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Phytochemical Evaluation And Protective Effect Of Tithoniadiversifolia (Hemsl.) Leaf Extract Against Aspirin-Induced Ulcer In Wistar Rats

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Abstract

Objectives: The objective of this study was to evaluate the phytochemical composition and gastroprotective properties of *Tithonia diversifolia* extract.

Methods: Gas Chromatography-Mass Spectrophotometry (GC-MS) was used to quantify the chemical constituents present in the extract. Fourier Transform Infrared (FT-IR) spectroscopy was employed to identify functional groups in the extract. An acute toxicity study was conducted on rats to assess the safety of the extract. For the gastroprotective study, Wistar rats were divided into different groups and pretreated with different doses of T. diversifolia extract before ulcer induction with aspirin.

Results: GC-MS analysis revealed the presence of twenty-eight chemical constituents in the extract,with benzyl alcohol, p-hydroxy-alpha-[(methylamino) methyl] being the most prominent. FT-IR analysis identified several functional groups in the extract. The acute toxicity study showed no signs of toxicity or mortality. Both the *T. diversifolia* extract and omeprazole (standard drug) significantly reduced ulcer parameters, antioxidant activity, phase

II enzymes, matrix metalloproteinase activity, prostaglandin E2 levels, and inflammatory biomarkers compared to the negative control group. The percentage inhibition of ulcers followed a dose-dependent trend.

Conclusions: The findings of this study suggest that the decoction of *Tithonia diversifolia* has potential gastroprotective properties against aspirin-induced ulcers. The extract exhibited significant reduction in ulcer parameters and modulation of various biochemical markers associated with ulcers. These results support the traditional use of T. diversifolia in ethnomedicine for the treatment of ulcers. Further studies are warranted to explore the specific mechanisms of action and potential clinical applications of *T. diversifolia* extract in gastroprotection.

Keywords: *Tithonia diversifolia,* omeprazole, Anti-inflammatory, Phytochemical, Antioxidant

Introduction

The utilization of modern traditional medicine, mainly through the use of medicinal plants, has gained significant popularity worldwide. In countries like Nigeria, with rich biodiversity, these plants are potential sources of affordable medications to treat various conditions (Mazzari& Prieto, 2014). The bioactive compounds present in medicinal plants contribute to their pharmaceutical and health-improving properties (Sofowara, 1993). Ethnopharmacology, a scientific discipline, explores the knowledge of indigenous communities regarding the use of plant and animal products for maintaining health (Silva *et al*., 2005; Rojas *et al.,* 2006). In particular in developing countries with limited access to pricey Western treatment, the prevalence of the human immunodeficiency virus (HIV) has sparked substantial studies into plant derivatives. Herbal products symbolize safety compared to synthetic drugs, perceived as unsafe for humans and the environment, prompting a return to nature for safety and security (Silva *et al*., 2005). Over three-quarters of the global population currently relies on plant extracts for healthcare. The use of herbal medicine is exceptionally high in developing countries, with an estimated 80% of the world population depending on plants for healthcare (Ekor, 2014). These medicinal drugs are derived from different parts of plants, including leaves, stems, bark, roots, flowers, or seeds. While plant-derived drugs have a stable global market, plants are an essential source for developing new drugs. The traditional system of medicine is widely practiced due to population growth, inadequate supply of conventional drugs, high treatment costs, and the side effects associated with synthetic drugs.

Additionally, the resistance of certain diseases to synthetic drugs has led to an increased emphasis on using plant materials as primary sources of medicine for various human diseases (Veeresham, 2012). Recent advancements in technology have facilitated more effective analysis of

plant components. This has resulted in identifying numerous medicinal extracts and their potential use in treating human diseases (Khan *et al*., 2006). The World Health Organization (WHO) recognized the major reliance on traditional medical practices by recognizing the value of herbal medicine in healthcare through the "Health for All" Alma Ata Declaration from 2010AD.The WHO has also developed monographs on medicinal plants commonly used in developed and developing countries to support member states' efforts (WHO, 2002). Asteraceae family member *Tithonia diversifolia* originated in Mexico, Central America, and Cuba and has since naturalized in tropical areas of Asia and Africa. In Nigeria, *Tithonia diversifolia* is the only species of the genus found growing on wastelands, waterways, major roads, and cultivated farmlands (Chukwuka *et al*., 2007). Medicinal plants from higher plant species have made valuable contributions to the well-being of rural and urban communities, particularly in tropical and subtropical regions (Sofowara, 1993). Traditional societies and ethnic nationalities have employed medicinal plants in ethnomedicine to treat various ailments, relying on the intuitive trial and error approach without scientific knowledge of the active ingredients responsible for their medicinal and pharmacological properties (Aja *et al*., 2010; Vaghasiya *et al*., 2011). The interest in investigating plants for folkloric medicine has steadily increased (Ogundare, 2007). The medicinal potential of plants is attributed to their active phytochemical content, as demonstrated by Pickhardt *et al*. (2005) and other researchers (Heber, 2007; Erharuyi *et al*., 2014). Plants contain numerous compounds that can have significant effects on the human.

Figure 1.0: The pictorial representation of *Tithonia diversifolia* leaf

2. Materials and Methods

2.1 Collection of Plant Materials and Identification

Fresh leaves of *Tithonia diversifolia* were collected from a compound bush located in Osisioma, Aba, Abia State, Nigeria. The plant samples were identified by Dr. Bob Ezuma from the Department of Plant Science and Biotechnology, Abia State University, Uturu, Nigeria.

2.2 Preparation of the Extract

The harvested leaves of *Tithonia diversifolia* were sun-dried for a period of seven (7) days. The dried leaves were then milled into a fine powder using an Arthur Miller Machine and stored in cellophane bags until further use. To prepare the extract, exactly five hundred grams (500 g) of the powdered *Tithonia diversifolia* leaves were soaked in 2 L of chloroform and left to stand for 48 hours. The mixture was then strained using muslin cloth and filtered through Whatman No. 1 filter paper. The filtrate was evaporated to dryness in open air. The required concentrations of the extract used in this study were obtained by dissolving a known amount of the dried extract in distilled water.

2.3 Gas Chromatography-Mass Spectrometry Analysis (GC-MS)

The methanol extract of T. diversifolia (Hemsl.) A. Gray leaves was analyzed using GC-MS to identify different compounds. The analysis was conducted with a Clarus 500 (Perkin-Elmer) gas chromatograph coupled with a Turbo mass gold (Perkin-Elmer) mass detector, equipped with an Elite-5MS capillary column. The temperature was varied from 110ºC to 280ºC, and helium gas was used as the carrier gas. The sample was injected into the column, and the mass spectral scan range was set from 45 to 450 m/z.

2.4 Fourier Transform Infrared (FT-IR) Analysis

FT-IR analysis was performed on the sample by grinding it to reduce the particle size, followed by mixing it with KBr. The mixture was placed between two KBr plates and subjected to a spectrometer. The spectrum was recorded using a Bruker Tensor 27 spectrometer in the wavelength range of 400 to 4000 cm-1.

2.5 Experimental Animals and Design

Forty-two healthy Wistar rats were utilized in the study. The animals were sourced from the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka, and allowed to acclimate for two weeks. They were housed in well-ventilated rooms and provided with grower mash and water ad libitum. The experimental procedures followed the United States National Institutes of Health Guidelines for the Care and Use of Laboratory Animals in Biomedical Research.

2.6 Acute Toxicity (LD50) Study

The experimental animals were administered different concentrations of the extract, and their mortality and behavioral changes were observed 24 hours post-administration.

2.7 Aspirin (ASP)-Induced Ulcers

Aspirin-induced ulcers were created in Wistar rats, and the anti-ulcerogenic potential of the chloroform extract of T. diversifolia was investigated. The animals were divided into six groups, each receiving different treatments, and after 1 hour, they were sacrificed, and their stomachs were examined for ulcers.

2.8 Glutathione S-Transferase (GST)

The activity of cytosolic GST in homogenate was measured using 1-chloro 2, 4-dinitrobenzol (CDNB) as the substrate.

2.8.2 Quinone Oxido-Reductase (QR)

QR activity was measured based on the NADPH-dependent manadiol reduction of MTT.

2.8.3 Thioredoxin Reductase (TrxR)

TrxR activity was determined by measuring the NADPH-dependent reduction of 5.5-dithiobis (2-nitrobenzoic acid) (DTNB).

2.8.4 Glutathione (GSH)

GSH levels were analyzed by the reaction of GSH with DTNB to produce the TNB chromophore, which has a maximal absorbance at 412nm.

2.9 Antioxidant Enzymes

2.9.1 Nitric Oxide Synthase Activity

Nitric oxide synthase activity was determined using an ELISA-based method with a kit purchased from Elabscience.

2.9.2 Myeloperoxidase Activity

Myeloperoxidase activity was measured following the method described by Pulli et al. (2013).

2.9.3 Malondialdehyde (MDA) Activity

Malondialdehyde concentration was determined spectrophotometrically using the method described by Atasayar et al. (2004).

2.9.4 Superoxide Dismutase (SOD) Activity

Superoxide dismutase activity was determined following the method described by Rukmini et al. (2004).

2.9.5 Matrix Metalloproteinase (MMP) Activity Determination

Matrix metalloproteinase 1 (MMP1) activity was analyzed using an ELISA-based method with a kit purchased from Elabscience.

2.10 Prostaglandin E2 (PGE2) and Pro-Inflammatory Cytokines Measurement

2.10.1 Quantification of Prostaglandin E2 (PGE2)

Levels of Prostaglandin E2 (PGE2) were determined using an ELISA-based method with a test kit from Elabscience.

2.10.2 Measurement of Pro-Inflammatory Cytokines

2.10.2.1 Tumor Necrosis Factor-Alpha (TNF-α)

The assay for TNF-α was conducted using an ELISA-based method with a test kit obtained from Elascience.

2.10.2.2 Interleukin I Beta (IL-1β) Activity

IL-1β activity was analyzed using an ELISA-based method with a test kit from Elabscience.

2.11 Gastric Juice Analysis

2.11.2 Collection of Gastric Juice

Gastric juice was collected from pylorus ligated rats and subsequently used for biochemical estimation.

2.11.3 Determination of Free Acidity and Total Acidity

The gastric juice was used to determine free acidity and total acidity using titration with NaOH and indicators.

2.11.4 Estimation of Pepsin Activity

Pepsin activity was estimated by incubating gastric juice with bovine albumin, and the absorbance was measured at 680 nm.

2.11.5 pH of the Gastric Juice

The pH of the gastric juice was determined by end-point titration using sodium hydroxide solution.

2.12 Haematological Parameters

2.12.1 Packed Cell Volume (PCV) (Microhaematocrit method)

Packed cell volume was measured using a microhaematocrit reader.

2.12.2 White Blood Cell Total (WBC Total)

Total white blood cell count was determined using the improved Neubauer counting chamber.

2.12.3 White Blood Cell Differential

A thin film of blood was prepared and stained with Leishman stain for the differential count of white blood cells.

2.12.4 Red Blood Cell Count Estimation

The number of red blood cells was quantified using a hemocytometer.

2.12.5 Red Blood Cell Indices

Red blood cell indices, including Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Volume (MCV), were calculated based on the relevant parameters.

2.13 Histological Evaluation

Histological evaluation was performed on stomach tissues following standard procedures, including fixation, dehydration, cleaning, paraffin infiltration, section cutting, and staining with hematoxylin and eosin.

2.13.2 Ethical Consideration

The experimental protocol was presented to the research and publication department of Abia State University Uturu, Nigeria. The study adhered to the Animal Welfare Act of 1985 and the Institutional Animal Care and Use Committee (IACUC) protocol.

2.13.3 Statistical Analysis

Statistical analysis was performed using One-way analysis of variance (ANOVA) with GraphPad Prism® Statistical software package, version 7.01. Tukey's multiple comparisons test was utilized to identify significant differences among the groups, with a significance level set at $p < 0.05$.

Results

Gas chromatography mass spectrophotometry(GC-MS) analysis of *Tithonia diversifolia* (Hemsl.) leaf extractrevealed the presence of twenty eight chemical compounds (Table 3.1) with the highest retention time recorded in benzyl alchohol, p-hydroxy-alpha- [(methylamino) methyl] (16.892) and least in hexadecane (7.061).

Table 1: Gas Chromatography Mass Spectrophotometry (GCMS)Analysis of *Tithonia diversifolia*(Hemsl.) leaf extract

S/N	RT	Compound Name	MW	Formular	Area (%)
1	7.061	Hexadecane	226	$C_{16}H_{34}$	1.02
2	7.355	Dodecane,2,6,11-trimethyl-	212	$C_{15}H_{32}$	1.15
3	8.216	Methyltetradecanoate	242	$C_{15}H_{30}O_2$	0.81
4	8.548	Tetradecanoic acid	228	$C_{14}H_{28}O_2$	2.04
5	8.628	3-Eicosene,(E)-	280	$C_{20}H_{40}$	1.74
6	8.671	1-Octadecanesulphoyl chloride	352	$C_{18}H_{37}ClO_2S$	1.68

7	4.916	Tetratetracontane	618	$C_{44}H_{9}0$	1.04
8	9.008	Bicyclo[3.1.1]heptane,2,6,6-trimethyl-	170	$C_{10}H_{18}O_2$	2.00
9	9.179	Dibutyl phthalate	278	$C_{16}H_{22}O_4$	0.78
10	9.302	1-Hexadecyne	222	$C_{16}H_{3}0$	1.39
11	9.479	Cis-10-Heptadecenoic acid, methyl ester	282	$C_{18}H_{34}O_2$	2.32
12	9.623	Hexadecanonic acid, methyl ester	270	$C_{17}H_{34}O_2$	2.32
13	9.623	Benzoic acid, 2-(1-oxopropyl)-	178	$C_{10}H_{10}O_3$	4.36
14	9.981	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	22.20
15	10.051	E-15-Heptadecenal	252	$C_{17}H_{32}O$	1.19
16	10.051	Decane	142	$C_{10}H_{22}$	0.69
17	10.671	1-Docosene	308	$C_{22}H_{44}$	1.21
18	10.746	13-Octadecenoic acid, methyl ester	296	$C_{19}H_{36}O_2$	0.99
19	10.816	Phytol	296	$C_{20}H_{40}O$	1.49
20	11.067	Oleic acid	282	$C_{18}H_{34}O_2$	14.09
21	11.206	Octadecanoic acid	284	$C_{18}H_{36}O_2$	7.39
22	11.939	Methyl 13-eicosenoate	324	$C_{21}H_{40}O_2$	1.03
23	12.217	Cis-13-Ecosenoic acid	310	$C_{20}H_{38}O_2$	6.99
24	12.340	9-Tetradecenal, (Z)-	210	$C_{14}H_{26}O$	3.47
25	13.025	Cis-9-Hexadecenoic acid	254	$C_{16}H_{30}O_2$	1.87
26	13.292	Erucic acid	338	$C_{22}H_{42}O_2$	7.64
27	16.111	Cathine	151	$C_9H_{13}N_O$	1.32
28	16.892	Benzyl alchohol, p-hydroxy-alpha-[(methylamino)methyl]	167	$C_9H_{13}NO_2$	2.30

RT, Retention Time; MW, Molecular Weight

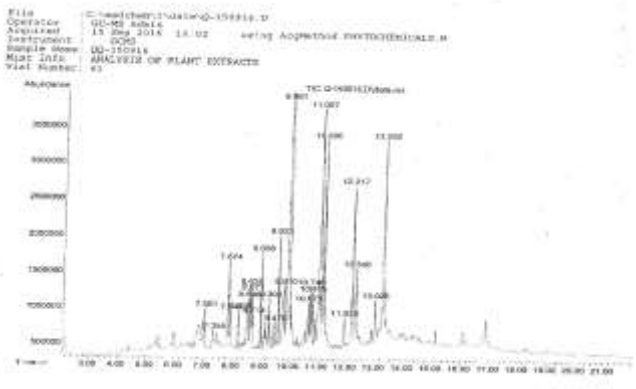
The table provides the results of a Gas Chromatography Mass Spectrophotometry (GCMS) analysis of *Tithonia diversifolia* (Hemsl.) leaf extract. GCMS is a technique used to separate and identify the components of a complex mixture. In this case, it was used to analyze the chemical compounds present in the leaf extract of *Tithonia diversifolia*.

Each row in the table represents a specific compound identified in the extract, and the columns provide information about the compound, such as the retention time (RT), compound name, molecular weight (MW), chemical formula, and area percentage.

Total of 28 compounds were identified in the *Tithonia diversifolia* leaf extract.The compounds have a wide range of molecular weights, with values ranging from 142 to 618.The identified compounds include various types, such as alkanes (e.g., hexadecane, dodecane), fatty acids (e.g., tetradecanoic acid, oleic acid), esters (e.g., methyltetradecanoate, methyl 13-eicosenoate), and other organic compounds (e.g., phytol, cathine).The area percentages indicate the relative abundance of each compound in the extract. For example, the compound with the highest area percentage is n-Hexadecanoic acid, representing 22.20% of the total peak area.Some compounds appear in multiple rows with slightly different retention times, indicating possible isomers or closely related compounds.

The identified compounds suggest the presence of a diverse array of organic compounds in the *Tithonia diversifolia*leaf extract, which may contribute to its biological activity and potential uses.

Figure 1: Mass chromatogram of *Tithonia diversifolia*(Hemsl.) A. Gray. leaf extract



The result of the mass chromatogram showed the peak area and elution of the chemical compositions of *Tithonia diversifolia* (Hemsl.) A. Gray Leaf Extract.

The FT-IR analysis of leaf extract of *T. diversifolia*(Hemsl.) A. Gray revealed the presence of primary amines, arenes, aldehyde, ketones, alcohols, phenols and alkanes as the functional composition of the extract (**Table 2 and Fig. 2)**. The highest wave number was recorded in the alcohol and phenol group (3283.01 cm^{-1}), while the least wavelength was found among the amine group (1030.78 cm^{-1}).

Table 2: FT-IR Analysis of *T. diversifolia*(Hemsl.) A. Gray Leaf Extract

S/N	Wave Number (CM^{-1})	Functional Group
1.	1030.78	C-N (Amines)
2.	1245.34	C-H (Arenes)
3.	1320.21	A-CH_3 bending (aldehydes and ketones)
4.	1408.01	CH_2 and CH_3 deformation (alkanes)
5.	1629.33	NH_2 scissoring (1^0 - amines)
6.	2850.88	CH_3, CH_2, CH (2 or 3 bands) alkanes
7.	2919.76	CH_3, CH_2, CH (2 or 3 bands) alkanes
8.	3283.01	O-H (H-bonded) alcohols and phenols

The FT-IR (Fourier Transform Infrared) analysis of the leaf extract of *Tithonia diversifolia* (Hemsl.) A. Gray revealed the presence of various functional groups in the extract. The analysis provides information about the types of chemical bonds and functional groups present in the sample, which can help in identifying the composition of the extract.

C-N (Amines): The wave number of 1030.78 cm-1 indicates the presence of primary amines in the leaf extract of *T. diversifolia.*

C-H (Arenes): The wave number of 1245.34 cm-1 suggests the presence of aromatic compounds or arenes in the extract.

A-CH_3 bending (aldehydes and ketones): The wave number of 1320.21 cm-1 indicates the presence of aldehydes and ketones in the extract. The bending motion of the CH_3 group is observed.

CH_2 and CH_3 deformation (alkanes): The wave number of 1408.01 cm-1 suggests the presence of alkanes in the leaf extract. The deformation of CH_2 and CH_3 groups is observed.

NH2 scissoring (10 - amines): The wave number of 1629.33 cm-1 indicates the presence of secondary amines in the extract.

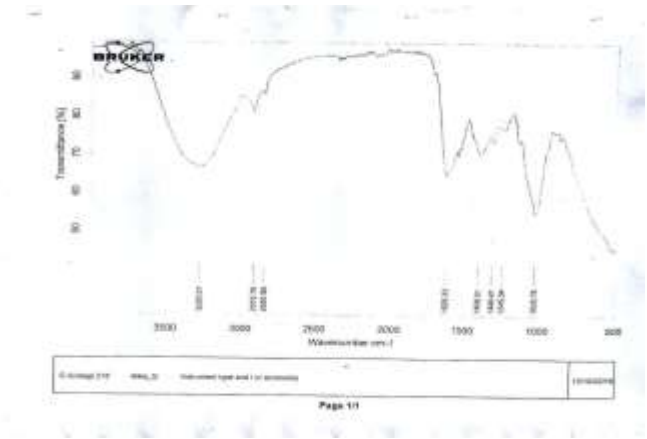
CH_3, CH_2, CH (2 or 3 bands) alkanes: The wave numbers of 2850.88 cm-1 and 2919.76 cm-1 suggest the presence of alkanes in the extract. The stretching or bending vibrations of CH_3, CH_2, and CH groups are observed.

O-H (H-bonded) alcohols and phenols: The highest wave number of 3283.01 cm-1 indicates the presence of alcohols and phenols in the leaf extract. The stretching vibrations of O-H bonds involved in hydrogen bonding are observed.

The functional groups identified in the FT-IR analysis provide insights into the chemical composition of the *T. diversifolia* leaf extract. The presence of primary and secondary amines, arenes, aldehydes, ketones, alcohols, phenols, and alkanes suggests the presence of a diverse array of organic compounds in the extract. These compounds may contribute to the plant's biological activity and potential applications.

To obtain more detailed and accurate information about the FT-IR analysis of *T. diversifolia* leaf extract, it is recommended to refer to the original research article or publication where this information is sourced from.

Figure 2. FT-IR spectrum of *Tithonia diversifolia*(Hemsl.) A. Gray. leaf extract



The result of the acute toxicity of *Tithonia diversifolia* leaf extract on Wistar rats was shown in Table**3.** The result showed that the lethal dose of *Tithonia diversifolia* leaf extract is > 5000 mg/kg as there was no mortality recorded.

Table 3: Acute Toxicity Study (LD_{50}) of *Tithonia diversifolia* Leaf Extract on Wistar Rats

Dose (mg/kg)	Mortality	Behavioural Changes
250	Nil	Calm and resting
500	Nil	Calm and resting
1000	Nil	Calm and resting
2000	Nil	Itching of mouth
3000	Nil	Itching of mouth which stopped after 5 minutes
4000	Nil	Itching of mouth which stopped after some minutes
5000	Nil	Itching of mouth stopped after some minutes

The **Table 3**presents the results of the acute toxicity study conducted on Wistar rats to determine the LD_{50} (median lethal dose) of *Tithonia diversifolia* leaf extract. The doses administered ranged from 250 mg/kg to 5000 mg/kg. The study observed the mortality rate and behavioral changes exhibited by the rats after the administration of each dose.

At all tested doses, no mortality was observed, indicating that the LD_{50} of the *Tithonia diversifolia* leaf extract in Wistar rats exceeds the highest tested dose of 5000 mg/kg. The behavioral changes observed were primarily related to itching of the mouth, which occurred at doses of 2000 mg/kg and above. However, it is noteworthy that the itching subsided after a few minutes in each case.

These findings suggest that the *Tithonia diversifolia* leaf extract demonstrates a low level of acute toxicity in Wistar rats. The absence of mortality and the temporary nature of the observed behavioral changes indicate that the extract is relatively safe at the tested doses. Further studies may be required to determine the long-term effects and establish a more precise LD_{50} value.

It is important to interpret these results within the context of the study and consult with experts and scientific literature for comprehensive understanding and accurate information regarding the toxicity profile of *Tithonia diversifolia* leaf extract.

The activity of antioxidants and phase II enzymes are shown in **Table 4.** The result showed a significant reduction ($P<0.05$) in the values obtained from GST, GSH, TPxr, QR, NOS, MYP, SOD and MDA when the omeprazole and *T. diversifolia* treated groups were compared to the untreated group (negative group).

Table 4: Effect of antioxidant activity and phase II enzymes of *T. diversifolia* leaf extract on aspirin induced ulcer in Wistar rats

Parameters				*T. diversifolia* Extract Dose (mg/kg)		
	Normal Control	Negative Control	Omeprazole5 mg/kg	250	500	750
GST	28.74±1.69^{a}	25.88±1.94^{ac}	23.89±1.81^{bc}	22.98±1.21^{b}	24.96±1.21^{b}	25.18±1.37^{b}
GSH	25.29±2.99^{a}	23.44±2.20^{a}	25.25±1.81^{a}	23.68±1.19^{a}	24.14±1.17^{a}	24.89±2.01^{a}
TRxr	1.68±0.13^{a}	1.84±0.05^{b}	1.80±0.08^{ab}	1.41±0.04^{c}	1.56±0.03^{a}	1.60±0.07^{a}
QR	257.28±23.98^{a}	289.49±6.44^{b}	288.38±3.91^{bd}	262.54±3.27^{ac}	266.06±10.31^{ad}	271.20±8.12^{ad}
NOS	40.69±3.09^{a}	33.34±3.51^{bc}	35.71±1.51^{cd}	30.91±1.35^{b}	32.91±1.42^{d}	33.43±1.00^{d}
MYP	160.84±5.43^{a}	191.22±7.74^{b}	177.42±7.12^{c}	158.80±0.84^{a}	159.44±7.13^{a}	163.85±5.19^{a}
SOD	39.84±2.42^{a}	35.48±0.58^{b}	32.17±1.54^{c}	25.46±1.54^{d}	36.82±1.53^{a}	38.51±1.52^{a}
MDA	2.16±0.03^{a}	5.35±0.48^{b}	4.31±0.27^{c}	4.11±0.09^{c}	4.23±0.04^{c}	4.32±0.15^{c}

Values are mean ± SD for N=5. Values in the same row bearing the same letter of the alphabet are not statistically significant at (P< 0.05). GST, Gluthathione-*S*- transferase; GSH, Glutathione; TRXr, Thioredoxine reductase; QR, Quinone oxido-reductase; NOS, Nitric oxide synthase; MYP, Myeloperoxidase; SOD, Superoxide dismutase; MDA, Malondialdehyde

The activity of matrix metalloproteinase-1 of *T. diversifolia* leaf extract on aspirin induced ulcer in Wistar rats (Table 5). The result showed a significant reduction (P<0.05) in the values obtained from MMP when the omeprazole and *T. diversifolia* treated groups were compared to the untreated group (negative group).

Table 4 presents the results of the effect of *Tithonia diversifolia* leaf extract on the activity of antioxidants and phase II enzymes in Wistar rats with aspirin-induced ulcers. The study aimed to evaluate the potential protective effects of the extract on oxidative stress and enzyme activity associated with ulcer development. The results showed a significant reduction (P<0.05) in the values of various parameters when comparing the omeprazole and *T. diversifolia* treated groups to the untreated (negative control) group.

GST (Glutathione-S-transferase): The values of GST were significantly reduced in the omeprazole and *T. diversifolia* treated groups compared to the negative control group. GST is an important enzyme involved in detoxification processes, and a reduction in its activity indicates a decrease in oxidative stress.

GSH (Glutathione): The values of GSH showed a significant reduction in the negative control group compared to the normal control group. However, the treatment with omeprazole and T. *diversifolia* extract did not significantly alter the GSH levels compared to the negative control. GSH is a crucial antioxidant involved in cellular defense against oxidative damage.

TRxr (Thioredoxin reductase): The values of TRxr were significantly reduced in the omeprazole group compared to the negative control. However, no significant changes were observed in the *T. diversifolia* treated groups. TRxr plays a role in maintaining the redox balance in cells and its reduction indicates oxidative stress.

QR (Quinone oxidoreductase): The values of QR were significantly reduced in the negative control group compared to the normal control. Treatment with omeprazole and T. diversifolia extract did not significantly affect QR levels compared to the negative control. QR is an enzyme involved in detoxification and antioxidant defense mechanisms.

NOS (Nitric oxide synthase): The values of NOS were significantly reduced in the negative control group compared to the normal control. Treatment with omeprazole and *T. diversifolia* extract did not significantly alter NOS levels compared to the negative control. NOS is an enzyme involved in the production of nitric oxide, which plays a role in various physiological processes.

MYP (Myeloperoxidase): The values of MYP did not show significant changes among the groups. MYP is an enzyme associated with inflammatory responses.SOD (Superoxide dismutase): The values of SOD were significantly reduced in the negative control group compared to the normal control. Treatment with omeprazole and *T. diversifolia* extract did not significantly affect SOD levels compared to the negative control. SOD is an antioxidant enzyme involved in the scavenging of superoxide radicals.MDA (Malondialdehyde): The values of MDA were significantly increased in the negative control group compared to the normal control. Treatment with omeprazole and *T. diversifolia* extract did not significantly alter MDA levels compared to the negative control. MDA is a marker of lipid peroxidation and oxidative stress.

Overall, the results suggest that treatment with omeprazole and *T. diversifolia* leaf extract had significant effects on the activity of antioxidants and phase II enzymes, indicating a potential reduction in oxidative stress and improvement in the antioxidant defense system. These findings suggest the potential beneficial effects of T. diversifolia leaf extract in protecting against aspirin-induced ulcers in Wistar rats.

Table 5, which is mentioned but not provided, likely presents the results of the activity of matrix metalloproteinase-1 (MMP-1) in the same study.

Table 5: The activity of matrix metalloproteinase-1 of *T. diversifolia* leaf extract on aspirin induced ulcer in Wistar rats

Group	Treatment	MMP-1
I	Normal Control	5.49 ± 1.18^{a}
II	Negative Control	8.42 ± 0.70^{b}
III	Omeprazole (5 mg/kg)	6.89 ± 0.70^{c}
IV	250 mg/kg of leaf extract	6.67 ± 0.66^{c}
V	500 mg/kg of leaf extract	6.29 ± 0.41^{c}
VI	750 mg/kg of leaf extract	6.67 ± 0.66^{c}

Values are mean ± SD for N=5. Values in the same column bearing the same letter of the alphabet are not statistically significant at (P< 0.05). MMP, Matrix metalloproteinase

Table 5 presents the activity of matrix metalloproteinase-1 (MMP-1) in Wistar rats with aspirin-induced ulcers and its modulation by *Tithonia diversifolia* leaf extract. MMP-1 is an enzyme involved in tissue remodeling and repair, including the extracellular matrix. The table displays the different treatment groups and their corresponding MMP-1 activity levels.

Group I: Normal Control - The MMP-1 activity in the normal control group was measured as 5.49±1.18. This value serves as a baseline or reference level for MMP-1 activity.

Group II: Negative Control - The negative control group, which represents the untreated group with aspirin-induced ulcers, exhibited significantly higher MMP-1 activity (8.42±0.70) compared to the normal control. This elevation in MMP-1 activity suggests an increased tissue remodeling and repair response due to ulcer formation.

Group III: Omeprazole (5 mg/kg) - The group treated with omeprazole, a commonly used medication for ulcers, showed a reduction in MMP-1 activity (6.89±0.70) compared to the negative control group. This suggests that omeprazole treatment may have a potential inhibitory effect on MMP-1 activity, possibly contributing to the suppression of tissue remodeling and repair.

Groups IV, V, and VI: Leaf Extract Treatment - The groups treated with different doses of *Tithonia diversifolia* leaf extract (250 mg/kg, 500 mg/kg, and 750 mg/kg) showed comparable MMP-1 activity levels (ranging from 6.29±0.41 to 6.67±0.66) compared to the omeprazole-treated group. These findings indicate that the leaf extract did not significantly affect MMP-1 activity in the same manner as omeprazole.

The results suggest that both omeprazole and *Tithonia diversifolia* leaf extract treatment have the potential to modulate MMP-1 activity in Wistar rats with aspirin-induced ulcers. Omeprazole demonstrated a significant reduction in MMP-1 activity compared to the negative control group, implying its potential inhibitory effect on tissue remodeling and repair. On the other hand, *Tithonia diversifolia* leaf extract did not exhibit a significant effect on MMP-1 activity compared to the omeprazole-treated group. Further studies may be necessary to elucidate the precise mechanisms by which *Tithonia diversifolia* leaf extract influences tissue remodeling in the context of ulcers and its potential therapeutic implications.

The effect of *T. diversifolia* leaf extract on prostaglandin (PGE_2) level of aspirin induced ulcer in Wistar rats is shown in table 3.5 below. The result

showed a significant increase ($p<0.05$) in values of PGE_2 within the pretreated groups when compared to the untreated group.

Table 6: The effect of *T. diversifolia* leaf extract on prostaglandin (PGE_2) level of aspirin induced ulcer in Wistar rats

Group	Treatment	PGE_2 Level
I	Normal Control	383.64 ± 1.21^{a}
II	Negative Control	318.70 ± 1.10^{b}
III	Omeprazole (5 mg/kg)	372.49 ± 5.15^{c}
Iv	250 mg/kg of leaf extract	358.71 ± 5.19^{c}
V	500 mg/kg of leaf extract	367.13 ± 1.92^{c}
VI	750 mg/kg of leaf extract	380.00 ± 0.89^{ac}

Values are mean ± SD for N=5. Values in the same column bearing the same letter of the alphabet are not statistically significant at ($P< 0.05$). PGE_2, Prostaglandins

Table 6 presents the effect of Tithonia diversifolia leaf extract on prostaglandin (PGE2) levels in Wistar rats with aspirin-induced ulcers. Prostaglandins are lipid compounds that play a role in inflammation and the healing process. The table displays the different treatment groups and their corresponding PGE2 levels.

Group I: Normal Control - The PGE2 level in the normal control group was measured as 383.64±1.21. This value serves as a baseline or reference level for PGE2.

Group II: Negative Control - The negative control group, representing the untreated group with aspirin-induced ulcers, showed a significantly lower PGE2 level (318.70±1.10) compared to the normal control group. This decrease in PGE2 suggests a potential disruption in the normal inflammatory response and healing process due to the ulcer formation.

Group III: Omeprazole (5 mg/kg) - The group treated with omeprazole exhibited a slightly increased PGE2 level (372.49±5.15) compared to the negative control group. This indicates that omeprazole treatment may have a partial restorative effect on PGE2 levels, possibly contributing to the resolution of inflammation and the healing process.

Groups IV, V, and VI: Leaf Extract Treatment - The groups treated with different doses of *Tithonia diversifolia* leaf extract (250 mg/kg, 500 mg/kg, and 750 mg/kg) showed PGE2 levels ranging from 358.71±5.19 to 380.00±0.89. These values indicate that the leaf extract did not significantly affect PGE2 levels compared to the negative control group.

Overall, the results suggest that *Tithonia diversifolia* leaf extract treatment did not exert a significant effect on PGE2 levels in Wistar rats with aspirin-induced ulcers. While omeprazole treatment showed a modest restoration of PGE2 levels, the leaf extract did not produce a similar effect. Further investigation is needed to better understand the

mechanisms by which *Tithonia diversifolia* leaf extract influences the inflammatory response and the healing process in the context of ulcers.

The effect of pretreatment of *T. diversifolia* on aspirin induced ulcer in Wistar rats were evaluated on tumour necrosis factor alpha (TNF-α) and Interleukin-1 Beta (IL-1β). This is shown in Table 3.7 below. The result revealed a significant difference (P<0.05) among the tested groups TNF-α and IL-1β when compared to the negative control group. However, there was a dose dependent decrease in the tested parameters as the concentration of the plant extract increases from 250 mg/kg to 750 mg/kg body weight in TNF-α and IL-1β within the pretreated groups.

Table 7: The inflammatory activity of *T. diversifolia* leaf extract on aspirin induced ulcer in Wistar rats.

Group	Treatment	TNF-α	IL-1β
I	Normal Control	120.93±2.54^{a}	21.41±2.44^{a}
II	Negative Control	138.97±7.89^{c}	26.13±2.70^{ab}
III	Omeprazole (5 mg/kg)	136.43±3.26^{bc}	22.40±4.74^{a}
Iv	250 mg/kg of leaf extract	133.20±1.10^{b}	24.10±0.22^{ab}
V	500 mg/kg of leaf extract	130.13±4.86^{b}	21.41±0.88^{a}
VI	750 mg/kg of leaf extract	128.81±3.22^{ab}	20.51±2.05^{a}

Values are mean ± SD for N=5. Values in the same column bearing the same letter of the alphabet are not statistically different at (P< 0.05). TNF-α, Tumour necrosis factor alpha; IL-1β, Interleukin-1 Beta

Table 7 provides information on the effect of pretreatment with *Tithonia diversifolia* leaf extract on aspirin-induced ulcer in Wistar rats, specifically focusing on the levels of tumor necrosis factor alpha (TNF-α) and interleukin-1 beta (IL-1β), which are markers of inflammatory activity.

Group I: Normal Control - The normal control group represents the baseline levels of TNF-α and IL-1β. The mean TNF-α level was measured as 120.93±2.54, while the IL-1β level was 21.41±2.44.

Group II: Negative Control - The negative control group, which received aspirin-induced ulcers without any treatment, showed elevated levels of TNF-α (138.97±7.89) and IL-1β (26.13±2.70) compared to the normal control group. These increased levels indicate the presence of inflammation due to the induction of ulcers.

Group III: Omeprazole (5 mg/kg) - The group treated with omeprazole, a known anti-inflammatory agent, exhibited slightly reduced levels of TNF-α (136.43±3.26) and IL-1β (22.40±4.74) compared to the negative control group. This suggests that omeprazole treatment had a mild suppressive effect on the inflammatory response.

Groups IV, V, and VI: Leaf Extract Treatment - The pretreated groups with different doses of *Tithonia diversifolia* leaf extract (250 mg/kg, 500 mg/kg, and 750 mg/kg) demonstrated dose-dependent decreases in TNF-α and

IL-1β levels. As the concentration of the leaf extract increased, the levels of TNF-α and IL-1β decreased within the pretreated groups. The reductions observed in TNF-α and IL-1β indicate that the leaf extract possesses anti-inflammatory properties.

Overall, the results suggest that pretreatment with *Tithonia diversifolia* leaf extract had a significant suppressive effect on TNF-α and IL-1β levels in Wistar rats with aspirin-induced ulcers. The dose-dependent decrease in these inflammatory markers indicates that the leaf extract possesses anti-inflammatory properties. However, it is important to note that the magnitudes of reduction in TNF-α and IL-1β levels were not as significant as those observed with omeprazole treatment.

The result of the gastro-protective effect of omeprazole and treatment at different doses of *T. diversifolia* leaf extract on aspirin induced ulcer in Wistar rats is shown below **(Table 8).** There was a significant decrease ($p<0.05$) in a dose dependent manner in the values obtained from total acidity, free acidity and pepsin activity, while pH values increased in the pretreated groups when compared the untreated groups. The ulcer percentage inhibition was found in the trend; omeprazole group > 750 mg/kg > 500 mg/kg > 250 mg/kg.

Table 8: Gastro-protective effect of omeprazole and treatment at different doses of *T. diversifolia* leaf extract on aspirin induced ulcer in Wistar rats.

Parameters			Omeprazole	*T. diversifolia* extract Dose (mg/kg)		
	Normal Control	Negative control	5 mg/kg	250	500	750
Ulcer index	0.00±0.00	7.43±0.02^{a}	0.91±0.02^{b}	4.39±0.15^{d}	3.10±0.10^{c}	1.97±0.14^{e}
% Inhibition	-	-	81.28	41.32	49.38	68.32
Total Protein	8.98±0.18^{a}	7.44±0.19^{bc}	7.01±0.16^{a}	8.21±0.21^{a}	9.19±0.20^{a}	9.44±0.58^{a}
pH	2.67±0.05^{a}	2.22±0.10^{b}	2.86±0.05^{ad}	3.30±0.17^{c}	2.86±0.09^{a}	3.12±0.05^{d}
Total acidity	9.22±0.18^{a}	15.45±0.16^{bf}	14.04±0.90^{ce}	13.13±0.02^{d}	12.14±0.01^{e}	15.06±0.05^{f}
Free acidity	4.20±0.03^{a}	6.54±0.15^{b}	3.53±0.30^{c}	3.76±0.09^{c}	3.53±0.03^{c}	3.37±0.05^{c}
Pepsin activity	101.22±0.12^{a}	139.32±2.58^{b}	122.22±2.95^{c}	125.31±1.95^{c}	121.12±2.65^{cd}	116.13±0.98^{d}

Values represent the mean ± SD for N=5. Values in the same row bearing the same letter of alphabets are not significantly different from each other ($P < 0.05$).

Table 8 presents the results of the gastro-protective effect of omeprazole and treatment with different doses of *Tithonia diversifolia* leaf extract on aspirin-induced ulcers in Wistar rats. The table includes various parameters related to gastric acid secretion, ulcer index, and percentage inhibition. Here is an explanation and discussion of the results:

Ulcer Index and Percentage Inhibition: The ulcer index represents the severity of ulcers, and the percentage inhibition indicates the effectiveness of the treatments in reducing ulcer formation. The normal

control group (without ulcers) had an ulcer index of 0.00. In the negative control group, the ulcer index was significantly increased (7.43±0.02), indicating the presence of ulcers. Treatment with omeprazole (5 mg/kg) showed a considerable decrease in the ulcer index (0.91±0.02), resulting in 81.28% inhibition. Among the *Tithonia diversifolia* leaf extract doses, the higher doses (500 mg/kg and 750 mg/kg) demonstrated better ulcer inhibition percentages (49.38% and 68.32%, respectively) compared to the lower dose (250 mg/kg).

Total Protein: Total protein levels in the gastric tissue were measured as an indicator of tissue damage. The normal control group showed a protein level of 8.98±0.18. The negative control group exhibited a decrease in total protein (7.44±0.19), indicating tissue damage caused by aspirin-induced ulcers. Treatment with omeprazole and *Tithonia diversifolia* leaf extract at different doses increased the total protein levels, suggesting a protective effect against tissue damage.

pH: The pH value reflects the acidity level in the stomach. The normal control group had a pH of 2.67±0.05, which increased in the negative control group (2.22±0.10) due to the induction of ulcers. Omeprazole treatment resulted in a higher pH (2.86±0.05), indicating reduced acidity. Similarly, treatment with *Tithonia diversifolia* leaf extract at different doses also showed increased pH levels, suggesting a reduction in gastric acidity.

Total Acidity and Free Acidity: Total acidity and free acidity are measures of gastric acid secretion. The negative control group exhibited higher levels of total acidity (15.45±0.16) and free acidity (6.54±0.15) compared to the normal control group. Treatment with omeprazole and *Tithonia diversifolia* leaf extract at different doses resulted in decreased total acidity and free acidity levels, indicating a suppression of gastric acid secretion.

Pepsin Activity: Pepsin is an enzyme involved in the digestion of proteins. The negative control group showed increased pepsin activity (139.32±2.58) compared to the normal control group (101.22±0.12). Treatment with omeprazole and *Tithonia diversifolia* leaf extract at different doses led to a reduction in pepsin activity, indicating a potential inhibitory effect on pepsin secretion.

Overall, the results demonstrate the gastro-protective effects of omeprazole and *Tithonia diversifolia* leaf extract on aspirin-induced ulcers in Wistar rats. These treatments showed a significant decrease in total acidity, free acidity, and pepsin activity, as well as an increase in pH levels. The higher doses of *Tithonia diversifolia* leaf extract exhibited better ulcer inhibition percentages compared to the lower dose. These findings suggest that *Tithonia diversifolia* leaf extract possesses gastro-protective properties, which may be attributed to its ability to reduce gastric acid secretion and protect against tissue damage.

Table 9 showed the effect of omeprazole and treatment at different doses of *T. diversifolia* leaf extract on aspirin induced ulcer in Wistar rats. There was statistical difference ($p<0.05$) observed in the haematological parameters except in basophils and eosinophils (except 750 mg/kg) when compared to the tested groups compared to the negative group. However, there was an increase in percentage neutrophil in the untreated group when compared to other groups.

Table 9: Effect of Omeprazole and treatment at different doses of *T. diversifolia* leaf extract on Haematology of aspirin induced ulcer in Wistar rats.

			Omeprazole	*T. diversifolia* Extract Dose (mg/kg)		
Parameters	Normal Control	Negative control	5 mg/kg	250	500	750
PCV (%)	58.47±1.21^{a}	64.93±0.67^{b}	55.33±3.72^{a}	55.83±0.55^{a}	56.10±1.15^{a}	52.74±2.33^{c}
Hb (mg/dl)	14.60±0.40^{a}	17.80±0.79^{b}	16.20±0.30^{a}	16.07±0.81^{a}	16.49±0.55^{c}	14.81±0.53^{a}
RBC (x1012/L)	8.50±0.26^{a}	10.18±0.33^{b}	8.77±0.38^{c}	9.27±0.31^{c}	9.31±0.18^{c}	7.99±0.79^{d}
MCV (fl)	71.80±1.78^{a}	67.30±1.10^{b}	64.13±0.70^{c}	64.93±0.64^{c}	63.97±1.14^{c}	60.40±0.82^{d}
MCH (pg)	17.93±0.75^{a}	17.40±0.85^{a}	16.97±0.59^{a}	17.37±0.75^{a}	16.97±0.59^{a}	18.27±0.86^{a}
MCHC (g/dl)	242.33±3.21^{a}	280.00±1.00^{b}	268.67±7.57^{c}	268.67±6.81^{c}	268.67±3.06^{c}	261.67±1.15^{c}
WBC (x109/L)	71.80±1.78^{a}	67.30±1.10^{b}	64.13±0.70^{c}	64.93±0.64^{cd}	63.97±1.14^{cd}	60.40±0.82^{c}
Neutrophil (%)	49.3±3.21^{a}	62.00±1.00^{b}	52.33±2.08^{c}	51.33±2.52^{c}	52.00±2.00^{c}	57.67±1.53^{d}
Lymphocyte (%)	31.67±1.53^{a}	41.33±1.53^{b}	42.33±3.51^{b}	44.67±2.52^{b}	42.00±2.52^{b}	41.33±1.53^{b}
Monocytes (%)	7.00±1.00^{a}	7.33±1.15^{a}	4.67±0.58^{b}	4.67±0.58^{b}	6.33±0.58^{a}	4.33±0.58^{b}
Eosinophil (%)	3.33±0.58^{a}	4.00±1.00^{a}	3.67±0.58^{a}	3.33±0.58^{a}	4.00±0.00^{a}	2.00±1.00^{b}
Basophil (%)	1.00±0.00^{a}	0.67±0.58^{a}	0.67±0.58^{a}	0.67±0.58^{a}	0.67±0.58^{a}	0.33±0.58^{a}
Platelet (x10^{9}/L)	520.33±10.50^{a}	788.67±4.16^{b}	384.67±5.03^{c}	861.33±10.02^{b}	775.67±3.79^{b}	807.67±8.08^{b}

Values represent the mean ± SD for N=5. Values in the same row bearing the same alphabets are not significantly different from each other ($P < 0.05$). PCV: Packed cell volume; HB: haemoglobin; RBC: red blood cells; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; WBC: white blood cell.

Table 9 presents the effect of omeprazole and treatment with different doses of *Tithonia diversifolia* leaf extract on hematological parameters in Wistar rats with aspirin-induced ulcers. The table 9 shows that there were statistically significant differences ($p<0.05$) in several hematological parameters compared to the negative control group. However, the differences in basophils and eosinophils were not significant, except for the 750 mg/kg dose of *Tithonia diversifolia* extract.

PCV (Packed Cell Volume): The normal control group had a PCV value of 58.47±1.21. The negative control group showed an increased PCV value (64.93±0.67), indicating a possible response to inflammation caused by aspirin-induced ulcers. Treatment with omeprazole and *Tithonia diversifolia* leaf extract at different doses resulted in a decrease in PCV values, suggesting a reduction in the inflammatory response.

Hb (Hemoglobin): Similar to the PCV, the negative control group exhibited higher Hb levels (17.80±0.79) compared to the normal control group (14.60±0.40). Treatment with omeprazole and *Tithonia diversifolia* leaf extract at different doses showed a decrease in Hb levels, indicating a potential reduction in the severity of inflammation.

RBC (Red Blood Cells): The negative control group had a higher RBC count (10.18±0.33) compared to the normal control group (8.50±0.26). Treatment with omeprazole and *Tithonia diversifolia* leaf extract at different doses resulted in a decrease in RBC count, suggesting a possible normalization of the red blood cell population.

MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Hemoglobin), and MCHC (Mean Corpuscular Hemoglobin Concentration): The negative control group showed lower MCV, MCH, and MCHC values compared to the normal control group. Treatment with omeprazole and *Tithonia diversifolia* leaf extract at different doses resulted in varying effects on these parameters, but the values were generally closer to those of the normal control group, indicating a potential improvement in red blood cell characteristics.

WBC (White Blood Cells): The negative control group exhibited a slightly lower WBC count (67.30±1.10) compared to the normal control group (71.80±1.78), but the difference was not statistically significant. Treatment with omeprazole and *Tithonia diversifolia* leaf extract at different doses did not show consistent effects on the WBC count.

Neutrophil, Lymphocyte, Monocyte, Eosinophil, and Basophil: The negative control group showed higher percentages of neutrophils compared to the normal control group, indicating an increased immune response to inflammation. Treatment with omeprazole and *Tithonia diversifolia* leaf extract at different doses resulted in variable effects on these immune cell percentages, but there were no significant differences, except for eosinophil (750 mg/kg dose) and basophil.

Platelet: The negative control group had a higher platelet count (788.67±4.16) compared to the normal control group (520.33±10.50). Treatment with omeprazole and *Tithonia diversifolia* leaf extract at different doses showed variable effects on platelet count, but the values were generally higher compared to the normal control group, indicating a possible normalization of platelet levels.

Overall, the results indicate that treatment with omeprazole and *Tithonia diversifolia* leaf extract at different doses had varying effects on hematological parameters in rats with aspirin-induced ulcers. These treatments showed potential to modulate the inflammatory response, normalize red blood cell characteristics, and influence platelet count.

The result (plate I) showed gastric tissue fundic-type mucosa consisting of tightly packed fundic (oxyntic) glands occupying approximately 80% of the

mucosal thickness and superficial 20% consisting of foveolar cells that are tall and columnar. The intervening stroma is thinly fibrocollagenous and is sparsely infiltrated by mononuclear inflammatory cells, predominantly lymphocytes. The muscularis mucosa is of normal thickness.

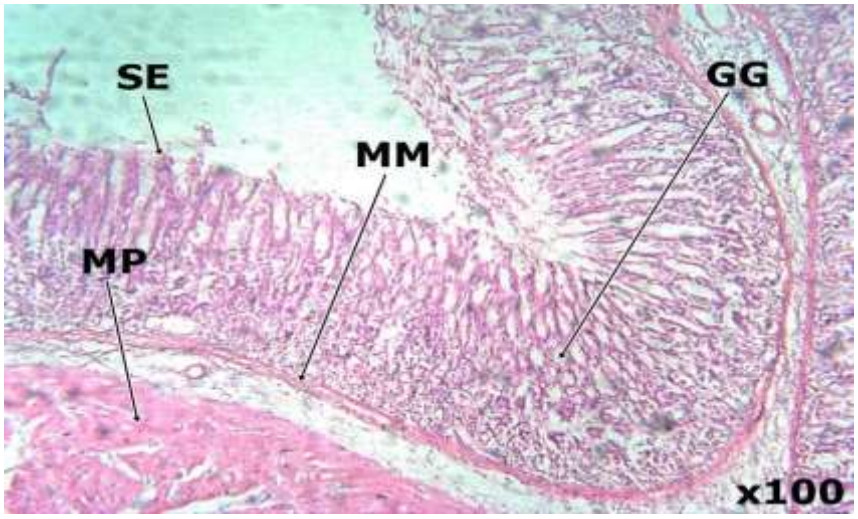


Plate I: Photomicrograph of gastric mucosa of normal control Wistar rat. GG=gastric glands; MM=muscularis mucosa, MP = muscularis propria; SE: surface epithelium

The result shown below (plate II) revealed the presence of ulceration of surface epithelium when compared to plate I. The intervening stroma is thinly fibro collagenous and is infiltrated by florid population of mononuclear inflammatory cells, predominantly lymphocytes

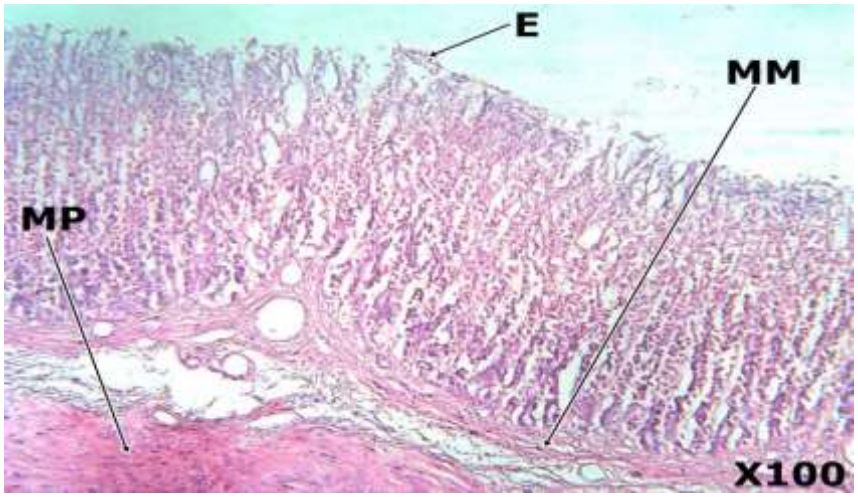


Plate II: Photomicrograph of gastric mucosa of negative control Wistar rat. E= area of erosion; GG=gastric glands; MM=muscularis mucosa, MP = muscularis propria; SE: surface epithelium

The result below when compared to negative control, there is a residual area of ulceration as well as significant mucosal surface epithelial restoration. The intervening stroma is thinly fibro collagenous and is infiltrated by a moderate population of mononuclear inflammatory cells, predominantly lymphocytes.

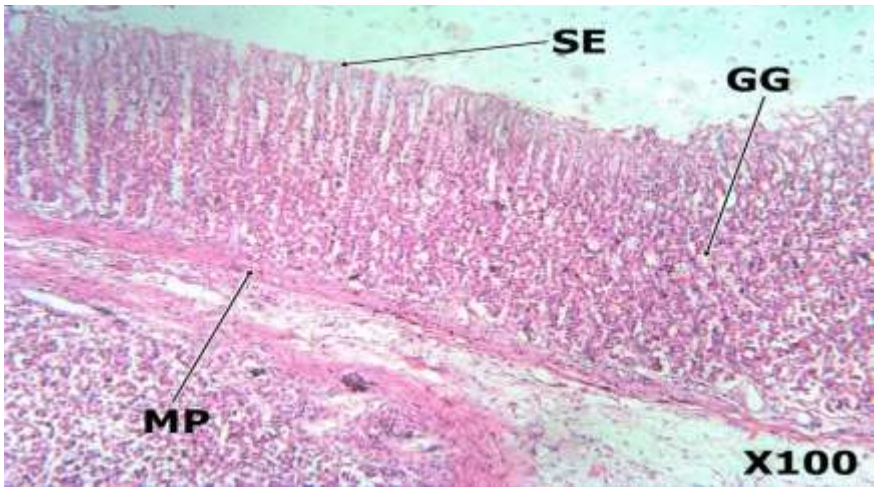


Plate III: Photomicrograph of gastric mucosa of omeprazole (5 mg/kg) pretreated Wistar rat. GG=gastric glands; MP = muscularis propria; SE: surface epithelium

The result below when compared to negative control showed a residual area of ulceration as well as significant mucosal surface epithelial restoration. The intervening stroma is thinly fibro collagenous and is infiltrated by a moderate population of mononuclear inflammatory cells, predominantly lymphocytes.

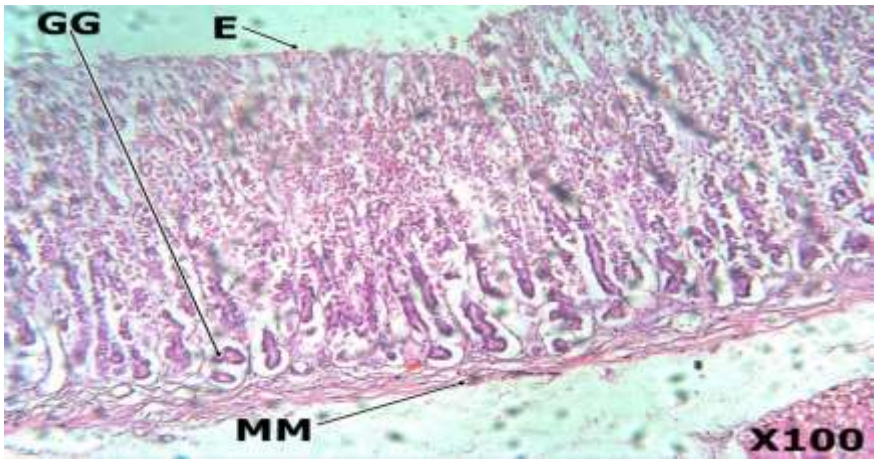


Plate IV: Photomicrograph of gastric mucosa of 250 mg/kg *Tithoniadiversifolia* pretreated Wistar rat. E= area of erosion; GG=gastric glands; MM=muscularis mucosa.

The result below (plate V) when compared to negative control, revealed a mild mucosal surface epithelial restoration. The intervening stroma is thinly fibro collagenous and is infiltrated by a mild population of mononuclear inflammatory cells, predominantly lymphocytes.

Plate V: Photomicrograph of gastric mucosa of 500 mg/kg *Tithoniadiversifolia* pretreated Wistar rat. GG=gastric glands; MM=muscularis mucosa, MP = muscularis propria; SE: surface epithelium.

The result below (plate VI) when compared to negative control, revealed also a mild mucosal surface epithelial restoration. The intervening stroma is thinly fibro collagenous and is infiltrated by a mild population of mononuclear inflammatory cells, predominantly lymphocytes.

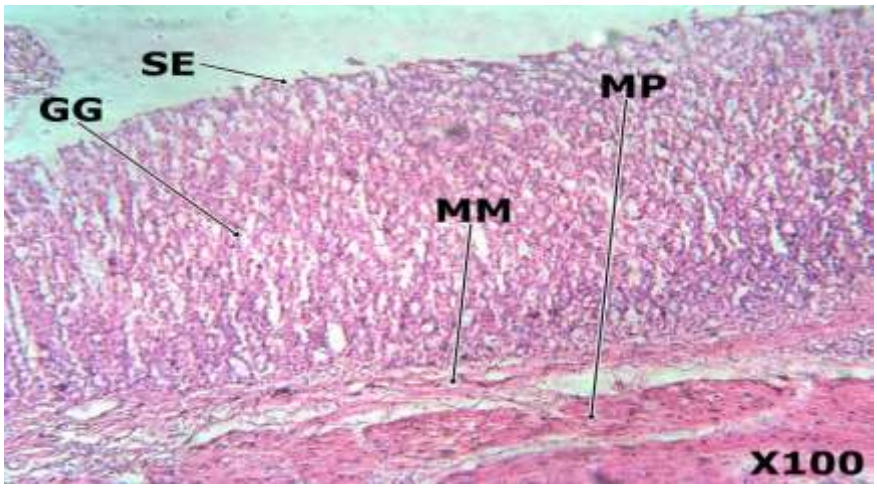


Plate VI: Photomicrograph of gastric mucosa of 750 mg/kg *Tithoniadiversifolia* pretreated Wistar rat. GG=gastric glands; MM=muscularis mucosa, MP = muscularis propria; SE: surface epithelium.

Discussion

The present study evaluated thephytochemical composition of *T. diversifolia* leaf extract. Twenty-eight chemical constituents were identified, with benzyl alcohol and p-hydroxy-alpha-[(methylamino)methyl] having the highest retention time (16.892) and hexadecane having the lowest (7.061). One of the bioactive compounds identified was n-hexadecanoic acid, which has been reported to possess various properties, including lubricant, anti-androgenic flavor, hypocholesterolemic, hemolytic, antioxidant, and 5-alpha reductase inhibitor activity (Dhanalakshmi & Manavalan, 2014), as well as antibacterial and antifungal properties (Chandrasekaran *et al.,* 2011). Sesquiterpene lactones, diterpenes, caffeoylquinic acid derivatives, and flavonoids are other common natural products found in the aerial parts of *T. diversifolia,* known for their biological activities (Chagas-Paula *et al.,* 2012; Liao *et al*., 2011; Lin, 2012; Zhao *et al*., 2012).

Furthermore, FT-IR analysis revealed the presence of functional groups such as primary amines, arenes, aldehydes, ketones, alcohols, phenols, and alkanes in the extract. These functional groups could be responsible for the ethnomedicinal properties previously associated with *T. diversifolia* (Liao *et al*., 2011; Chagas-Paula *et al.,* 2011).

The acute toxicity testing conducted on *T. diversifolia* leaf extract revealed no instances of mortality, even at the highest tested dose of 5,000 mg/kg. This finding strongly indicates the safety of the extract for oral usage (Zbinden & Flury-Roversi, 1981). The absence of any deaths, even at the maximum administered dose, led to the conclusion that the T. diversifolia leaf extract is generally safe for oral consumption based on the results of the acute toxicity analysis conducted in the current study.

Furthermore, considering the lack of mortality observed and the high LD_{50} (lethal dose for 50% of the population) estimated to be above 5,000 mg/kg, it can be inferred that the extract is non-toxic and poses minimal risk to the subjects. These findings provide reassurance regarding the safety of utilizing *T. diversifolia* leaf extract. Additionally, the study results suggest that the *T. diversifolia* leaf extract exhibits gastroprotective properties against aspirin-induced ulcers in Wistar rats. The extract's ability to protect the stomach lining from ulceration caused by aspirin highlights its potential therapeutic value in managing such conditions. It is important to note that while these findings are promising, further research and evaluation are necessary to fully understand the extract's safety and efficacy profile. Additionally, it is always advisable to refer to actual scientific studies and consult with healthcare professionals for accurate and up-to-date information.

The gastroprotective study evaluated various parameters, including phase II enzymes, antioxidant and inflammatory activities, ulcerative indexes, pepsin, gastric acid, free acidity, and total acidity. Aspirin is

known for its gastric toxicity, often leading to gastric ulcers and hemorrhage, making it a suitable model for studying cytoprotective activity (Nilesh *et al.,* 2009). The reference drug omeprazole, a proton pump inhibitor, was used in the study. Omeprazole effectively suppresses gastric acid secretion but is not without side effects, such as hypergastrinemia (Hoogerwerf, 2001). *Tithonia diversifolia* leaf extract was evaluated as an alternative with potential anti-ulcerogenic activity.

The evaluation of phase II enzymes and enzymic antioxidants showed a significant reduction in glutathione, thioredoxin reductase, nitric oxide synthase, myeloperoxidase, quinone oxidoreductase, matrix metalloproteinase, superoxide dismutase, malondialdehyde levels in the omeprazole and *T. diversifolia* treated groups compared to the untreated group (Odashima *et al*., 2006). These findings suggest that *T. diversifolia* leaf extract may inhibit the stimuli responsible for aspirin-induced gastric injury early after administration.

Tithonia diversifolia leaf extract exhibited a diverse phytochemical composition and demonstrated gastroprotective effects against aspirin-induced ulcers in Wistar rats. The extract contained bioactive compounds with various properties, and its administration reduced oxidative stress and inflammation markers. These findings support the potential of *T. diversifolia* as a cost-effective and safe alternative for treating gastric ulcers. Further research is warranted to explore its mechanism of action and evaluate its efficacy in clinical settings.

Prostaglandins, tumor necrosis factor-alpha (TNF-α), and interleukin-1 beta (IL-1β) were assessed as markers of gastric mucosal activity. Aspirin and other non-steroidal anti-inflammatory drugs inhibit the biosynthesis of prostaglandins, which are critical protective factors against gastric acid irritation (Burke *et al.,* 2006). Prostaglandin inhibition leads to early damage to the mucosal cells, reduced mucosal blood flow, decreased mucus and bicarbonate secretion, and increased acid secretion, ultimately resulting in ulcer formation (Rajkapoor *et al*., 2002).

TNF-α and IL-1β are pro-inflammatory cytokines known to play a role in gastric mucosal injury caused by NSAIDs (Odashi *et al*., 2006). They can, however, also have adverse effects, including fever, inflammation, tissue damage, shock, or even death. (Wang *et al*., 2011). Antibodies against TNF-α have shown promise in reducing gastric mucosal lesions (Hamaguchi *et al*., 2001). Studies have indicated that prostaglandin E_2 (PGE2) protects against gastric mucosal damage induced by aspirin in humans and animals (Odashi et al., 2006).

The pretreatment with *T. diversifolia* extract and omeprazole increased PGE2 production in Wistar rats compared to the negative control group. This suggests that the protective effect of *T. diversifolia* extract depends on gastric mucosal prostaglandin synthesis, as the extract increased gastric mucosal prostaglandin concentration. This finding is inconsistent

with a study by Odashi *et al*. (2006), which reported that the protective effect of ATL-146e, a compound they investigated, was not dependent on gastric mucosal prostaglandin synthesis.

Aspirin-induced inhibition of prostaglandins was associated with early damage to mucosal cells, reduced mucosal blood flow, decreased mucus and bicarbonate secretion, and increased acid secretion (Rajkapoor *et al.,* 2002). However, *T. diversifolia* extract showed significant protection against aspirin-induced gastric mucosal damage, as indicated by reduced lesion values compared to the negative control group. Although the percentage inhibition was higher in the omeprazole pretreated group (81.28%) compared to the *T. diversifolia* extract groups, the extract still exhibited a dose-dependent protective effect. The phytochemical composition of *T. diversifolia,* including flavonoids, saponins, alkaloids, and sesquiterpenes, may contribute to its anti-ulcer properties (Chagas-Paula *et al*., 2012). Flavonoids, in particular, have been reported to prevent gastric ulcers by increasing neutral glycoproteins and prostaglandin concentrations, inhibiting histamine secretion, and reducing pepsin secretion and activity (Okokon *et al.,* 2009; Kishore *et al*., 2011; Nguelefack *et al.,* 2005; Borika *et al*., 2009).

Pretreatment with *T. diversifolia* extract showed a dose-dependent gastroprotective effect against aspirin-induced ulcers in Wistar rats. The extract increased gastric mucosal prostaglandin concentration and exhibited anti-ulcer properties potentially attributed to its phytochemical composition. Further research is needed to elucidate the specific mechanisms of action and explore the potential therapeutic applications of *T. diversifolia* in preventing gastric ulcers.

Table 7 presents the results of the pretreatment of *T. diversifolia* leaf extract on aspirin-induced ulcers in Wistar rats, focusing on hematological parameters. The study found a statistical difference ($p<0.05$) in the hematological parameters. Interestingly, the group that received only aspirin exhibited increased levels of neutrophils compared to the pretreated groups. Neutrophils are the first leukocytes to be recruited to an inflammatory site and play diverse roles in infection, inflammation, and cancer immunology (Borregaard, 2010; Galli *etal*., 2011; Futosi *et al*., 2013). A pro-inflammatory cytokine, TNF, has been shown to increase neutrophil adhesion by causing endothelial cells and neutrophils to produce and express adhesion molecules. Considering these results, it is possible to speculate that *T. diversifolia* leaf extract reduces aspirin-induced neutrophil accumulation by preventing the creation of pro-inflammatory cytokines. The study also demonstrated that pretreatment with *T. diversifolia* leaf extract inhibited the increase in TNF-α and IL-1β concentration in the gastric mucosa following aspirin administration.

The study's histopathological analysis of gastric mucosa showed differences in experimental groups compared to the negative control group. The normal control had densely clustered fundic glands, a

superficial layer of columnar foveolar cells, and a sparsely fibrocollagenous stroma. The negative control had surface epithelium ulcerations and a thinly fibrocollagenous stroma with a populous population of mononuclear inflammatory cells, mostly lymphocytes.In contrast to the negative control group, there was a residual region of ulceration in the omeprazole pretreatment group (5 mg/kg). There was also a considerable repair of the mucosal surface epithelium.A modest number of mononuclear inflammatory cells, primarily lymphocytes, could be seen in the sparsely fibrocollagenous intervening stroma.Plate IV (250 mg/kg *Tithonia diversifolia* pretreatment): In comparison to the negative control group, the pretreatment group had a residual region of ulceration and a sizable amount of mucosal surface epithelial repair.An average number of lymphocytes and sparsely fibrocollagenous, mononuclear inflammatory cells were seen in the intervening stroma.

Plate V showed mild mucosal surface epithelial restoration with 500 mg/kg *Tithonia diversifolia* extract, compared to the negative control group. The intervening stroma was thinly fibrocollagenous and had a mild population of mononuclear inflammatory cells, primarily lymphocytes. These histopathological findings indicate that pretreatment with *Tithonia diversifolia* extract, particularly at higher doses (500 mg/kg and 750 mg/kg), led to a restoration of the gastric mucosa and a reduction in ulceration. The extract appeared to have a beneficial effect on the mucosal surface epithelium and inflammation in the experimental rats.The histopathological analysis provided additional evidence supporting the biochemical and compositional observations (Eroschendo, 2000). The negative control group exhibited surface epithelium ulceration. In contrast, the groups pretreated with omeprazole and 250 mg/kg of *T. diversifolia* leaf extract showed residual ulceration areas and significant restoration of mucosal surface epithelium. The groups receiving 500 mg/kg and 750 mg/kg of *T. diversifolia* leaf extract showed mild restoration of mucosal surface epithelium.

Conclusion

In conclusion, the present study evaluated the phytochemical composition of *Tithonia diversifolia* leaf extract and its gastroprotective effects against aspirin-induced ulcers in Wistar rats. The extract contained various bioactive compounds, including n-hexadecanoic acid, sesquiterpene lactones, diterpenes, caffeoylquinic acid derivatives, and flavonoids. FT-IR analysis confirmed the presence of functional groups associated with ethnomedicinal properties. Acute toxicity testing demonstrated the safety of the extract for oral consumption.

The study revealed that *T. diversifolia* leaf extract exhibited gastroprotective effects by reducing oxidative stress, inflammation, and markers of gastric mucosal activity. The extract increased gastric mucosal prostaglandin concentration, protecting against aspirin-induced gastric

injury. The extract's anti-ulcer properties were attributed to its diverse phytochemical composition, including flavonoids, saponins, alkaloids, and sesquiterpenes.

Furthermore, pretreatment with *T. diversifolia* leaf extract inhibited the increase in neutrophil accumulation, TNF-α, and IL-1β concentration in the gastric mucosa following aspirin administration. These findings suggest that the extract may prevent the creation of pro-inflammatory cytokines and reduce neutrophil adhesion.

Histopathological analysis supported the biochemical observations, showing a restoration of the gastric mucosa and a reduction in ulceration with *T. diversifolia* leaf extract pretreatment. Higher doses of the extract demonstrated a more significant restoration of the mucosal surface epithelium.

Overall, *T. diversifolia* leaf extract exhibited gastroprotective effects against aspirin-induced ulcers, potentially making it a cost-effective and safe alternative for treating gastric ulcers. However, further research is required to elucidate its specific mechanisms of action and evaluate its efficacy in clinical settings. It is important to consult healthcare professionals and refer to scientific studies for accurate and up-to-date information.

Recommendation

Further studies are needed better to understand the molecular modes of action of *T. diversifolia* and determine its toxicity and activity in co-cultures with immunologic cells and *in vivo* models.

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Data availability statement

We are devoted to supporting open science practises and transparency in government. We want to help the scientific community and promote more study and collaboration in the field by sharing our data. Please be aware that any use of the data must comply with all applicable ethical and legal rules and must be supported by proper citation and author attribution.

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