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ANTIUROLITHIATIC POTENTIAL OF *Parkia biglobosa, Lannea humilis* STEM BARK METHANOL EXTRACT AND KO-888 TONIC

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Abstract: **Kidney stones affect several biological processes, including urine volume, pH, calcium oxalate, and urates. The aim of the study was to evaluate the antiurolithiatic potential of *Parkia biglobosa*, *Lannea humilis* stem bark and KO-888 (Jigsimur). These plants were extracted with methanol using soxhlet extraction. The phytochemical screening was carried out using standard procedures. Renal calculi were induced using ethylene glycol in 55 rats and treated with the extracts and KO-888 for 14 days. The phytochemical contents showed the presence of tannins, saponins, flavonoids, steroids, hydrogen cyanides, glycosides, alkaloids, phenols, and terpenoids with copious concentrations of glycosides, alkaloids, and terpenoids in the plant extracts. While, steroids, hydrogen cyanides, alkaloids, and terpenoids were absent in K0-888 compared to the plant extracts. The MDA level decreased significantly ($p<0.05$) at all doses in the treatment groups. The SOD activity showed a significant ($p<0.05$) increase in *Lannea humilis* and KO-888 at all doses and low dose mixture of *Lannea humilis* and *Parkia biglobosa* (50:50). The CAT activity showed a significant ($p<0.05$) increase in all treated groups. While GPx activity showed a significant ($p<0.05$) increase in *Lannea humilis* at a low dose and *Parkia biglobosa* at a high dose. These antioxidant enzyme effects were less than the standard. The blood urea nitrogen showed a significant ($p<0.05$) reduction in all treatment groups and the creatinine level showed a significant ($p<0.05$) decrease in *Lannea humilis* and KO-888 at all doses, similar to the standard. Hence, they could be used for the protective management of kidney diseases.**

Keywords: **antioxidant, blood urea nitrogen, creatinine, kidney stone, phytochemicals, traditional medicine.**

1. INTRODUCTION

Kidney stone formation is one of man's oldest and widest-spread diseases [1]. Urinary stones are polycrystalline aggregates composed of various organic components and crystalline matrices with lipids being an important component in the stone matrix where it is only 2–3% of the dry weight from urolith [2]. The formation of calcified renal stone is a physiochemical event leading to crystal nucleation, aggregation and its growth assisted by many biological processes including urine volume, pH, increased calcium oxalate or sodium oxalate, and urates [3].

Numerous therapeutic and prophylactic approaches exist to combat the occurrence of urolithiasis but due to the complexity of its pathogenesis, the success rate of its therapy remains low [4]. Most of the remedies used in traditional medicine systems are taken from plants with positive effects on patients, especially some composite plants and herbal drugs such as *Herniaria hirsuta*, *Bergenia ligulata*, *Piper nigrum*, *Dolichos biflorus* and *Plantago major* where responses showed that their extracts reduce the crystal size [2]. The mechanisms used to achieve this include maintaining crystalloid-colloid balance by decreasing the excretion of urinary calcium, oxalate, uric acid, phosphorus and protein in urolithiasis, improving the renal

function by increasing the excretion of urea and creatinine, antioxidant activity reducing renal tubular epithelial cell injury when exposed to oxalate and/or CaOx, so that the loss of membrane integrity subsequently facilitates the retention of calcium oxalate crystals and growth of stones in renal tubules, and diuretic and angiotensin-converting enzyme (ACE) inhibition activity [5].

Parkia biglobosa, commonly known as "African locust beans", which belongs to the family of Mimosaceace, is one of such medicinal plant which is widely distributed in Guinea and Sudan Savannah and its phytochemical evaluation has shown that it contains alkaloids, cardiac glycosides, high amino acids and proteins [6]. *Lannea humilis* is a deciduous shrub with medicinal properties including; Antihelmintic, Antibacterial, Antidirrheal, Anti-inflammatory, Anti-bacterial, and Antioxidants [7]. These plants *P. biglobosa* and *L. humilis* are used traditionally in north central Nigeria for the treatment of ulcers, microbial infection and fever. KO-888 is an approved herbal drug in Nigeria by the National Agency for Food and Drug Administration and Control (NAFDAC) with registration number: A7-2720L. It is brewed from the sap of the Aloe Ferox plant from South Africa and has been harvested by the indigenous people for the 'bitter sap' that exudes from the cut end of the leaf, which is boiled in cauldrons and solidifies on cooling to form 'aloe bitters' [8].

It is on the basis of the medicinal properties of *P. biglobosa* and *L. humilis* that this research was designed to evaluate their antiurolithiatic properties.

2. MATERIALS AND METHODS

2.1 Preparation of plant extract

The stem bark of *P. biglobosa* and *L. humilis* was cut into small pieces, dried under laboratory conditions, and pulverized to a coarse powder. 500g each of the *P. biglobosa* and *L. humilis* powders were successively extracted with methanol using soxhlet extraction method. The extracts were concentrated using a rotary evaporator under reduced pressure. The methanol extracts obtained were used for the preliminary phytochemical screening and pharmacological studies. The extracts were administered to the animals by dissolving them in distilled water, after calculating dosages using the Ratio and Proportion Method [9].

2.2 Qualitative phytochemical analysis

The extracts of *P. biglobosa* and *L. humilis* were subjected to preliminary qualitative phytochemical analysis using standard procedures. Tannins, flavonoids, phenols, saponins, hydrogen cyanides, and glycosides were determined using the method of Trease and Evans, [10], while, alkaloids, terpenoids, and steroids were determined using the method of Sofowora, [11].

2.3 Quantitative phytochemical analysis

The quantitative phytochemical analysis was determined using the Folin Denis colorimetric method of Nwaokonkwo, [12] for tannins. Flavonoids, alkaloids, saponnins, hydrogen cyanide, phenols, terpenoids, steroids and glycosides were determined according to the method of El-Olemy *et al.*, [13].

2.4 Determination Free Radical Scavenging

Activity The free radical scavenging activity of the methanol extracts was measured using 1,1- diphenyl-2-picryl-hydrazyl (DPPH) assay according to the method of Jain *et al*., [14]. A solution of 0.2 mM DPPH in methanol was prepared. 1.0 mL of this solution was mixed with 3 mL of extract in methanol containing 0.001-0.2 mg/mL of the extract. The mixture was vortexed thoroughly and left in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm. Ascorbic acid and Vitamin E were used as the reference standards.

Experimental Animals

Fifty-five adult Albino rats of both sexes weighing 150-200 g were assigned randomly for the study. The rats were housed in cages of 5 rats each and allowed acclimatization to laboratory status for two weeks before the experiment commenced. Animals were maintained at room temperature and with a 12h light/12h dark cycle and allowed *ad libitum* access to water.

Induction of Nephrolithiasis/Urolithiasis

The rats were grouped into eleven (11) groups of 5 each. Ethylene glycol (0.75%) was dissolved in drinking water and administered to group II- XI for the induction of renal calculi for 14 days. Group III received 10 ml/kg distilled water as a

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standard antiurolithiatic drug. Group IV-IX received (extracts) of KO-888, *P. biglobosa* and *L. humilis* at high and low doses (each) and X –XI received *P. biglobosa* and *L. humilis* 50/50 at high and low-dose (each) from day 15th to 28th.

Collection of Blood and Tissue Homogenates

After the experimental period, the rats were sacrificed, and blood was collected in a plain sample bottle and centrifuge for kidney function tests. The kidneys were harvested, homogenized and centrifuged for antioxidant assay.

2.5 Determination of MDA and Antioxidant

Superoxide dismutase (SOD) was determined using Cayman's Superoxide Dismutase Assay Kit (No: 706002). Catalase (CAT) activity was determined using the Cayman Catalase Assay Kit (No: 707002). Glutathione Peroxidase (GPx) was determined using the Cayman Glutathione Peroxidase Assay Kit (No: 703102). Reduce glutathione (GSH) was determined using the Cayman Glutathione Assay Kit (703002). Lipid Peroxidation was determined using Cayman TBARS Assay Kit (No:100090550).

2.6 Determination of Kidney Function Markers

Urea concentration was determined using the method of Bartels and Bohmer [15] as described in Randox Kit. Urea in serum is hydrolyzed to ammonia in the presence of urease. The ammonia is then measured spectrophotometrically.

The serum creatinine was determined using the method of Bartels and Bohmer [15] as outlined in the Randox kit.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Qualitative Phytochemical screening of *Parkia biglobosa, Lanea humulis* and KO-888

The results of the qualitative phytochemical analysis are shown in Table 1. The table showed the presence of tannins, saponins, flavonoids, steroids, hydrogen cyanides, glycosides, alkaloids, phenols, and terpenoids in *P. biglobosa* extract and *L. humilis*, while KO-888 showed the presence of tannins, saponins, flavonoids, alkaloids, and phenols.

Table 1: Qualitative Phytochemical screening of *Parkia biglobosa, Lanea humulis* and KO-888

Phytochemicals	*P. biglobosa*	*L. humilis*	KO.888
Tannin	++	++	++
Saponin	+	+	+
Flavonoid	++	++	++
Steroid	+	+	-
HCN	+	+	-
Glycoside	+++	+++	-
Alkaloid	+++	+++	+
Phenol	++	++	+
Terpenoid	+++	+++	-

+ = present, - = absent

3.1.2 Quantitative Phytochemical screening of *Parkia biglobosa, Lanea humulis* and KO-888

Figure 1 showed a significant (p <0.001) copious amount of glycosides, alkaloids, and terpenoids in *P. bioglobosa* and *L. humilis.* Tannins, flavonoids, and phenols were observed in significant (p<0.01) amounts in *P. biglobosa*, *L. humilis,* while others were in low amounts. KO-888 showed a significant (p <0 .05) amount of flavonoids (p<0.01), tannins, and phenol (p<0.05) in a moderate amount.

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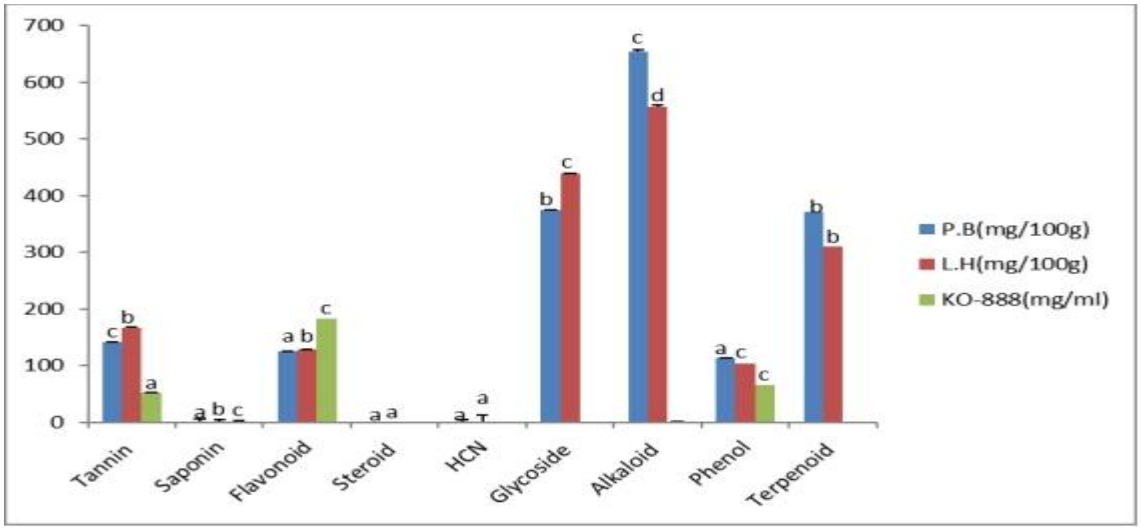

Figure 1: Quantitative Phytochemical screening of *Parkia biglobosa, Lanea humulis* and KO-888

Results are presented in Mean ± SD, (N = 3), mean values with different letters as superscripts down the groups are considered significant at b = p <0.05, c = p<0.01, d = p<0.001

3.1.3 Free Radical Scavenging Activity of *P. biglobosa, L. humilis* and KO-888

The result of the radical scavenging assay with (DPPH) is presented in Figure 2. The percentage (%) inhibition of the methanol extracts of *P. biglobosa* and *L. humilis* was 97.23 to 85.98 % at 25 to 800 µg/ml. KO-888 showed 63.49 to 92.05 % at 25 to 800 µg/ml. While, the standard (vitamin C) showed 100 to 98.67 at 25 to 800 µg/ml, respectively.

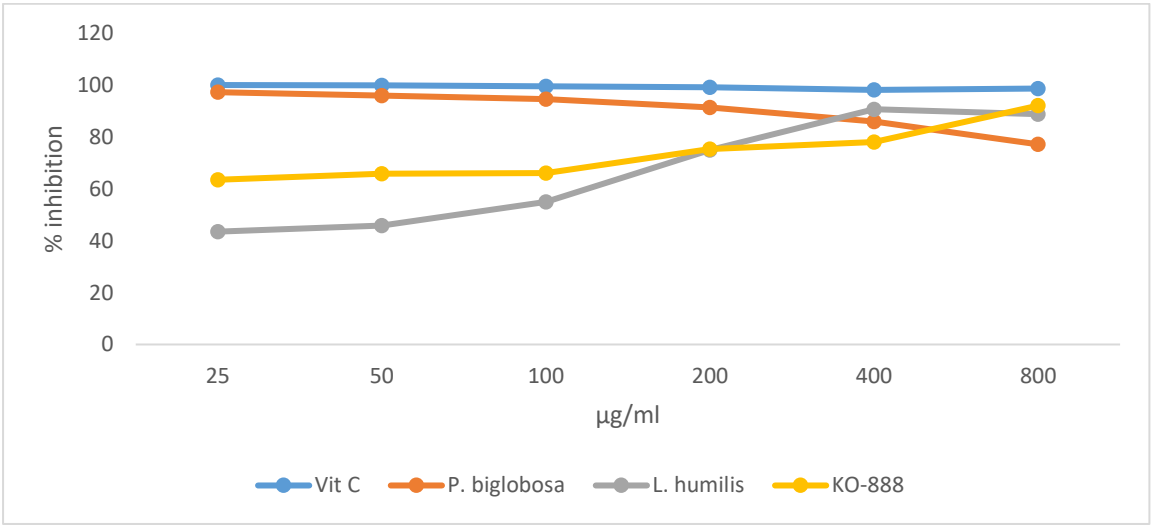

Figure 2: Free Radical Scavenging Activity of *P. biglobosa, L. humilis* and KO-888

Results are presented in percentage Mean ± SD, (N = 3)

3.1.4 Antioxidant Activity of *P. biglobosa, L. humilis* and KO-888

The result of the MDA levels in Figure 3 showed that there was a significant (p<0.05) increase in the MDA level in the negative control group compared to the normal control group. However, SOD and GPx showed a significant (p<0.01) decrease, while, CAT and GSH showed significant (p<0.001 and p<0.05) decrease in negative control compared to the normal control group. Treatment with the extracts, KO-888 and the standard drug showed a significant (p<0.05) MDA decrease in *L. humilis*, *P. biglobosa*, KO-888 and the standard drug groups. There was a significant (p<0.05) SOD increase in *L. humilis* and KO-888 at all doses, and low dose *L. humilis/P. biglobosa* (50:50) groups compared to negative control. CAT activity showed a significant (p<0.05) increase in *L. humili*, *P. biglobosa* and KO-888 groups at all doses compared

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to control. GPx activity showed a significant ($p<0.05$) increase in *L. humilis* low dose and *P. biglobosa* high dose. The standard drug group showed similar MDA and antioxidant activity similar to the normal control.

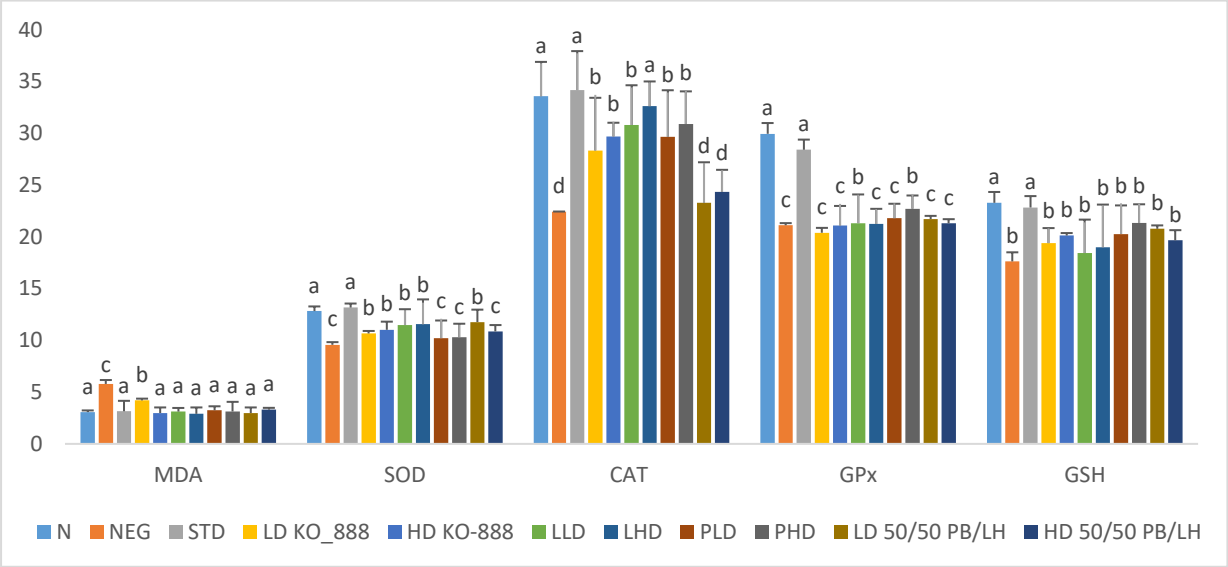

Figure 3: Antioxidant Activity of *P. biglobosa, L. humilis* and KO-888

Results expressed in mean ± SD (n = 5). Letters assigned as superscripts different from 'a' showed significant differences. b = ($p<0.05$), c = ($p<0.01$) and d = ($p<0.001$). N= normal control, Neg= negative control, STD= standard, PB= *P. biglobosa*, LH= *L. humilis*, LD= low dose, HD= high dose.

3.1.5 Effect of *P. biglobosa, L. humilis* and KO-888 on Kidney Function

The result of the blood urea nitrogen (BUN) levels in Figure 3 was significantly ($p<0.05$) increased in the negative control group compared to the normal control group. Treatment with the *P. biglobosa*, *L. humilis* extracts, KO-888 and standard drug showed a significant ($p<0.05$) decrease in *L. humilis and P. biglobosa* treated groups at all doses. KO-888 showed a significant ($p<0.05$) decrease in (BUN) at a high dose.

The result of the creatinine levels in Figure 4 showed a significant ($p<0.05$) increase in the creatinine level in the negative control group compared to the normal control group. Treatment with the extracts, KO-888 and the standard drug showed a significant ($p<0.05$) decrease in creatinine level in *L. humilis*, KO-888 and standard drug at all doses.

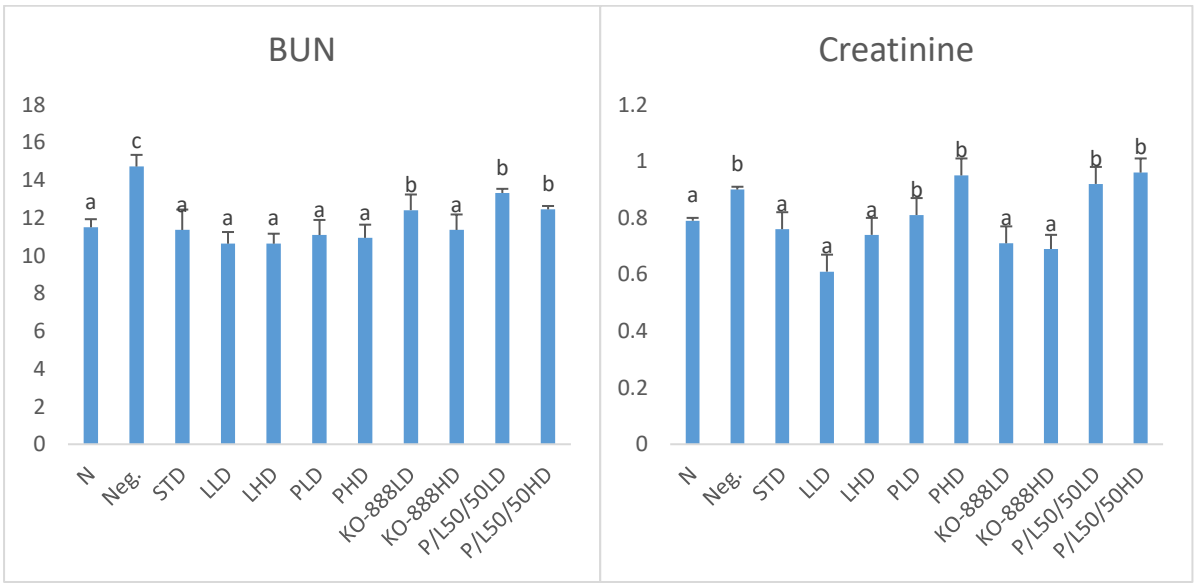

Figure 4: Effect of *P. biglobosa, L. humilis* and KO-888 on Kidney Function

Results expressed in mean ± SD (n = 5). Letters assigned as superscript different from 'a' showed significant different. b = (p <0.05), c = (p <0.01) and d = (p <0.001). N= normal control, Neg= negative control, STD= standard, PB= *P. biglobosa*, LH= *L. humilis*, LD= low dose, HD= high dose.

3.2 Discussion

This study was designed to evaluate the antiurolithiatic activity of *Parkia biglobosa, Lannea humilis* plants extracts and KO-888 to ascertain scientific rationales behind their ethnomedicinal uses therapeutically. Medicinal plants have the ability to synthesize almost an unlimited number of chemical substances and in many cases, these chemicals serve in plant defense mechanisms against microorganisms, insects, and herbivores [2].

The results of the phytochemical screening showed the presence of tannins, saponins, flavonoids, steroids, hydrogen cyanide, glycosides, alkaloids, phenols, and terpenoids with copious concentrations of glycosides, alkaloids, and terpenoids in *P. biglobosa* and *L. humilis*. While, steroids, hydrogen cyanides, alkaloids, and terpenoids were absent in K0-888. Flavonoids, phenolic compounds, saponins and tannins have been reported to inhibit urinary stone formation *via* inhibition of calcium oxalate crystallization in urine as well as crystal deposition, disaggregating the suspension of mucoproteins, which are the promoters of crystallization [3].

Production of various crystallization modulators which are involved in the inhibition of crystal nucleation, growth and aggregation is also regulated by the reactive oxygen species (ROS) which also regulate osteogenesis in both the renal tubular epithelial and vascular endothelial cells [16]. The *P. biglobosa* extract exhibited substantial dose-dependent decreased antioxidant activity against DPPH (97.23 %) at 25 µg/ml. *L. humilis* and KO-888 possessed a dose-dependent increased antioxidant activity (88.75 and 92.05 %) at 800 µg/ml comparable to vitamin C (100 %). This effect may be linked to the presence of phenolic, flavonoid, alkaloid, and terpenoid compounds in the extract since they can readily donate a hydrogen atom to the radical to neutralize it [17].

The increase in MDA level and decreased SOD, CAT, GPx and GSH after administration of ethylene glycol is an indication that the exposure of renal epithelial may have caused the production of reactive oxygen species (ROS) by the renal epithelial cells and therefore responsible for the renal injury [16]. However, the significant ($p<0.05$) decrease in MDA levels after treatment with the extracts and KO-888 compared to the untreated group may have reversed the adverse situation by inhibiting lipid peroxidation in the treated animals implying that it may possess antioxidant capacity [18]. The significant ($p<0.05$) increase in SOD activity in *P. biglobosa*, *L. humilis* (low and high doses), low dose KO-888 and 50:50 low dose *P. biglobosa/L. humilis*, CAT activity in low and high doses of *P. biglobosa*, *L. humilis* doses) and KO-888, GPx activity in low-dose *L. humilis* and high-dose *P. biglobosa* extracts treated groups compared to the untreated group supported the antioxidant effect of the extracts and KO-888 tonic which may have seen mediated by its antioxidant properties [19]. The results of the present study showed higher levels in blood urea nitrogen and creatinine levels in the untreated group indicating that the ethylene glycol stimulated possible protein metabolism leading to high release of the nitrogenous waste products which was released into the bloodstream and transported to the kidneys for excretion. The elevation in creatinine levels may overwhelm the kidney leading to kidney damage [18]. The decrease in blood urea nitrogen (BUN) and creatinine level after treatment with *P. biglobosa*, *L. humilis* (low and high doses) and KO-888 low dose is evidence of the nephroprotective effect of the extracts, which was similar to the standard drug used. However, the significant ($p<0.05$) increase in BUN and creatinine is a sign of renal damage which was shown by low creatinine clearance and an increase in BUN [20].

The anti-urolithiasis properties of the plant extracts and KO-888 may be attributed to the inhibition of calcium oxalate monohydrate (COM) aggregation, which could be due to the copious amount of flavonoids, phenolic compounds, saponins and tannins present in the extracts, confirming the earlier report of Aryal *et al*., [3].

4. CONCLUSION

Findings from this study showed the presence of several phytochemicals in *Parkia biglobosa* and *Lannea humilis* extracts at levels higher than KO-888 tonic. All the extracts and KO-888 exhibited significant antioxidant activity at low doses. However, *L. humilis* and KO-888 possessed a better anti-urolithialic effect at all doses by marked reduction of blood urea nitrogen and creatinine levels. These effects may be due to the presence of the phytoconstituents in *P. biglobosa, L. humilis*, and KO-888. Hence, they could be used for the protective management of kidney diseases. Further studies will be required for investigations of the fractions of these plants in order to isolate compounds for prophylaxis and therapeutic use.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All experimental procedures were made according to Standard Operating Procedure for Nasarawa State University Keffi Animal Care and Use Research Ethics Committee (NSUK-ACUREC).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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