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Original Research Article

Antimicrobial, Antioxidant and *In Vivo* Toxicity Studies of *Triumfetta rhomboidea* Jacq Leaf Extract in Male Wistar Rats

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ABSTRACT

Although *Triumfetta rhomboidea* (TR) has found its place in folklore medicinal applications, the scientific basis of these applications is poorly understood. This study examined the antioxidant and antibacterial properties of TR and its potential alteration of the renal and liver functions. The chemical constituents and antioxidant capacity of the ethanol extract of TR leaves were determined using FTIR, GC-MS and standard methods, and the agar well diffusion method was used to measure the antimicrobial activity. For the toxicity study, fifteen rats were divided into three groups: A – C; control, 200 mg/kg and 300 mg/kg ethanol TR extract per body weight administered orally for fourteen days. Biochemical assays were performed on the plasma, liver and kidney homogenates, and histo-pathological changes were examined in the tissues. Results showed that TR leaves contain total flavonoids (0.1316 ± 0.0001 mg/ GAE/100g) and total phenols (0.13 ± 0.0002 mg GAE/100g). GC-MS analysis of the leaf extract showed the presence of 44 major compounds. The extract demonstrated significant inhibitory activity against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*. Biochemical assay revealed no significant effect on the liver and renal function changes compared to the control group, except for the critical ($p< 0.05$) increase in Na. The antioxidant parameters in both the kidney and liver homogenates of TR-treated rats were not significantly altered except for reduced SOD levels at 200 mg/kg. Histopathological assay revealed relative degenerative changes in the two organs. TR leaves have antimicrobial and antioxidant properties, but could be toxic to the liver and kidneys at high doses.

Keywords: *Triumfetta rhomboidea*, Antibacterial, Anti-inflammatory, Antimicrobial-resistant bacteria, Histopathology.

Introduction

Triumfetta rhomboidea Jacq (TR) belongs to the family Tiliaceae. It is an undershrub, widely distributed in tropical and subtropical India, Ceylon, the Malay Peninsula, China, Africa and America.[1] It is a perennial herb that is essential in ancient therapy. Various parts of the plant are used therapeutically: fruit, flower, leaves, bark, and roots. The root has tonic, styptic, galactagogue, aphrodisiac, cooling, and diuretic properties, and it is also helpful in treating dysentery. Pounded roots are utilised in the treatment of intestinal ulcers. The leaves, flowers, and fruit of TR are mucilaginous, demulcent, astringent, and also helpful in treating gonorrhea, hormonal imbalance and infertility. [2 3] The ethnobotanical and traditional uses of TR as antibiotics and analgesics suggested its antibacterial, anti-inflammatory and antioxidant importance. Antioxidants are compounds known to protect the body from the harmful effects of free radicals, and also help to combat oxidative stress and alleviate some disease conditions associated with oxidative stress.

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Generally, metabolic processes in aerobic organisms produce free radicals/ reactive oxygen species, which are highly reactive since they contain unpaired electrons in their outer shells. These free radicals interfere with critical physiological processes in the body by combining with some biomolecules, including lipids, proteins, and nucleic acids, thus leading to their dysfunction, which can, as a consequence, lead to an array of diseases in the body.[4] A significant effect occurs when these free radicals are produced in excessive amounts that overwhelm the body's antioxidant defence, leading to a condition known as oxidative stress. The aetiology has been connected to oxidative stress and pathogenesis of various diseases such as liver damage, cardiovascular diseases, kidney damage, cancer, diabetes mellitus, and neurodegenerative diseases.[5,6,7,8]

Similar to the antioxidant-linked diseases are those caused by infectious agents, whose prevalence is increasing, particularly due to the antibiotic resistance of microbes against existing synthetic drugs. Some specific groups of pathogenic bacteria are responsible for numerous microbial infections.[9] The diseases caused by microorganisms are usually treated using antibiotics, which typically present with their side effects, such as organ damage. Recently, the increased resistance of pathogenic organisms to antibiotics has necessitated the dire need for novel antimicrobial compounds from natural sources. Therefore, there has been an increased interest in using natural plants like herbs due to their availability, safety, effectiveness and biodegradability compared to currently available antibiotics with high side effects.[10] Essentially, most plant phytochemicals, such as phenolic acids, alkaloids, glycosides, flavonoids, tannins, stilbenes, coumarins and anthocyanins, possess antioxidant and antimicrobial properties. [11, 12]

It is known that most plants with therapeutic principles could still be toxic to humans when consumed in excess amounts. The liver and the kidneys are the most vulnerable organs to damage from toxins because they are actively involved in the metabolism and excretion of various

xenobiotics from different sources.[13] However, despite the numerous medicinal uses attributed to TR, there are few pharmacognostic and pharmacological reports on this plant and also few studies on the potential toxicity of the plant on the kidney and liver, as there is the risk of being abused by consumers. [14] Hence, this study was designed to investigate the antimicrobial, antioxidant and potential toxicity of TR on the liver and kidney. The outcome of this study shall shed more light on the antimicrobial and antioxidant activities of TR and its probable effect on the liver and kidney, which shall enable consumers to make informed choices about the amount to be consumed without any consequences.

Materials and Methods

Plant Collection and Identification

The leaves of *Triumfetta rhomboidea* were collected from Redeemer's University Campus in Ede, Osun State, Nigeria, in January 2023. The plant was taxonomically identified by Prof Ernest Durugbo, a Botanist in the Department of Biological Sciences, Redeemer's University, Ede, Osun State, Nigeria. The plant was initially identified, and a voucher specimen was deposited at the University of Lagos herbarium, where the voucher number LUH 9506 was assigned. The plant materials were ground with a grinder after being allowed to air dry at ambient temperature.

Chemicals and reagents

Chloro-2-4-nitrobenzene (CDNB) and five 5'-Dithio-bis-2-nitrobenzoic acid (DTNB) were obtained from Sigma Chemicals, USA. Alanine aminotransferase kit, alkaline phosphatase kit, and aspartate aminotransferase were from Randox UK. Ethanol was obtained from Honeywell, and other chemicals used were of analytical grade.

Preparation of Ethanol Leaf Extract of TR

The air-dried powdered leaves 250 g were soaked in ethanol 750 ml and put in a shaker for 72 h, and filtered afterwards into a flask to obtain the filtrate, which was then placed in a rotary evaporator to get a semi-solid mass.

Phytochemical Screening for Metabolites

With minor modifications, the method of Olaniyi et al. was used to test for saponin, alkaloids, tannin, phenol, and phlobatannins in TR leaf powder.[15]

Evaluation of the in vitro antioxidant properties of the TR leaf

The total phenolic content, total flavonoid content, nitric oxide (NO) scavenging activity, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity, ferric reducing power assay, and total antioxidant capacity (TAC) of TR leaf powder were determined. The Singleton and Rossi method was employed to determine the phenolic acid content, while the Marinowa et al technique was used to detect total flavonoids.[16,17] NO scavenging activity assay was carried out using the method of Alhakmani et al.[18] The DPPH scavenging activity of the TR leaf was determined using the technique of Marcocci et al. [19] The reducing power was determined using the method of Gulcin et al. [20] The total antioxidant capacity (TAC) was calculated as described by Prieto et al. [21] All the experiments were carried out in triplicate.

Fourier-transform infrared spectroscopy (FT-IR) and GC-MS analyses of TR ethanol leaf extract

The FTIR spectrophotometer used for the characterisation was the SHIMADZU FTIR-8400S. The functional groups and positions in the plant extract were examined in the 4000 to 400 cm^{-1} range. The Perkin-Elmer Clarus 680 system (Perkin-Elmer Inc., the USA) was used for the TR extract GC-MS analysis. The mass spectra of the phytochemicals in the extract were compared to those in the National Institute of Standards and Technology (NIST) library's spectrum database of authentic substances, and the phytochemicals in the extract were identified. [8, 22]

TR extract antibacterial activities and minimum inhibitory concentration

Using agar well diffusion methods, the antibacterial properties of TR leaf extract were assessed against two Gram-positive bacteria, *Bacillus cereus* (ATCC10876) and *Staphylococcus aureus* (ATCC25923)[23] and three Gram-negative bacteria- *Escherichia coli* (DSM10974), *Pseudomonas aeruginosa* (ATCC9077) and *Salmonella typhimurium* (ATCC13311). The antibacterial activities were measured as the zone of inhibition with a Vernier calliper. The minimum inhibitory concentration of the TR leaf extract was also determined using the agar well diffusion method; this was used to establish the effective concentrations of the extract on pathogens.

Toxicity study using rat models

TR's potential toxicity was assessed *in vivo* with 15 male albino rats. The rats, which ranged in weight from 160 to 190 g, were given rat chow (Ladokun Feeds, Ibadan, Nigeria), free access to water, and sufficient care following the standards outlined by the Redeemer's University Committee on Ethics for Scientific Research. Rats were divided into three groups (A, B, and C; 5 rats per group). The Redeemer's University Committee on Ethics for Scientific Research approved the study and assigned the approval number, RUN/BCH/17/17013.

Group A: The control animal was given normal saline
Group B: received 200 mg/kg TR.
Group C: received 300 mg/kg TR.

The medication was administered orally to the rats for 14 days, after which the rats were sacrificed by cervical dislocation. Blood samples were drawn from the inferior vena cava and centrifuged for 10 minutes at 6500 rpm. The plasma samples were separated and stored at -20°C until required for analysis. The liver and kidneys were carefully separated, cleaned to remove debris, and weighed. The tissues were then preserved for micro-morphological examination with a formalin solution.

Biochemical analysis of plasma

Most of the biochemical analyses were done with Randox kits (Randox Laboratories Limited, Crumlin, U.K.); the following parameters were measured as per the manufacturer's instructions: aspartate aminotransferase (AST), alanine aminotransferase (ALT), cholesterol, albumin, total bilirubin, creatinine, urea, uric acid, calcium, potassium, and sodium.

Determination of the antioxidant status of the liver and kidney homogenates

The level of reduced glutathione (GSH) was estimated using the method of Jollow et al. [24]. The levels of glutathione peroxidase (GPx) and glutathione S-transferases (GST) were as described by Rotruck et al. and Habig et al., respectively [25,26]. Superoxide dismutase (SOD), catalase (CAT) activities, and malondialdehyde (MDA), which is an index of lipid peroxidation levels, were also evaluated using techniques described by Misra et al., Clairborne et al. and Varshney and Kale, respectively; and the total protein content was calculated using the Lowry method.[27,28,29,30]

Histopathology Examination of Rats' Tissues

Liver and kidney tissues were embedded in paraffin after being dehydrated in graded alcohol (80%) and fixed in 10% formaldehyde. The tissues were divided into sections (4-5 mm thick) using a Reichert-Jung rotary microtome, stained differently with hematoxylin, eosin, and periodic acid Schiff, and then examined under a light microscope.

Statistical analysis

The results were expressed as mean ± S.D. One-way analysis of variance (ANOVA) followed by a post hoc Tukey test was utilised to analyse differences between groups, and the significance was set at $p \leq 0.05$ using GraphPad Prism.

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Results and Discussion

Phytochemical Screening for Secondary Metabolites and Antioxidant Activity

The screening result for the secondary metabolites showed that the leaf extract of TR contained saponin, alkaloids, tannin, phenol and phlobatannins. Total flavonoids (0.1316± 0.0001 mg gallic acid equivalent [GAE] / 100g), total phenol (0.13 ±0.0002 mg GAE/100g), DPPH Scavenging activity (20.78 ± 0.92 %), nitric oxide inhibition activity (21.1 ± 1 %), total antioxidant capacity (28.98 ± 1.31 %) and ferric reducing power (1.203 ± 0.28 %) were obtained in the quantitative phytochemical and antioxidant analysis.

Phytochemicals are biologically active compounds that are not conventional nutrients but contribute radically to protection against degenerative diseases. [31] Results obtained from the phytochemical screening revealed that saponin, alkaloids, tannin, phenol, phlobatannins and flavonoids were found in the leaves of *Triumfetta rhomboidea*, which agreed with other studies.[2,32] The *in vitro* antioxidant assay results suggested that TR extract has relatively free radical scavenging activities. These activities might be attributed to its phenolic and flavonoid content. Flavonoids exert anti-inflammatory, antimicrobial, and antioxidant effects. [33] Previous reports have confirmed TR's free radical scavenging and antioxidant activity. [2, 32]

Fourier-transform infrared spectroscopy (FTIR)

The FTIR result of *Triumfetta rhomboidea* leaf extract (**Figure 1)** shows six major and minor peaks, along with some stretches. The broad 3441.12 cm-1 and the sharp 2826.11 cm-1 with a 2854.74 cm-1 shoulder peak are prominent among these peaks. Other peaks are 1737.92, medium 1637.77 and 1633.76. The 1458.30, 1384.94, 1240.27, 1163.11 and 1074.39 are less well-known. Finally, there are groups of peaks between the 912 and 416 cm-1 peaks. According to Coates, the absorption at 3441.12 cm^{-1} is due to the stretching of hydroxyl groups in the extract.[34] The bands at 2926.11 cm^{-1} and 2854.74 cm^{-1} are due to the extract's symmetric methylene (CH_2) group extension. The bands at 2355.16 cm^{-1} and 1456.3 cm^{-1} are assigned to be those of carbonate ions. The band at 2015.68 cm^{-1} suggests the presence of cyanide, thiocyanate and other related ions.

GC-MS analysis of TR extract

The GC-MS analysis of the ethanol leaf extract of *Triumfetta rhomboidea* revealed the presence of a total of 128 compounds. **Figure 2** displays roughly six conspicuous peaks and some lesser peaks. The first small peak corresponds to formamide, N-methoxy- with retention time of 3.6703 and peak area of 0.02 %; prominent peaks are neophytadiene with retention time of 14.505 (4.26%), Cyclohexane, 1-methyl-4-(1-methylethenyl)-, with retention time of 14.857 (1.77 %), 1,7-Octadien-3-one, 2-methyl-6-methylene- with retention time of 15.1862 (0.15 %), n-Hexadecanoic acid with retention time of 15.6714, (2.20 %), 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- with retention time of 17.0863 (5.91 %), trans-Geranylgeraniol with retention time of 21.6084 (1.07 %), Tris(trifluoromethylthio)methane with retention time of 22.5786 (2.32 %), vitamin E with retention time of 23.3352 (7.51 %), campesterol with retention time of 24.0918 (2.53 %), stigmasterol with retention time of 24.3459 (3.39 %), gamma-Sitosterol with retention time of 24.8541 (5.99 %), lanosterol with retention time of 25.2179 (1.31 %), 9,19-Cyclolanost-24-en-3-ol, (3.beta.) with retention time of 25.5356 (4.40%), and friedelan-3-one with retention time of 26.8581 (5.18 %).

The infrared spectrum of the FTIR is essential for structural analysis and was used to identify the functional group of the active components based on the peak value in the infrared region. As the results show, the extract contained hydroxyl groups, methylene groups and some other inorganic ions. Further characterisation was done using GC-MS to identify the specific compounds in the extract. Most of the compounds in the extract have been reported to possess one biological activity or another.[35] Vitamin E, the most abundant compound, has antioxidant and anti-inflammatory activities. [36] Gamma-sitosterol, the extract's second most abundant compound, has been reported to possess hypolipidemic and anti-viral properties. [37] Another compound, 9, 12, 15 15-Octadecatrienoic acid, has anti-inflammatory, cancer preventive, hepatoprotective, antioxidant and hypocholesterolemic activities. [38]

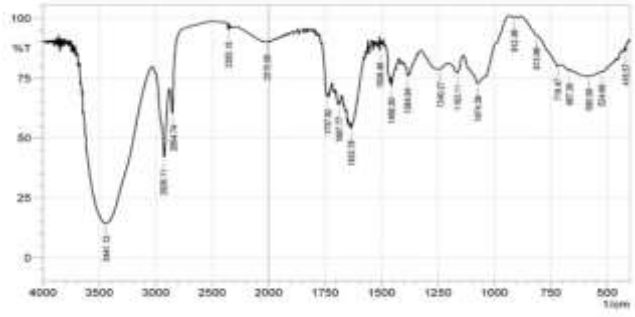

Figure 1: FTIR spectrum of the ethanol leaf extract of *Triumfetta rhomboidea*

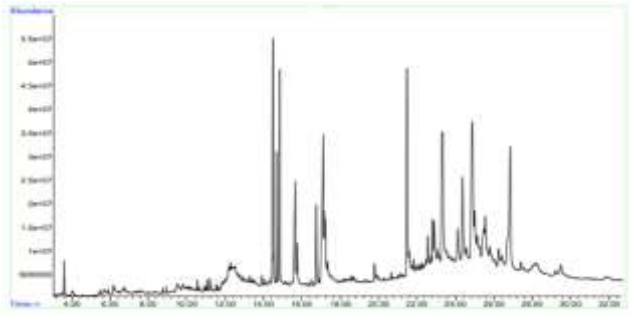

Figure 2: GC-MS spectrum of the ethanol leaf extract of *Triumfetta rhomboidea*

Antibacterial activities of ethanol leaf extract of Triumfetta rhomboidea

The results of the antibacterial activity of (*Triumfetta rhomboidea*) extracts investigated on pathogenic bacteria revealed that the *Triumfetta rhomboidea* leaf extract effectively inhibited Gram-positive and Gram-negative bacteria by showing inhibition zones. The inhibition zones on all test bacteria are shown in Figure 3 as compared with the performance standard for antimicrobial susceptibility testing. The extract was observed to have the highest zones of inhibition on *Salmonella typhi, 27 mm, followed by Bacillus cereus, 24 mm, Staphylococcus aureus, 27 mm, Escherichia coli, 20 mm and Pseudomonas aeruginosa,* 18 mm. The result of the minimum inhibitory concentration revealed that 50 mg/mL of *Triumfetta rhomboidea* extract was the minimum concentration to inhibit *Escherichia coli, Staphylococcus aureus,* and *Bacillus cereus,* while the minimum concentration of the extract to inhibit *Salmonella typhi* and *Pseudomonas aeruginosa* was 100 mg/mL. The antimicrobial activities of TR could be ascribed to the presence of compounds such as friedelan-3-one, 9,19-cyclolanost-24-en-3-ol, stigmasta-5,24(28)-dien-3-ol,4-dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene), neophytadiene, stigmasterol, and certain naphthalene derivatives, which have been reported with antimicrobial activities. [39] Gamma-Tocopherol antioxidants, cellular signalling, gene regulation, membrane processes, and nerve functions, n-hexadecanoic acid has antioxidant, hypocholesterolemic, nematicide, pesticide, anti-androgenic, hemolytic, 5-alpha reductase inhibitor, and anti-inflammatory activities. [40]

The *in vitro* antibacterial study of TR showed that the extract effectively inhibited the growth of Gram-positive and Gram-negative bacteria, thus demonstrating the broad-spectrum antibacterial nature of the extract's active components and the possibility of using the extract in treating antimicrobial-resistant bacteria. The low inhibition zones obtained with TR application on the strains of *P. aeruginosa* and *E. coli* in this study suggested that the organisms were less susceptible to the extract, which inhibited their growth substantially.[23]

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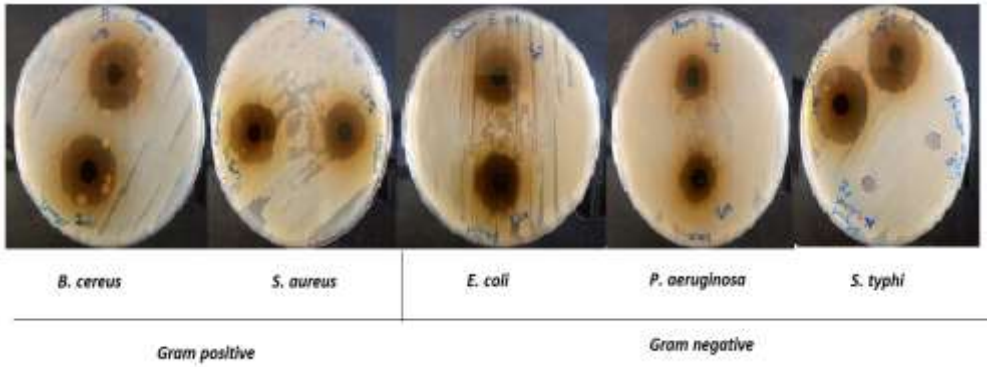

Figure 3: Plate view of antibacterial activities of *Triumfetta rhomboidea* leaf extract on pathogens.

The results of the minimum inhibitory concentration of TR extracts on the reported pathogens were similar to those of Hongfei et al.[41]

Effect of TR extract on plasma biochemical parameters

The effects of the ethanol leaf extract of *Triumfetta rhomboidea* on liver and kidney function parameters: plasma albumin, cholesterol, AST, ALT, total bilirubin, uric acid, urea, creatinine, calcium, sodium and potassium levels in treated rats are depicted in Table 1. Ethanol leaf extract of TR at 200 mg/kg and 300 mg/kg lowered both AST, ALT, cholesterol, uric acid, urea and creatinine plasma levels significantly ($p<0.05$) compared to the control group. TR extract at 300 mg/kg slightly lowered the total bilirubin level, but this was not statistically significant. TR extract also impacted the electrolytes; calcium (Ca) and sodium (Na) levels in the plasma were elevated significantly at $p<0.05$ in both the 200 mg/kg and 300 mg/kg groups compared to the control group. However, the plasma potassium (K) level is lowered significantly ($p<0.05$) in the treated rats.

Table 1: Effect of the ethanol leaf extract of *Triumfetta rhomboidea* on biochemical parameters

Parameter	A	B	C
Albumin (g/dl)	2.96± 0.40	3.99± 0.30*	4.53± 0.60*
Aspartate aminotransferase (U/I)	20.4± 0.89	17.6± 1.82*	15.8± 1.48*
Alanine aminotransferase (U/I)	26.4± 1.34	13.8± 1.10*	15.00± 0.71*
Total bilirubin (mg/dl)	0.85± 0.14	0.85± 0.07	0.76± 0.24
Uric acid (mg/dl)	63.58± 2.57	26.59± 1.98*	43.09± 2.05*
Urea (mg/dl)	14.46± 1.20	7.71± 1.15*	11.15± 0.74*
Creatinine (mg/dl)	7.99± 1.68	3.67± 0.71*	4.58± 1.14*
Calcium (mg/dl)	2.63± 0.68	4.09± 0.42*	5.82± 0.80*
Sodium (mEq/l)	157.88± 3.41	190.24± 6.73*	207.26± 5.83*
Potassium (mEq/l)	6.07± 0.50	5.33± 0.68	7.22± 0.80
Cholesterol (mg/dl)	0.66± 0.10	0.32± 0.08*	0.77 ± 0.17

Values are expressed as mean ± S.D. * significantly different from control ($p< 0.05$), A – Control group, B – Group given 200 mg/kg *Triumfetta rhomboidea*, *C* -Group given 300 mg/kg *Triumfetta rhomboidea*

Effect of TR extract on the antioxidant parameters in the liver and kidney

The effect of the extract on the antioxidant parameters in liver and kidney homogenates is shown in Table 2. There were no significant ($p< 0.05$) differences in the catalase, GST, NO and MDA levels in the liver homogenates of the treated rats compared to the control group. Similarly, there were no significant differences in the GSH, GST, NO and MDA levels in the kidney homogenates of the treated rats compared

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to the control group. However, a significant reduction ($p< 0.05$) was reported in the SOD levels in the liver and the kidney homogenates of the rats that received 200 mg/kg extract of TR compared to rats in the control group.

Table 2: Effect of ethanol leaf extract of TR on the antioxidants and non-antioxidant parameters in liver and kidney homogenates

Organ	Group	GSH	GPx	CAT	SOD	GST	NO	MDA
Liver	NOR	0.44± 0.06	0.48± 0.10	11.07± 0.95	0.31± 0.03	0.21± 0.05	0.05± 0.08	2.01± 0.03
	TR200mg	0.82± 0.11*	0.77± 0.07*	10.11± 6.08	0.22± 0.02*	0.24± 0.32	0.07± 0.01	2.06± 0.03
	TR300mg	0.94± 0.06*	1.15± 0.04*	11.81± 0.32	0.28 ± 0.01	0.19± 0.05	0.07± 0.01	2.08± 0.03
Kidney	NOR	0.84± 0.14	1.24± 0.11	2.31± 0.57	0.15± 0.02	0.02± 0.02	0.05± 0.01	0.0002± 0.0
	TR200mg	0.84± 0.05	1.09± 0.11	2.34± 0.61	0.10± 0.01*	0.02± 0.01	0.08± 0.01	0.0003± 0.0
	TR300mg	0.98± 0.12	1.73± 0.25*	3.59± 1.08*	0.14± 0.02	0.03± 0.02	0.06± 0.02	0.0002± 0.0

*Significant difference at $p< 0.05$ when compared to NOR (the control group)

Key: GSH- Reduced glutathione (µ mol/g tissue), GPx- Glutathione peroxidase (units/mg protein), CAT –Catalase (µ mol/ hydrogen peroxide (H_2O_2) consumed), SOD- Superoxide dismutase (units/mg protein), GST- Glutathione S transferase (µ mol CDMB-GSH complex formed/mg protein)- GSH-reduced glutathione (units /mg protein), NO-nitric oxide(unit/ mg protein) MDA –malondialdehyde (µ mol MDA formed/ mg protein).

Histopathological analysis

The photomicrographs of the liver and kidney homogenates of the experimental rats are shown in Figures 4 and 5, respectively. The liver of rats treated with the ethanol extract of *Triumfetta rhomboidea* showed dilated central venules with congestion, and the morphology of the hepatocytes presented mild pyknosis. Also, the sinusoids appeared mildly infiltrated and congested, while a slight haemorrhage was observed across profiles. As shown in Figure 5, the kidneys of normal rats showed standard architecture; rats treated with 200 mg/kg Triumfetta rhomboidea leaf extract showed mild degenerative architecture. The glomerulus appeared mildly sclerotic with a more expansive capsular space. Also observed in this group were well-outlined renal parenchymal cells. In addition, the kidneys of rats treated with 300 mg/kg TR leaf extract showed severe degenerative changes, characterised by congested renal tubules (proximal and distal convoluted tubules), infiltrated renal parenchyma by red inflammatory cells and signs of bleeding.

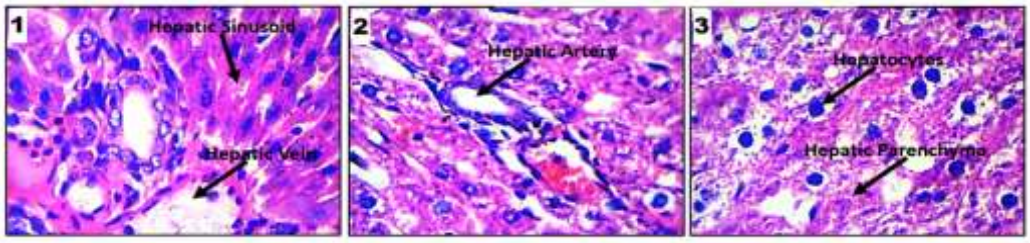

Figure 4: Magnified views of a liver micromorphological section demonstrated by Haematoxylin and Eosin staining at high magnification (x400). The hepatocytes, sinusoids, and portal triad (hepatic vein, hepatic artery and bile duct) are visible across the various groups. 1: CTR, 2: TR200mg, 3: TR300mg

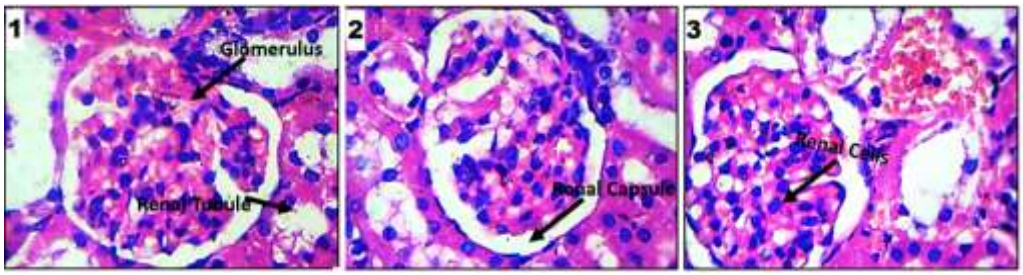

Figure 5: Haematoxylin and Eosin staining at high magnification demonstrated magnified views of the micromorphological kidney section (X400). The renal cortex, renal tubules, glomeruli, mesangial cells, and proximal and distal renal convoluted tubules are all visible across the various groups. 1: CTR, 2: TR200mg, 3: TR300mg

Some critical biochemical indicators that serve as toxicity markers were monitored in an in vivo study to study the safety of TR administration. A prominent predictor of organ damage is the leaching of biomolecules from tissue or organs into the blood, resulting in an elevated concentration of such biomolecules. Elevated plasma ALT, AST and bilirubin are indicators of hepatocyte injury, while urea, uric acids and creatinine are indicators of renal injury. [42]

The *in vivo* study showed a decrease in the value of AST and ALT in the animals treated with the plant extract at the two dosages used in this study compared to the control group. [43] There was also a slight, non-significant reduction in the bilirubin level of the animals treated with 300 mg/kg ethanol leaf extract of the TR. The depression in plasma levels of these liver biomarkers suggested the hepatoprotective effect of the plant extract at the doses used and that the plant may be able to restore liver injury. Similarly, reduced plasma urea, creatinine, and uric acid levels could indicate the renal protective activity of the TR extract. Previous studies have shown different plant materials with hepatoprotective and nephroprotective activities.[44 45] TR also caused a reduction in plasma calcium and sodium levels, but elevated potassium levels are associated with kidney function decline. [46] This suggested electrolyte acid-base imbalance, the type associated with NSAIDs, such that the water and chlorides are retained in the glomeruli. [47] This situation has been linked to exercise and over-hydration with hypotonic fluids.

Still, in the *in vivo* study, the ability of TR to initiate oxidative stress was examined in the liver and kidney tissues. Oxidative stress is associated with damaged organs such as the liver and kidney, during which antioxidant markers such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione (GSH) are generally elevated to prevent damage induced by reactive oxygen species (ROS).[48,49] While GPx was elevated in both the liver and kidney, GSH was elevated in the liver alone and CAT in the kidney alone, thus suggesting that the TR extract elicited the production of specific antioxidant proteins to curb the ROS produced, probably from ageing. [50] Other markers, such as the SOD, nitric oxide and Malondialdehyde (MDA), were within the normal range. MDA occurs due to lipid peroxidation and is a good indicator of oxidative stress in the body. [50] The fact that the MDA levels were not altered in the treated animals' livers and kidneys implies that TR did not induce lipid peroxidation at those concentrations. Dilated central venules in the liver and expansive capsular space in the kidney, as shown in the histopathological analysis of the liver and kidney of rats treated with TR extract, confirmed the retention of fluid in the extracellular matrix of tissues resulting from electrolyte imbalance similar to those caused by prostaglandin modulation.[49]

Conclusion

The leaf extract of *Triumfetta rhomboidea* showed potent antioxidant and broad-spectrum antibacterial effects against common bacterial pathogens, linked to its chemical components identified using GC-MS. It appeared to have a protective effect on the liver based on plasma toxicological markers. However, electrolyte imbalances (low calcium and sodium, high potassium) were observed in the plasma. Mild liver toxicity was indicated by tissue markers and histopathology, showing liver and kidney structural changes at high extract usage. The antibacterial action involves anti-inflammatory effects similar to NSAIDs, which may affect kidney fluid balance. While the extract is promising for treating bacterial infections and free radical-based tissue damage, caution is advised due to potential liver and kidney toxicity with excessive or long-term use. Further study is needed on its impact on electrolyte balance during extended use.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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