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Research article

Antioxidant Potentials of *Mitragyna Inermis* Stem-Bark Methanol Extract in Sodium Oxalate-Induced Urolithiasis in Rats

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Abstract: The development of urolithiasis has been closely linked to oxidative stress and the resulting cellular damage within the urinary system. Several medicinal plants exhibit strong antioxidant activities due to their high phenolic content. These properties may help reduce oxidative stress associated urolithiasis. This research aimed to assess antioxidant potentials of *Mitragyna inermis* stem-bark methanol extract in sodium oxalate induced urolithiasis in rats. In vitro assay was conducted using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging and ferric reducing power procedures. Sodium oxalate 70mg/kg, i.p was used to induced oxidative stress in in vivo studies and six groups, each consisting of six animals (n = 6): Group I served as vehicle control, received normal saline (5ml/kg p.o.), Group II served as lithiatic control, received sodium oxalate 70mg/kg, i.p., Group III served as positive control, received standard drug Cystone (500mg/kg, p.o.) + Sodium oxalate 70mg/kg, i.p., and Groups IV, V and VI received Sodium oxalate 70 mg/kg, i.p.,+ extract 50, 100 and 200mg/kg, p.o. respectively. The extract demonstrated dose-dependent antioxidant activities, though Vitamin C consistently showed higher activities, with significant ($p<0.05$) differences observed only in DPPH scavenging at 500 µg/ml. In vivo, the extract and standard drug (cystone) improved superoxide dismutase levels in treated rats, with some groups reaching values comparable ($p>0.05$) to normal controls. Although treatments significantly ($p<0.05$) reduced elevated serum levels of catalase, glutathione, malondialdehyde, and vitamins A, C, and E, none fully restored normal levels. These findings suggest that *M. inermis* stem-bark has notable antioxidant potential, which may be beneficial in managing oxidative stress associated with urolithiasis.

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Introduction

Urinary stones are polycrystalline aggregates composed of various organic components and crystalline matrices [1]. The stone matrix, which makes up only 2–3% of the stone's dry weight, includes lipids, proteins (64%), nonamino sugars (9.6%), hexosamine as glucosamine (5%), water-binding elements (10%), and ash [2]. The increasing prevalence and pain of stone formation are strongly linked to lifestyle and nutritional factors. Stone formation, or lithiasis, is classified as nephrolithiasis when occurring in the kidney, and urolithiasis when present in the bladder, ureter, or any urinary tract region outside the kidney [3].

Antioxidants are molecules that protect the body by neutralizing various reactive oxygen species (ROS) and other free radicals, thereby preventing cellular and molecular damage [4-6]. The body's endogenous antioxidant defenses, which include enzymes and small molecules, often require support from dietary and external sources, especially under conditions of elevated oxidative stress [7]. Among these, medicinal

plants have emerged as significant sources of natural antioxidants, owing to their rich content of compounds such as phenolics, flavonoids, and vitamins [4, 8]. The increasing interest in plant-based antioxidants is linked to their potential for reducing the risk of chronic diseases and mitigating the adverse effects of oxidative stress. The significance of medicinal plants as antioxidants is further emphasized by their accessibility, safety, and multipurpose therapeutic effects, making them valuable alternatives or complements to synthetic antioxidants [9].

Mitragyna inermis, commonly known as Giyya in Hausa land, is a multi-stemmed deciduous shrub with a wide range of documented medicinal uses. Ethnobotanical research shows that preparations from the bark, leaves, and roots are traditionally used to treat conditions such as fever, malaria, gastrointestinal disorders, skin infections, pain, and inflammation [10]. Phytochemical investigations confirm the presence of alkaloids, flavonoids, steroids, tannins, and saponins in the stem bark, which contribute to the plant's antioxidant and antimicrobial activities [11, 12].

Materials and Methods

Experimental animals

The albino rats used in this study were bought from Animal House, Usman Danfodiyo University, Sokoto, in February 2024. They were brought to the Animal House, Faculty of Life Science, Abdullahi Fodio University of Science and Technology, Aliero, in well-ventilated cages. Before the trial started, the rats were kept in a clean cage and given fourteen days to acclimatize. The rats were fed a typical rat diet and given unlimited access to water.

Collection, preparation and extraction of plant material

The plant extracts were prepared according to a modified method of Dupont *et al*. (2006) [13]. The collected plant parts were washed with clean water and air-dried in the shade and pulverized using a pestle and mortar. One hundred grams (100g) each of the powdered plant parts was measured and soaked in 100 mL of 99% methanol. The mixture was then kept at room temperature for 24 h and filtered twice; initially with a muslin cloth and later with Whatman filter paper No. 1. The filtrate was evaporated to dryness at 45^{o}C using a rotary evaporator.

In vitro antioxidant assay

Determination of antioxidant potential by ferric reducing antioxidant power assay (FRAP)

The potassium ferricyanide- ferric chloride assay was used to determine the ferric reducing power of the extract [14]. Approximately 1ml of extract and standard drug. Vitamin C at different concentrations (1, 2, and 3mg/ml) was introduced into 2.5mL of potassium ferricyanide in individual test tubes, followed by incubation at 50^{o}C for 20 minutes. Subsequently, 2.5mL of trichloroacetic acid was incorporated into the mixture, which was then centrifuged at 650 × g for 10 minutes. From the resulting 2.5mL of supernatant, an equal volume (0.5ml) of distilled water and ferric chloride solution was added. The absorbance was recorded at a wavelength of 700nm using a spectrophotometer, and the reducing capacity was determined as outlined.

Reducing power = $\frac{AM}{AC} \times 100$

AM = Absorbance of reaction mixture,

AC =Absorbance of control mixture (distilled water).

Determination of antioxidant potentials by DPPH assay

The methodology from a previous study [14] was used to assess the DPPH scavenging effect of the plant extract. Extract concentrations and ascorbic acid (1000, 2000, and 3000µg/ml) were prepared from the concentration of the pooled fraction and Vitamin C, respectively, in 20mL of methanol. Subsequently, 0.004% DPPH solution (2mL) in methanol was combined with 1mL of the various concentrations of the pooled chromatographic fraction and the standard (ascorbic acid) and further incubated at 25^{o}C for 30 minutes, after which the absorbance of the tube was measured against a blank at 517nm using a spectrophotometer. The percentage of antioxidant activity was determined using the formula provided below:

% Inhibition=$\frac{Acontrol-Asample}{Acontrol} \times 100$

Determination of hydrogen peroxide radical scavenging

The ability of plant extracts to scavenge hydrogen peroxide is determined according to the method described in a previous study [14]. A 20Mm solution of hydrogen peroxide was prepared in buffer saline (P_H 7.4). 1ml of various concentrations of plant extract and standard ascorbic acid solution (100, 200, 400, 800µg/ml in methanol) was added to 1ml of hydrogen

peroxide solution. For making a concentration of 100µg/ml, 10ml of this stock is added to 90ml of water. Absorbance of hydrogen peroxide at 230nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. A 20 mM solution of H_2O_2 in phosphate buffer saline (P_H 7.4). From this solution, we will take 2ml solution of H_2O_2 $_{solution}$ and 1ml of extract solution. Whereas the standard stock is prepared by adding 10mg ascorbic acid (0.01) in 100ml. From various dilutions, we take 1ml solution with 1ml solution of H_2O_2.

The percentage inhibition was calculated using the following formula:

$$\text{Percentage Inhibition} = \frac{A0 - AT}{A0} \times 100$$

Induction of urolithiasis

The sodium oxalate-induced urolithiasis model was used to evaluate antiurolithiatic effect in rats. The Wistar albino rats were divided into six groups, each consisting of six animals (n = 6): Group I served as Vehicle Control, received normal saline (5ml/kg p.o.), Group II served as lithiatic control, received Sodium oxalate 70mg/kg, i.p., Group III served as positive control, received standard drug Cystone (500 mg/kg, p.o.) + Sodium oxalate 70 mg/kg, i.p., and Groups IV, V and VI received Sodium oxalate 70 mg/kg, i.p.,+ extract 50, 100 and 200mg/kg, p.o. respectively. The above treatment was repeated up to 14 days [15].

Experimental design for In vivo antioxidant assay

Twenty-eight albino rats were randomly assigned into seven groups, with each group containing four rats. The venom administration was intraperitoneal (i.p.), and oral extract treatment is outlined below.

Table 1. Grouping criteria.

Groups	Treatments
Group 1	Received oral administration of distilled water and served as normal control
Group 2	lithiatic control, received Sodium oxalate 70 mg/kg, i.p
Group 3	Received standard drug Cystone (500 mg/kg, p.o.) + Sodium oxalate 70 mg/kg, i.p
Group 4	Received Sodium oxalate 70 mg/kg, i.p.,+ extract 50mg/kg, p.o.
Group 5	Received Sodium oxalate 70 mg/kg, i.p.,+ extract 100mg/kg, p.o.
Group 6	Received Sodium oxalate 70 mg/kg, i.p.,+ extract 200 mg/kg, p.o.

The same volume of preparations was administered to all the groups. Twenty-four hours after the last treatment, the animals were sacrificed, and blood samples were collected for biochemical analysis.

In vivo antioxidant assay determination of lipid peroxidation

Lipid peroxidation (Malondialdehyde level) was assessed spectrophotometrically, following the method from a previous study [16]. The assay for SOD and catalase activity was performed as described [17]. GSH was quantified based on the procedures of [18].

Data analysis

The data generated from the study are presented as mean ± SEM and subjected to one-way analysis of variance (ANOVA), and statistical differences between the means were separated using New Duncan's Multiple Range Test at $p<0.05$ with the aid of a statistical package (IBM SPSS Statistics 20).

Results

DPPH scavenging effect of M. inermis stem-bark methanol extract

The DPPH scavenging effect of *M. inermis* stem-bark methanol extracts is presented in **Table 2**. The results revealed a dose-dependent scavenging activity in both extracts and Vitamin C extracts. However, Vitamin C exhibited higher DPPH scavenging activities at all concentrations (250, 500, 750, and 1000µg/ml) compared to the extracts. However, the differences were only significant ($p<0.05$) at the 500µg/ml concentration.

Table 2. DPPH Scavenging Effect of *M. inermis* Stem-bark Methanol Extract.

Concentrations (µg/ml)	% Inhibition		
	Extract	Vitamin C	p value
250	18.00±1.00	36.67±1.15	0.609
500	26.67±4.62	40.00±0.00	0.016*
750	38.67±0.58	56.33±0.58	1.00
1000	49.33±0.58	62.33±0.57	1.00

Values are presented as mean ± SEM. Values in asterisks are significantly different, analyse using a t-test with the aid of SPSS version 20.0.

Ferric reducing antioxidant effect of M. inermis stem-bark methanol extract

The ferric reducing antioxidant effect of *M. inermis* stem-bark methanol extracts is presented in **Table 3**. Dose-dependent ferric reducing antioxidant potentials were observed in both extracts and Vitamin C. However, Vitamin C exhibited a non-significant ($p>0.05$)

higher ferric reducing effect at all concentrations (250, 500, 750, and 1000µg/ml) compared to the extract

Table 3. Ferric Reducing Antioxidant Effect of *M. inermis* Stem-bark Methanol Extract.

Concentrations (µg/ml)	% Inhibition		
	Extract	Vitamin C	p value
250	0.67±0.06	5.00±2.00	0.259
500	12.67±0.58	16.67±1.53	0.154
750	26.00±1.00	32.33±0.58	0.561
1000	33.00±1.00	45.00±2.65	0.116

Values are presented as mean ± SEM. Values in asterisks are significantly different, analysed using a t-test with the aid of SPSS version 20.0.

Hydrogen peroxide scavenging effect of M. inermis stem-bark methanol extract

The hydrogen peroxide scavenging effect of *M. inermis* stem-bark methanol extracts is presented in **Table 4**. The results revealed a dose-dependent scavenging activity in both extracts and Vitamin C extracts. However, Vitamin C exhibited a non-significant (p>0.05) higher hydrogen peroxide scavenging activity at all concentrations (250, 500, 750, and 1000µg/ml) compared to the extract."

Table 4. Hydrogen peroxide Scavenging Effect of *M. inermis* Stem-bark Methanol Extract.

Concentrations (µg/ml)	% Inhibition		
	Extract	Vitamin C	p value
250	2.33±0.58	6.33±2.08	0.089
500	45.67±1.53	53.00±1.00	0.442
750	55.00±1.00	62.00±1.00	1.00
1000	66.67±0.58	70.33±1.53	0.184

Values are presented as mean ± SEM. Values in asterisks are significantly different, analysed using a t-test with the aid of SPSS version 20.0.

Table 5. Antioxidant Effect of *M. inermis* Stem-bark Methanol Extract on Sodium Oxalate-Induced Urolithiasis in Albino Rats.

Dose Administered mg/kg	SOD (unit/ml of enzyme)	CAT (unit/ml of enzyme)	GSH (mg/dL)	MDA (nmole/mL)	Vitamin A (mg/dl)	Vitamin C (mg/dl)	Vitamin E (mg/dl)
Normal control	1.46±0.08d	0.13±0.02a	213.04±1.09a	33.76±2.25a	25.09±0.41b	21.26±0.29b	175.80±1.61b
Induced control	0.41±0.07a	0.25±0.03c	339.94±0.27f	172.22±2.59f	38.88±0.23c	46.69±031f	268.54±2.42f
Standard drug control (100mg/kg)	0.94±0.11b	0.14±0.01ab	280.70±0.55d	81.62±1.96c	26.20±0.30b	36.36±0.22e	181.45±1.62c
M. inermis Extract (100mg/kg)	1.18±0.13c	0.16±0.01b	279.43±0.42b	106.19±6.33e	23.90±0.45b	16.31±0.29a	166.39±1.23a
M. inermis Extract (200mg/kg)	1.29±0.18cd	0.15±0.01ab	313.76±0.42e	97.86±1.61d	27.21±5.62bb	34.92±0.25d	186.82±1.23d
M. inermis Extract (400mg/kg)	1.47±0.17d	0.13±0.00ab	276.90±0.55b	75.42±0.98b	16.80±0.34a	29.85±0.64c	214.78±2.46e

Values are presented as mean ± SEM (n = 4). Values having the same superscript are not significantly different at (p>0.05) analyzed using One-Way ANOVA, followed by Duncan multiple comparison test with SPSS version 20.0. SOD= superoxide dismutase, CAT= catalase, GSH= glutathione, and MDA= malondialdehyde.

In vitro antioxidant effect of M. inermis stem-bark methanol extract on sodium oxalate-induced urolithiasis in albino rats

The antioxidant effect of *M. inermis* stem-bark methanol extract on kidney function parameters of sodium oxalate-induced urolithiasis albino rats is presented in **Table 5**. The result revealed significant (p<0.05) decreases in SOD in the induced control compared to the normal control. Treatment with extract and standard drug (Cystone) was able to increase SOD concentration, with only groups treated with extract 200 and 400 comparable (p>0.05) with the normal control. A significant increase in CAT, GSH, MDA, vitamins A, C and E was observed in induced control compared to normal control. Although standard drug and extract treatment significantly reduced the serum concentrations of these parameters compared to induced control, none of the treatments was able to reverse the alteration close to normal control.

Discussion

Oxidative stress plays a pivotal role in the initiation and progression of urolithiasis by promoting renal tubular epithelial injury, crystal nucleation, aggregation, and retention within the urinary tract [19]. ROS generated

during hyperoxaluria can overwhelm endogenous antioxidant defenses, leading to lipid peroxidation, cellular dysfunction, and inflammation [7]. Therefore, agents with potent antioxidant properties may offer therapeutic benefits by attenuating oxidative damage and preserving renal function. The present study demonstrated that the methanol stem-bark extract of *M. inermis* possesses appreciable antioxidant activity both in vitro and in vivo, supporting its potential role in mitigating oxidative stress associated with sodium oxalate-induced urolithiasis.

Antioxidant assays such as DPPH, FRAP, and hydrogen peroxide scavenging are widely used to assess the free-radical scavenging abilities of plant extracts. These methods measure a plant's capacity to neutralize reactive oxygen species and reduce oxidative stress in biological systems. By mitigating oxidative damage in renal tissues, these antioxidant activities help prevent the formation and aggregation of kidney stones, thereby contributing to the anti-urolithiatic potential of medicinal plants [20, 21]. The dose-dependent DPPH, FRAP, and hydrogen peroxide scavenging effect observed in *M. inermis* stem-bark methanol extract in the present study is directly attributed to the potent antioxidant properties of this plant.

In the present study, an elevated level of MDA, GSH and CAT, and reduced SOD activity were observed following kidney stone induction; however, promising normalization potentials were observed following *M. inermis* stem-bark methanol extract post-treatment. According to [20, 21], plant bioactive compounds can increase the levels of catalase, SOD, and GSH, thereby enhancing the kidney's natural defense mechanisms. At the same time, these antioxidants reduce MDA levels, indicating decreased lipid peroxidation and cellular injury. Through these actions, antioxidant-rich plant extracts help protect renal tissues, promote healing, and restore biochemical balance during urolithiasis.

The sodium oxalate-induced urolithiasis model successfully produced oxidative stress, as evidenced by alterations in both enzymatic and non-enzymatic oxidative stress biomarkers. Administration of *M. inermis* extract significantly improved SOD activity, indicating enhancement of endogenous antioxidant defense mechanisms. SOD constitutes the first line of antioxidant defense by catalyzing the conversion of superoxide radicals into hydrogen peroxide, thereby limiting oxidative injury [12]. Restoration of SOD activity toward normal values suggests that the extract may protect renal tissues against oxalate-induced oxidative damage.

Treatment with the extract also significantly reduced the elevated levels of catalase, glutathione, MDA, and antioxidant vitamins compared with the untreated lithiatic group. Elevated MDA is a widely recognized indicator of lipid peroxidation and membrane damage [16]; therefore, its reduction indicates attenuation of oxidative injury. Although the antioxidant parameters did not completely return to the levels observed in normal control animals, the significant improvement relative to untreated animals indicates partial restoration of the oxidant-antioxidant balance. Similar findings have been reported for several medicinal plants rich in polyphenolic compounds, where antioxidant activity contributes to protection against experimentally induced nephrolithiasis [9, 10].

The protective effects observed in this study were generally comparable to those produced by Cystone, a well-established polyherbal antiurolithiatic formulation [20]. This finding suggests that *M. inermis* may possess bioactive constituents capable of modulating oxidative pathways involved in stone formation. Nevertheless, the inability of the extract to completely normalize all oxidative stress biomarkers indicates that oxidative injury may persist despite treatment or that higher doses, prolonged treatment duration, or combination therapy may be required to achieve complete biochemical recovery.

The observed antioxidant activity likely represents one of several mechanisms through which *M. inermis* may exert protective effects in urolithiasis. Besides antioxidant properties, medicinal plants with high phenolic content often exhibit anti-inflammatory, membrane-stabilizing, and nephroprotective activities that collectively reduce crystal-induced renal injury. However, the present study focused primarily on antioxidant parameters; therefore, additional investigations are warranted to elucidate the precise molecular pathways involved, including modulation of inflammatory mediators, apoptotic signaling, and crystal adhesion molecules such as osteopontin and kidney injury molecule-1.

Study limitations: Several limitations should be acknowledged. The study did not quantify the total phenolic or flavonoid contents of the extract, nor were individual bioactive constituents characterized. Furthermore, molecular analyses of antioxidant gene expression and histopathological correlation were not performed, limiting mechanistic interpretation. Future studies should include phytochemical profiling, isolation of active compounds, histopathological evaluation of renal tissues, and assessment of

oxidative stress-related signaling pathways to further validate the therapeutic potential of *M. inermis*.

Conclusion

The present study revealed that *M. inermis* stem-bark methanol extract exhibits potent DPPH, hydrogen peroxide scavenging effect, and ferric reducing antioxidant power in *in vitro* studies. Similarly, *in vivo* studies also showed that the extract has lipid peroxidation neutralization and enzyme complementary effects. Hence, this study validates antioxidant activity as one of the mechanisms exerted by the *M. inermis* plant in managing diseases.

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Conflicts of Interest: The authors declare no conflict of interest.

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