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DIURETIC ACTIVITY OF AQUEOUS DECOCTION EXTRACT AND ETHYL ACETATE FRACTION OF *LANNEA MICROCARPA* ENGL. AND K. KRAUSE (ANACARDIACEAE) TRUNK BARKS IN WISTAR RATS

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ABSTRACT

Medicinal plants are highly used in developing countries. In Burkina Faso, the *Lannea microcarpa* trunk barks extracts have been used for long time by the local community that evoked its various medicinal properties. However, no scientific evidence is hitherto available to support highlighted effects. To study the beneficial actions of *Lannea microcarpa* extracts as a cardiovascular and renal protective agent, we investigated the diuretic and saluretic properties of these extracts in normotensive rats. The experimental animals were divided into nine (09) groups of six (06) rats each. The rats were pretreated with isotonic saline (0.9% NaCl, 25 mL/kg body weight), to impose an uniform water and salt balance. Subsequently, different groups of rats were treated orally with LMaq or LMAE (10, 50 and 100 mg/kg), or furosemide (15 mg/kg) or amiloride (5 mg/kg). Rats urine were collected using a graduated and transparent tubes and its volume was measured at 2 h, 4 h, 6 h, 12 h and 24 h after starting experimentation. Cumulative urine excretion was calculated in relation to body weight and expressed as mL/100 g of body weight. The electrolytes Na^+ and K^+ concentrations were measured using an automate "Humalyte plus 3." Aqueous decoction showed a diuretic dose-dependent potential with

respect to its ethyl acetate fraction. At a dose of 100 mg/kg LMaq, urinary excretion is more intense compared to control. Interestingly, the extracts eleminate Na^+ while stabilizing the excretion of K^+. Their mechanism of action was closer to furosemide than amiloride. Thus, *Lannea microcarpa* extracts could be benefit for the therapeutic management of cardiovascular and renal pathologies.

KEYWORDS: *Lannea microcarpa*, diuretic potential, electrolyte excretion, Rat wistar.

1- INTRODUCTION

Medicinal plants are highly used in developing countries. In some cases, extracts of medicinal plants are used by traditional healers as diuretics drugs for treatment of many patients suffering from hypertension, heart failure and other diseases involving the cardiovascular system.[1-3] Nowadays, several vegetal drugs are studied due to their ability to increase the volume of urine and electrolyte excretion, and therefore help the body to reduce fluid buildup. The most common disease treated with diuretics agents is high blood pressure. Indeed, the current drugs have still not overcome the high blood pressure. In this context, the search for new therapeutic alternatives for cardiovascular and renal disorders is extremely necessary, both to broaden the available pharmacological options and to prove the effectiveness of preparations used in folk medicine.[4] In Burkina Faso, the practice of traditional medicine relies mainly on the use of medical plants. *Lannea microcarpa* Engl. and K. Krause (Anacardiaceae) commonly known as African grape is one of these commonly used plant. Traditional remedies prepared from its leaves, bark, roots and fruits are used to treat many diseases such as mouth blisters, rheumatism, sore throats, dysentery, conjunctivitis, stomatitis, skin eruptions, ulcers [5-7] and high blood pressure.[8] Its trunk barks extracts are known to be practically nontoxic even at high dose in rats.[9,10]

Lannea microcarpa trunk barks extracts have been used for long time by the local community that evoked its various medicinal properties. Evidence of the beneficial properties of *Lannea microcarpa* extracts as a cardiovascular and renal protective agent has been reported. However, its potential effect as a diuretic agent in pre-clinical trials remain unknown. Considering the described biological effects of *Lannea microcarpa* extracts as an antihypertensive agent and in view of the relationship of volumie, as well as the importance, of diuretic drugs in clinic to reduce blood pressure, this present study therefore aimed to investigate (i) the diuretic and saluretic properties of *Lannea microcarpa* extracts in normotensive rats, and (ii) elucidates its main mode of action.

2- MATERIALS AND METHODS

2.1- Plant material

The *Lannea microcarpa* Engl. and K. Krause (Anacardiaceae) trunk barks were collected on January 2015 in the area of Loumbila (zone of savannah), located at 20 km in the Northeast of Ouagadougou (Burkina Faso). The plant sample was authenticated at "Herbier National du Burkina (HNBU)" located at "Département Environnement et Forêt / Centre National de la Recherche Scientifique et Technologique" (DEF-CNRST), Ouagadougou (Burkina Faso) where the voucher specimen has been deposited under number HNBU 361.

The collected sample was air-dried deprived of solar light, dust and was powdered using a mechanical grinder. The obtained powder was used to prepare the extracts for biological investigation.

2.2- Preparation of the lyophilized aqueous decoction and fractions with dichloromethane and ethyl acetate

Two hundred grams (200 g) of stem barks powder from Lannea microcarpa were extracted by decoction using 1 L water distilled during 30 min. The aqueous solution was filtered and then centrifuged at 650 g (centrifugal force) for 5 min. One part of supernatant was lyophilized and the other part was fractionnated. The sequential extraction method with the aqueous decoction was used with two organic solvents based on the polarity. A fractionation of the aqueous decoction (200 mL) was carried out starting with dichloromethane (DCM), followed by ethyl acetate (AcOEt). Fractions with dichloromethane (LMDCM, 50.4 mg) and with ethyl acetate (LMAE, 915 mg) were obtained after exhaustion with DCM (3 x 100 mL) and AcOEt (3 x 100 mL) respectively followed by rotary evaporator concentration and dry evaporation (35-40°C). These fractions were used for further pharmacological investigations.

2.3- Animals

Healthy male Wistar rats (4-5 months, weighing 280-320 g) were used in this study. They were procured from the animal house of University Ouaga I Pr Joseph KI-ZERBO and acclimated in the pet Shop of the "Institut de Recherche en Sciences de la Santé" (IRSS), Ouagadougou, Burkina Faso. The animals were randomly selected, marked for individual identification and housed in animal cages with free access to water and standard laboratory pellet enriched with proteins (29%). All animals were maintained in temperature controlled room of 22-25°C with a natural light and dark cycle. Experience was carried out in

accordance with international standard protocols [Guidelines set by the European Union on the protection of animals (CEC Council 86/609)] and adopted by IRSS, Burkina Faso.[10]

2.4- Screening for acute diuretic activity

The diuretic method with slight modification was used to assess the diuretic activity of all test substances.[11,12] In these experiments, the rats were deprived from food and water overnight (12 h). The experimental animals were divided into nine (09) groups of six (06) rats each. The rats were pretreated with isotonic saline (0.9% NaCl, 25 mL/kg body weight), to impose an uniform water and salt balance. Subsequently, different groups of rats were treated orally with decoction aqueous lyophilised extract (LMaq) or its ethyl acetate fraction (LMAE) of *Lannea microcarpa* trunk barks (10, 50 and 100 mg/kg), or furosemide (15 mg/kg) or amiloride (5 mg/kg). All the drugs were freshly prepared. Control rats received equal volume of isotonic saline, the vehicle used to dissolve the extracts. Immediately after treatment, the rats were individually placed in metabolic cages provided with a wire mesh bottom and a funnel retaining faeces while allowing urine passage. During this period, no food and water were made available to the animals. Rats urine were collected using a graduated and transparent tubes and its volume was measured at the timepoints 2 h, 4 h, 6 h, 12 h and 24 h after starting experimentation. Cumulative urine excretion was calculated according to body weight and expressed as mL/100 g of body weight. Urines were stored at 4°C for electrolyte analysis. The electrolytes Na^+ and K^+ concentrations were measured using an automate "Humalyte plus 3". The instrument was calibrated with standard solutions containing different concentrations of Na^+ and K^+.

Diuresis and electrolytes parameters were calculated as previously described[13], according to the following formula:

Urinary excretion = (V_o/Vi) x 100 where V_o is the total urinary output and V_i is the total volume of fluid administered.

Diuretic index = Vt/Vc where V_t is the mean urine volume of test group and V_c is the mean urine volume of negative control group.

Diuretic activity = Vt/Vr where V_t is the mean urine volume of test group and V_r is the mean urine volume of reference group.

Saluretic index = Ct/Cc where C_t is the concentration of electrolyte in the urine of test group and C_c is the concentration of electrolyte in the urine of negative control group.

Natriuretic activity = Na^+/K^+ratio = C_n/C_k where C_n is the concentration of Na^+ in the urine of a group and C_k is the concentration of K^+ in the urine of the same group.

2.5- Statistical analysis

Data from each group were summarized by mean ± SEM (n=6). Statistical difference between treated and control groups were tested by one-way analysis of variance (ANOVA) using GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA) followed by Bonferroni multiple comparison tests. $P<0.05$ was considered statistically significant.

3- RESULTATS

3.1- Effect on urine volume

The decoction extract and ethyl acetate fraction of the *Lannea microcarpa* trunk bark produced a small dose-dependent diuresis during the 6th hour (Figures 1A-C). In fact, at a dose of 10 mg/kg, 50 mg/kg and 100 mg/kg of LMaq and LMAE, the values of urinary excretion after 2 hours were between 0.10±0.04 and 0.37±0.12 mL/100 g of body weight. The highest excretion was observed at a dose of LMaq 100 mg/kg and 0.19±0.08 mL/100 g of body weight for the control group. Positive control groups produced respectively 1.33±0.27 and 0.70±0.11 mL/100 g of body weight for furosemide (15 mg/kg) and amiloride (5 mg/kg) (Figure 1A). At 4 h experimentation with the same doses of LMaq and LMAE (10, 50 and 100 mg/kg), control groups and standard groups, the urine excretion values of extracts were 0.14±0.06 to 0.56±0.17 mL/100 g. The control group produced 0.22±0.08 mL/100 g and respectively for furosemide and amiloride produced 1.93±0.35 and 0.91±0.15 mL/100 g of body weight (Figure 1B). At the end of the time (6 h) of cumulatively urine excretion volume for the first part, the groups control were produced 1.26±0.09 mL/100 g, extracts groups between 0.68±0.25 and 1.75±0.31 mL/100 g of body weight and positive groups respectively 3.05±0.20 mL/100 g and 2.15±0.19 mL/100 g for furosemide and amiloride (Figure 1C). For the three conditions, highest excretion of LMaq 100 mg/kg was important compared to control. However, these excretion were comparable to the excretion of amiloride but not to furosemide.

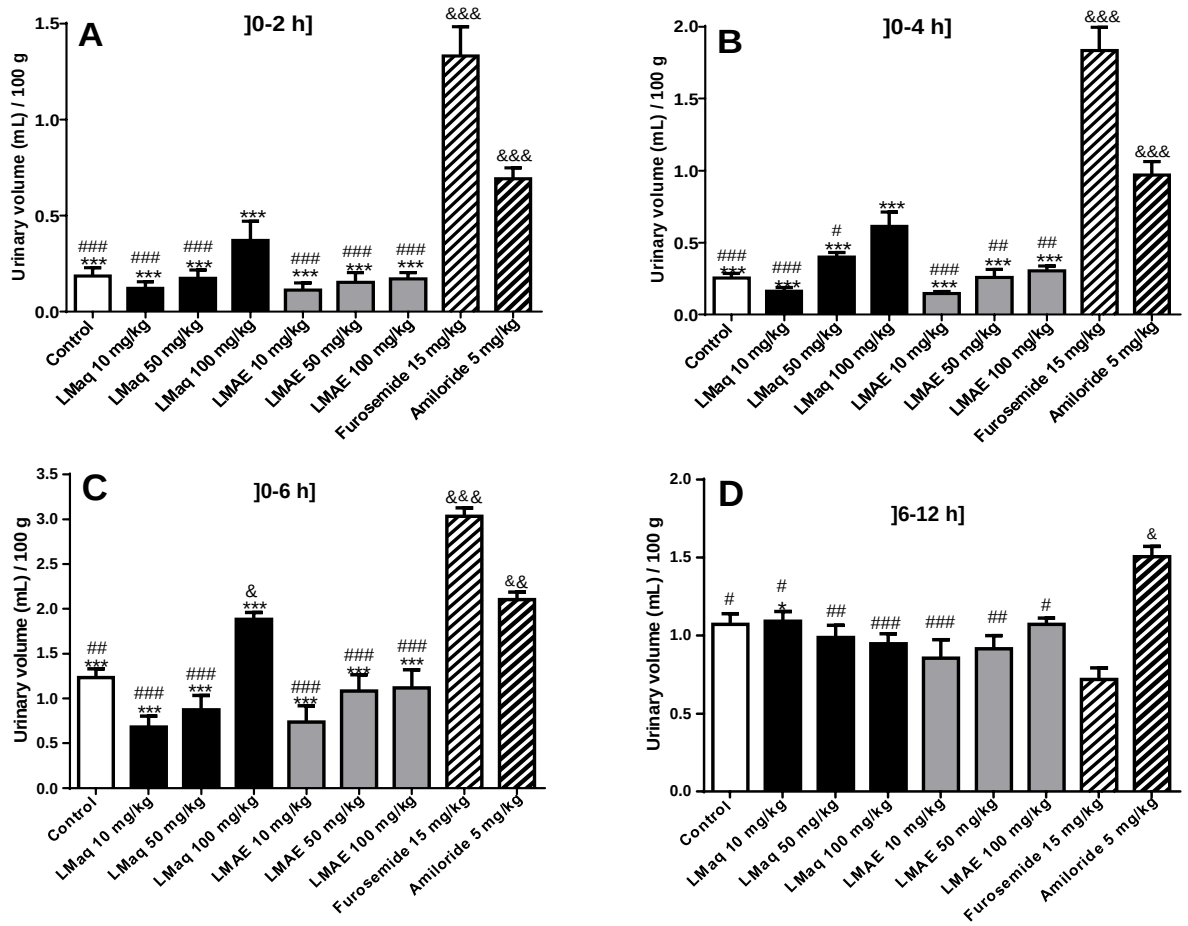
For the period of 6 h to 12 h, highest urine excretion of control and extracts doses were not statistically different to amiloride group excretion. Exceptionally, highest LMaq 10 mg/kg dose excretion was statistically different to furosemide excretion. Others doses of LMaq and LMAE have produced lowers volumes**.** These volumes were statistically differents compared to furosemide excretion (Figure 1D).

For the period of 12 h to 24 h, highest urine excretion of control and extracts doses were not statistically different to furosemide and amiloride group excretion. Exceptionally, highest LMaq 10 mg/kg dose excretion was statistically different to furosemide excretion (Figure 1E).

For the period of 24 h, urine excretion of LMaq and LMAE doses were not statistically different to control group excretion. Exceptionally LMaq 100 mg/kg of body weight (bw) excretion that was not statistically different to furosemide and amiloride excretion, others doses of LMaq and LMAE were produced lowers volumes that were statistically differents compared to furosemide and amiloride excretion (Figure 1F).

3.2- Effect on diuretic excretion, diuretic index and diuretic activity

As shown in Table 1, results have showed significant diuretic activity by increasing urinary output at the dose of 100 mg/kg of body weight (bw) when compared with control or the reference drugs, furosemide (15 mg/kg) and amiloride (5 mg/kg). The effect of *Lannea microcarpa* extracts was found to be dose dependent, i.e., among the three doses studied of LMaq and LMAE, higher doses produced more effect as depicted in Table 1.



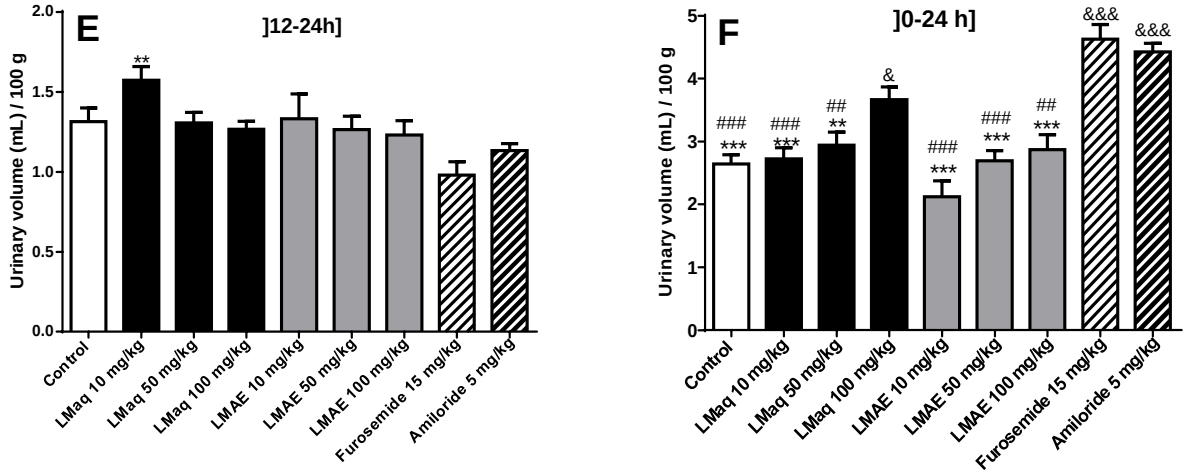


Figure 1: Evolution of urinary excretion volume as function of decoction extract (LMaq) and ethyl acetate fraction (LMAE) of the *Lannea microcarpa* trunk barks doses administration and time A,]0-2 h] ; B,]0-4 h] ; C,]0-6 h] ; D,]6-12 h] ; E,]12-24 h] ; F,]0-24 h]. Values are means ± SEM, n = 6, $^{\&\&\&}$p < 0.001 vs Control ; ***p < 0.001 vs Furosemide ; $^{\#\#\#}$ p < 0.001 vs Amiloride.

Table 1: Effects of the aqueous decoction extract and ethyl acetate fraction of *Lannea microcarpa* trunk barks on the diuretic excretion, diuretic index and diuretic activity in 24 h (n = 6).

Groups	Diuretic excretion	Diuretic index	Diuretic activity	
			With Furosemide	With Amiloride
Control	1.06	1.00	0.71	0.74
LMaq 10 mg/kg	1.09	0.91	0.64	0.67
LMaq 50 mg/kg	1.18	0.95	0.62	0.57
LMaq 100 mg/kg	1.40	1.28	0.84	0.87
LMAE 10 mg/kg	0.86	0.73	0.52	0.53
LMAE 50 mg/kg	1.14	0.97	0.69	0.72
LMAE 100 mg/kg	1.05	0.98	0.66	0.68
Furosemide 15 mg/kg	1.75	1.53	1.04	1.00
Amiloride 5 mg/kg	1.71	1.65	1.00	0.96

3.3- Effect on urinary electrolytes excretion

As shown in Figures 2A-C, furosemide, a standard drug, significantly increased urinary excretion of sodium and potassium ($p < 0.001$) during the first 6 hours of dosing. The sodium excretion was remained statistically higher until 12 hours from the start of the experiment compared to furosemide. However, beyond these periods, the excretion of Na^+ and K^+ is no longer significantly different from those excreted by the control. For the different measurements of electrolytes in urine in a 24-hour study, amiloride, a standard drug, significantly increased urinary sodium excretion and decreased potassium levels ($p < 0.001$) relative to the controls, LMaq and LMAE (Figures 2A-H). During the 6 hours of administration of LMaq 100 mg/kg, the Na^+ elimination effect of extract was comparable to furosemide and amiloride (Figure 2A). As shown in Figure 2, LMaq and LMAE excretion of

sodium and potassium were increased slightly. The effect of LMaq and LMAE was dose-dependent with a better effect for LMaq. Of the three doses studied of LMaq and LMAE, the higher dose (100 mg/kg) has produced more electrolyte removal effect.

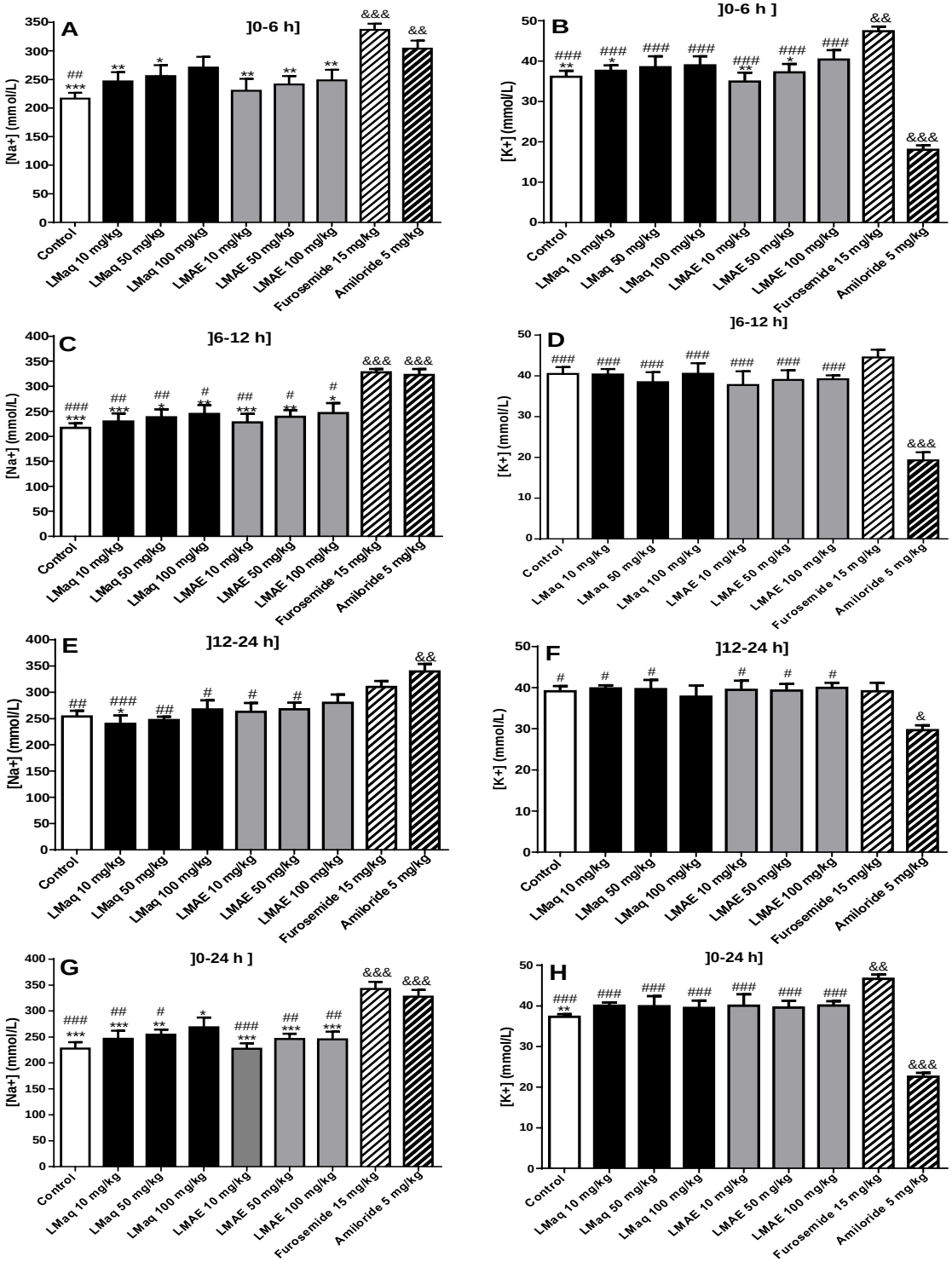


Figure 2: Evolution of urinary excretion of electrolytes (Na^+ and K^+) as function of decoction extract (LMaq) and ethyl acetate fraction (LMAE) of *Lannea microcarpa* trunk barks doses administration and time, A-B,]0-2 h] ; C-D,]6-12 h], E-F,]12-24 h] and G-H,]0-24 h]. Values are means ± SEM, n = 6, $^{\&\&\&}$p < 0.001 vs Control ; ***p < 0.001 vs Furosemide ; $^{\#\#\#}$ p < 0.001 vs Amiloride.

3.4- Effect of LMaq and LMAE and diuretics standard on saluretic index and natriuretic Activity

Table 2 shows the activity of saluretic and natriuretic after administration of extracts and reference substances. The dose of LMaq 100 mg/kg showed an increase in saluretic and natriuretic activities but not statistically significant when compared to the negative control group.

Table 2: Effects of the aqueous decoction extract and its ethyl acetate fraction of *Lannea microcarpa* trunk barks on the saluretic index and natriuretic activity.

Groups	Saluretic index		Natriuretic activity
	Na^+	K^+	Na^+/K^+
Control	1.00	1.00	6.04 ###
LMaq 10 mg/kg	1.04	1.03	6.11 ###
LMaq 50 mg/kg	1.03	1.10	5.57 ###
LMaq 100 mg/kg	1.08	1.06	6.18 ###
LMAE 10 mg/kg	1.10	1.03	6.42 ###
LMAE 50 mg/kg	0.99	1.00	6.63 ###
LMAE 100 mg/kg	0.99	1.03	5.81 ###
Furosemide 15 mg/kg	1.04	1.02	6.12 ###
Amiloride 5 mg/kg	1.12	0.56	12.08 &&&

$p < 0.001$ vs Amiloride ; &&& $p < 0.001$ vs Control ; n = 6

4- DISCUSSION

The use of herbal medicines is rise continuously as a result of increased acceptance and adherence in both developing and developed countries.[14] The soaring interest in traditional medicine is attributable to either failure of modern medicine or the research of news molecules to alleviate many chronic illnesses.[15] *Lannea microcarpa*, a plant highly appreciated for its nutritional and medicinal properties, is used for the management of hypertensive and kidney-related disorders.[8,10,16] The present study reports on the pharmacological characterization of the diuretic and natriuretic effect of *Lannea microcarpa* extracts. The diuretic activity of the decoction extract and the ethyl acetate fraction of furosemide and amiloride was compared. Both are potent diuretic used in clinical practice.[17,18]

The onset of diuretic action of both the extract of the *Lannea microcarpa* mimics the trend observed with the standard diuretic drug furosemide, which is known to have an onset of action lowly after oral administration, reaching its peak effect within 2-3 hours.[13] The aqueous crude extract of *Lannea microcarpa* trunk barks and ethyl acetate fraction exhibited

both an increase in urine volume in dose-dependent manner during the first six hours of administration with a better action in favor of LMaq. Therefore, it can be inferred that the subtle difference in the diuretic potential between the two arises the difference in phytoconstituent content. These phytoconstituents might have arisen from difference in the concentration of active principles following the fractionation of aqueous decoction by ethyl acetate, a moderately polar solvent. Interestingly, the diuretic action of these two extracts increased with the dose and even beyond 6 hours for the fraction. In addition, LMaq 100 mg/kg has an action similar to amiloride (5 mg/kg) because there is no significant difference in terms of the quantity of urine excreted at this dose up to 6 h and even in the cumulative 24 hours.

The analysis of the natriuretic index showed similar dose values between LMaq, LMAE and Furosemide. However, these values differed significantly from amiloride values. These results suggest a similarities in the mechanism of action of *Lannea microcarpa* and furosemide. Indeed, the loop diuretics such as furosemide act in the ascending branch of Henle's loop by blocking the Na^+-K^+/2Cl^- cotransporter. They induce a strong increase in diuresis, the excretion of sodium (natriuretic) and potassium (kaliuretic), chlorid (Cl^-), magnesium (Mg^{2+}) and calcium (Ca^{2+}).[19, 20] The extracts caused a significant increase in excretion of K^+ compared to amiloride (5 mg/kg), suggesting LMaq and LMAE of the *Lannea microcarpa* trunk barks do not act as the amiloride.

Interestingly, in both extracts, no change in the urine potassium was observed while furosemide caused hypokalemia. These findings might suggest a potential diuretic effect without hypokalemia for both extracts requiring further investigations. This is a common finding with the use of loop diuretics. It is suggested that the active principle (s) in both extracts may have a potassium-sparing effect. For all doses of LMaq and LMAE, natriuretic activity (Na^+/K^+) was comprised between 2.0 and 10.0 indicating a favorable natriuretic effect, and not a significant K^+-sparing effect.[21] In addition, if the diuretic index value is > 1.50, it shows a good diuretic activity, whereas the diuretic index values ranging from 1.00-1.50 and 0.72-0.99 demonstrate moderate and mild diuretic activity, respectively. Furthermore, diuretic index value < 0.72 indicates no diuretic activity.[11,12] In the present study, the diuretic index values of the maximum doses was 1.18 and 0.97 respectively for LMaq and LMAE. These results shows that LMaq and LMAE have moderate and mild

diuretic activity, respectively. Indeed, higher doses of LMaq may have important diuretic activities, especially since LMaq is practically without toxicity.[10]

5- CONCLUSION

This study provided evidence that the aqueous extract trunk barks of *Lannea microcarpa* have moderate and dose-response diuretic in experimental animal model compared to the ethyl acetate fraction. *Lannea microcarpa* extracts could be beneficial for the therapeutic management of different pathological conditions though there is a deficit in the generation of relaxing mediators. Therefore this study support previous reports confirming that, *Lannea microcarpa* extracts could be a promising alternative for the treatment of cardiovascular and renal pathologies.

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