, Verma PR, et al. Antidiabetic potential of unripe Carissa carandas Linn . fruit extract. J Ethnopharmacol 2011; 135: 430–433.

[17] Hegde K, Joshi AB. Hepatoprotective effect of Carissa carandas Linn root extract against CCl 4 and paracetamol induced hepatic oxidative stress. 2009; 47: 660–667.

[18] Kaunda JS, Zhang Y-J. The Genus Carissa : An Ethnopharmacological , Phytochemical and Pharmacological Review. Nat Products Bioprospect 2017; 7: 181–199.

[19] Sahreen S, Khan MR, Khan RA, et al. Cardioprotective role of leaves extracts of Carissa opaca against CCl 4 induced toxicity in rats. 2014; 1–9.

[20] Kpodar MS, Karou SD, Katawa G, et al. An ethnobotanical study of plants used to treat liver diseases in the Maritime region of Togo. J Ethnopharmacol 2016; 181: 263–273.

[21] If L, Foa L, Op A, et al. Ethnobotanical assessment of the plant species used in the treatment of diabetes in the Sudano- Guinean zone of Benin. J Anim &Plant Sci 2015; 26: 4108–4123.

[22] Adjagba M, Awede B, Osseni R, et al. Antihypertensive effect of extracts from Crateva adansonii DC.ssp. adansonii in the Wistar rats. Int J Biol Chem Sci 2018; 11: 2604.

[23] Parolari A, Camera M, Sci B, et al. Systemic Inflammation After On-Pump and Off- Pump Coronary Bypass Surgery : A One-Month. Cardiovascular 2007; 1–6.

[24] Shah SL, Zambare GN, Bodhankar SL. Original Article Cardioprotective Activity of Methanol Extract of Fruit of Trichosanthes cucumerina on Doxorubicin-induced Cardiotoxicity in Wistar Rats. 2012; 167–173.

[25] Bachhav SS, Bhutada MS, Patil SD, et al. Effect of Viscum articulatum Burm . (Loranthaceae) in N o -nitro- L - arginine methyl ester induced hypertension and renal dysfunction. J Ethnopharmacol 2012; 142: 467–473.

[26] Adjagba M, Awede B, Nondichao K, et al. Antihypertensive Activity of Different Fractions of Tridax Procumbens Crude Aqueous Extract in Wistar Rats. J Physiol Pharmacol Adv 2015; 5: 713.

[27] Adjagba M, Awede B, Osseni R, et al. Effects of crude aqueous extracts of Heliotropium indicum Linn (Boraginaceae) on blood pressure in Wistar rats. Int J Pharmacol Res 2017; 7: 165–169.

[28] Osseni R, Akoha S, Adjagba M, et al. In vivo Toxicological Assessment of the Aqueous Extracts of the Leaves of Carissa edulis (Apocynaceae) in Wistar Rats. Epub ahead of print 2016. DOI: 10.9734/EJMP/2016/26083.

[29] Adomou A, Yedomonhan H, Djossa B, et al. Etude Ethnobotanique des plantes médicinales vendues dans le marché d'Abomey-Calavi au Bénin. Int J Biol Chem Sci 2012; 6: 745–772.

[30] Eliezer D, Townsend SSM, Sawyer PJ, et al. System-justifying beliefs moderate the relationship between perceived discrimination and resting blood pressure. Soc Cogn 2011; 29: 303–321.

[31] Begum S, Syed SA, Siddiqui BS, et al. Phytochemistry Letters Carandinol : First isohopane triterpene from the leaves of Carissa carandas L . and its cytotoxicity against cancer cell lines. Phytochem Lett 2013; 6: 91–95.

[32] Snedden W, Fernandez PG, Nati C. High performance liquid chromatography analysis of nifedipine and some of its metabolites in hypertensive patients1. can J physiol pharmacol 1986; 64: 290–296.

[33] Liu Q. R2D2 , a Bridge Between the Initiation and Effector Steps of the Drosophila RNAi Pathway. 1921. Epub ahead of print 2014. DOI: 10.1126/science.1088710.

[34] Capie S. China ' s pharmaceutical revolution. 2015; 4: 98–105.

[35] Iwatashi KH, Hirakawa HS, Ori KH, et al. Reduction of Blood Pressure by Soybean Saponins , Renin Inhibitors from Soybean , in Spontaneously Hypertensive Rats. 2010; 74: 2310–2312.