Section A-Research paper

Therapeutic Effects of Chromolaena odorata Leaf Extract on Aspirin-Induced Gastric Ulcers in Rat Models

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Abstract

The present study investigated the therapeutic effects of Chromolaena odorata leaf extract on aspirin-induced gastric ulcers in rat models. The phytochemical constituents of the extract were analyzed using GC-MS, and its functional groups were studied using FTIR. Acute toxicity tests were performed to assess its safety profile. Albino rats were divided into six groups, receiving different pre-treatments before ulcer induction, while a separate set of rats underwent acute toxicity testing. After ulcer induction, various physiological and biochemical parameters were evaluated to assess the extract's impact. Enzyme activity, pro-inflammatory cytokines, hematological parameters, and serum total protein were also analyzed. Histological examination provided insights into the extract's effects on ulcer healing. The results indicated low toxicity at lower doses of the extract but mild toxicity at higher doses. The extract exhibited dose-dependent inhibition of ulcers, with the highest dose showing the greatest effect. It also regulated various parameters related to gastric mucosal health, antioxidant enzyme expression, and inflammation. Histological examination revealed varying degrees of restoration and inflammation, suggesting the extract's potential in promoting epithelial restoration. Overall, the study suggests that Chromolaena odorata leaf extract has therapeutic potential in managing gastric ulcers by promoting tissue repair, reducing inflammation, and inhibiting ulcer formation. Further research is needed to explore the underlying mechanisms and validate its efficacy in clinical settings.

Key words: Chromolaena odorata, Peptic ulcers, Phytochemicals, Pro-inflammatory, prostaglandins

1.0 INTRODUCTION

Peptic ulcers, affecting the gastric and intestinal walls, are a prevalent pathological condition, affecting about 10 to 15% of the population (Soll *et al.*, 1990). They result from an imbalance between aggressive factors (e.g., HCL, pepsin, bile, reactive oxygen species) and cyto-protective mechanisms (e.g., prostaglandins, nitric oxide) in the stomach and intestines Robert *et al*., 1983

Various factors, such as stress, alcohol consumption, smoking, Helicobacter pylori infection, and Nonsteroidal Anti-inflammatory Drugs (NSAIDs) use, can cause peptic ulcers (Malfertheiner *et al*., 2009)

In traditional medicine, plants have been used to treat various ailments, including peptic ulcers, due to their bioactive compounds like flavonoids, alkaloids, terpenoids, and phenolic acids, contributing to their therapeutic effects (Patwardhan *et al*., 2005).

Chromolaena odorata, a large multi-stemmed perennial flowering shrub of the Asteraceae family, is native to South and Central America (Holm *et al*., 1979). Introduced to Nigeria in the 1930s, it has since spread throughout West and Central Africa. The plant grows up to 2.5m in open areas but can reach up to 10m in shady conditions when it behaves as a creeping plant. Its leaves are hairy and glandular, emitting a pungent aromatic odor when crushed (Holm *et al*., 1979). C. odorata regenerates through its hairy seeds, which can be dispersed by wind or clinging to fur, clothes, and machinery (Holm *et al*., 1979). The plant is characterized by triangular leaves with serrated edges and produces white or pale pink flowers in clusters at the ends of branches (Odugbemi., 2008). It is commonly found in open fields and farms in southeastern Nigeria.

Chromolaena odorata has been traditionally used as a medicinal plant for wound healing in Nigeria and Thailand. Its leaves are crushed and applied to treat skin wounds, and it has been used in traditional medicine to alleviate intestinal pains, coughs, colds, diabetes, and other ailments(Odugbemi., 2008). The plant's therapeutic properties have been attributed to the presence of phyto-compounds such as chromomoric acid, which induces the transcription factor NFE2L2 (NrF2), responsible for defensive, anti-inflammatory, and detoxifying functions (Odugbemi., 2008). The aim of the present study was to evaluate the potential therapeutic effects of Chromolaena odorata leaf extract on aspirin-induced gastric ulcers in rat models. This investigation sought to explore the extract's pharmacological properties and understand its underlying mechanisms by evaluating its phytochemical constituents using Gas Chromatography-Mass Spectrometry (GC-MS) and analyzing its functional groups using Fourier Transform Infrared Spectroscopy (FTIR). By conducting these analyses, the study aimed to provide valuable insights into the extract's composition and its potential benefits in managing

gastric ulcers, which could pave the way for future research on natural and alternative treatments for this prevalent gastrointestinal condition.

2.0 MATERIALS AND METHODS

2.1 Collection and Identification of Plant

Leaves of Chromolaena odorata were collected from a bush in Okigwe, Imo state, Nigeria. The plant was identified and authenticated by a taxonomist at the Department of Plant Science and Biotechnology, Abia State University, Uturu.

2.2 Sample Preparation

The collected leaves were thoroughly washed with clean water to remove dust and earthy materials, and then air-dried under shade. Once dried, the leaves were ground into a fine powder using a mechanical homogenizer and stored in an airtight container for further use.

2.3 Preparation of Plant Extract

Fifty grams (50g) of the powdered leaf sample were subjected to extraction using a soxhlet extractor with chloroform as the solvent. The extraction was carried out at a temperature of 70°C for 48 hours. The resulting extract in solution was then dried at a low temperature (40°C) in a hot air oven, yielding a dark green solid extract weighing 2.1 grams, representing a percentage yield of 4.2%. The extraction process was repeated three more times to obtain sufficient extract for the experiment. The obtained extract was preserved at low temperature in a refrigerator until required for use.

2.4 Phytochemical Studies

The freshly-prepared crude extract was subjected to qualitative analysis for the presence of phytochemical constituents using Gas Chromatography-Mass Spectrometry (GC-MS) and Fourier Transform Infrared (FTIR) spectroscopy.

2.4.1 GC-MS Analysis

For GC-MS analysis, 3 grams of the extract sample powder was dissolved in chloroform solvent. The analysis was performed using a GC-MS-QP 2010 SHIMADZU instrument. The column oven and injector temperatures were set at 80°C and 200°C, respectively. A split injection mode with a split ratio of 20 was used, and helium gas with 99.9995% purity was employed as the carrier gas at a flow rate of 1.46 ml/min. The temperature program for the column oven was set at 80°C with a 2-minute hold time, followed by a gradual increase to 300°C with a 10-minute hold time. The mass-to-charge (m/z) ratio ranged from 40 to 700.

2.4.2 FT-IR Analysis

For FT-IR analysis, 3 grams of dried leaf powder was mixed with 10 mg of FTIR-grade KBr. The mixture was compressed to form a pellet, which was then analyzed using a SHIMADZU FTIR spectrometer.The experimental procedures for phytochemical studies aimed to identify and characterize the chemical constituents present in the Chromolaena odorata leaf extract, providing valuable information for further investigation of its potential therapeutic properties.

Laboratory Animals and Experimental Design

Albino rats, aged 8 weeks and weighing between 130-140g, were used for this study. The rats were housed in the animal facility at the Department of Biochemistry, Abia State University, Uturu. They were allowed to acclimatize for 14 days (2 weeks) under standard laboratory conditions with access to commercial feed and water ad libitum. All animal experiments were conducted following the NIH guidelines for the care and use of laboratory animals, as stipulated by the Organization for Economic Cooperation and Development (OECD) in 2001.

After the acclimatization period, the rats were divided into six (6) groups, with 5 animals in each group. The acute toxicity test was performed on 12 albino rats using Lorke's method. The plant extract was prepared by dissolving 5g of the extract in 0.03ml of Tween 80, and then made up to 50ml with distilled water, resulting in a stock concentration of 0.1g/ml (100mg/ml).

The administration of the extract was calculated using the formula:

Volume from stock = (Dose × body weight) / (Concentration × 1000)

The groups were treated as follows:

Group 1: Positive control rats received distilled water with 0.03ml of Tween 80.

Group 2: Negative control rats received only 1000mg/kg body weight aspirin.

Group 3: Rats pre-treated with 20mg/kg body weight standard drug compound (Omeprazole) before ulcer induction with aspirin.

Group 4: Rats pre-treated with 250mg/kg body weight of leaf extract before ulcer induction using aspirin.

Group 5: Rats pre-treated with 500mg/kg body weight of leaf extract before ulcer induction using aspirin.

Group 6: Rats pre-treated with 750mg/kg body weight of leaf extract of Chromolaena odorata before ulcer induction using aspirin.

The acute toxicity test (Median Lethal Dose, LD50) was carried out in two phases. In phase 1, nine animals were divided into three groups and administered doses of 10, 100, and 1000 mg/kg of the test substance, while phase 2 involved three animals in three groups receiving higher doses of 2000, 4000, and 5000mg/kg.

After the acclimatization period, rats in groups 2 to 6 were starved for 48 hours but had access to clean water. Groups 4, 5, and 6 were pre-treated with 250, 450, and 750mg/kg body weight of leaf extract, respectively. Group 3 rats were pre-treated with 20 mg/kg body weight of Omeprazole as the standard drug. After 30 minutes, 1000mg/kg body weight of aspirin was orally administered to rats in groups 2 to 6 using a gavage tube.

Four hours after administering aspirin, the animals were anesthetized using chloroform and sacrificed. Blood samples were collected by cardiac puncture, some in EDTA bottles and others in clot tubes for biochemical analysis. Tissues from the duodenum and stomach were collected and preserved in 10% saline. Tissues for histological analysis were preserved in 10% formalin solution. Gastric juice was collected by excising the stomach and opening it along the greater curvature to enhance collection.

The physicochemical evaluation included the measurement of the ulcer index, determined using Main and Wittle's method, as well as the estimation of free acidity, total acidity, and pepsin

activity based on the methods proposed by Lawrie et al. and Gary and Billings, respectively. Assay reagents were purchased from Randox Laboratories Ltd.

2.10 ESTIMATION OF COX-1- CYCLOOXYGENASE 1 CONCENTRATION

Cox-1 levels were quantified using an ELISA based method (Zhangand- An, 2007). The test kits were purchased from Elabscience and the procedure and instruction given on kit was followed.

2.11 PROSTAGLANDIN ENDOPEROXIDE (PGE) DETERMINATION

PGE2 levels where quantified by using an ELISA based method(Zhangand- An, 2007). The test kit were purchased from Elabscience and the instructions stated on kit was followed

2.12 Antioxidant Assay

2.12.1 Myeloperoxidase Activity

Myeloperoxidase activity was determined following the method described by Bradley *et al.* (1982). Pre-weighed tissue samples were homogenized (1:10 w/v) in 50mM potassium phosphate buffer (pH 6.0) containing 0.5% hexadecyl trimethyl ammonium bromide and sonicated in an ice bath for 20 seconds. Three freeze cycles were performed, followed by sonication. The supernatant obtained after centrifugation at 1700g for 5 minutes at 4°C was used to assay myeloperoxidase activity. The assay involved mixing 0.1ml of supernatant with 2.9ml of 50mM potassium phosphate buffer containing 0.167g/L of dianisidine dihydrochloride and 0.0005% hydrogen peroxide (H2O2). The change in absorbance at 460nm was measured using a UV-visible spectrophotometer.

2.12.2 Superoxide Dismutase (SOD) Activity

SOD activity was determined following the method of Marklund and Marklund (1974). The assay is based on the inhibition of nitroblue tetrazolium (NBT) reduction. The reaction mixture

consisted of phosphate buffer (pH 7.8), methionine, NBT, riboflavin, and serum in a total volume of 3ml. Illumination of the solution in a 10ml beaker was carried out in an aluminum foil-lined box using a 15W fluorescent lamp for 10 minutes. The absorbance was measured at 560nm, and one unit of SOD activity was defined as the amount of enzyme required to inhibit NBT reduction by 50% under the specified conditions.

2.12.3 Malondialdehyde (MDA) Activity

The concentration of malondialdehyde (MDA) was determined spectrophotometrically using the method described by Buege and Aust (1978). MDA reacts with thiobarbituric acid (TBA) to form a red or pink colored complex that absorbs maximally at 532nm. In this assay, 0.1ml of serum was mixed with water, trichloroacetic acid (TCA), and thiobarbituric acid (TBA) in sodium hydroxide solution. The mixture was then boiled, cooled, and treated with sodium dodecyl sulfate (SDS). The absorbance at 532nm was measured against the blank without the enzyme source.

2.13 Inducible Nitric Oxide Synthase (iNOS) Activity

The activity of inducible nitric oxide synthase (iNOS) was determined using an ELISA-based method following the instructions provided by the manufacturer (Zhang and An, 2007). The ELISA kit for iNOS was purchased from Elabscience.

2.14 Phase II Enzyme Activities

2.14.1 Glutathione (GSH)

The level of glutathione (GSH) was analyzed using the method documented by Rahman et al. (2007).

2.14.2 Glutathione S-Transferases (GST)

GST activity was determined according to the method described by Keen et al. (1976). The supernatant of tissue homogenate was used as the source of GST, and 1-chloro 2, 4-

dinitrobenzene (CDNB) was added as the substrate. The test principle was based on the activity of cytosolic GST in the homogenate, acting to transfer GSH to the substrate CDNB in vitro.

2.14.3 THIOREDOXIN REDUCTASE (TrxR)

This was determined by the method described by Hill *et al.,* (1997). The measurement of TrxR activity was based on the NADPH dependent reduction of 5.5-dithiobis (2-nitrobenzoic acid) (DTNB). The activity was corrected for non TrxR dependent DTNB reduction by inhibiting the enzyme TrxR using auranofin. Tissue homogenate was incubated with auranofin (5µm) for 20 minutes at 37^{o}C.

2.14.4 NADPH QUINONE OXIDOREDUCTASE (QR)

QR activity was measured by the method described by Gerhauser *et al.,* (1997). Test principle is based on kinectics of NADPH dependent manadiol reduction of 3- (4, 5- dimethyl -2-thiazyl) -2, 5-dipenyl-2H-tetrazolium bromide (MTT). NADPH is a coenzyme for g-6-p dehydrogenase (glucose -6-phosphate dehydrogenase) and this made part of reaction mixture.

2.15 MATRIX METALLOPROTEINASE (MMP) ACTIVITY DETERMINATION

Matrix metalloproteinase 1 (MMP1) was analyzed by an ELISA based method, following the instructions included in kit manual(Zhangand An, 2007). The kit was purchased from Elabscience.

2.16 PRO-INFLAMMATORY CYTOKINE DETERMINATION

2.16.1 TUMOR NECROSIS FACTOR-ALPHA (TNF -$\propto$)

An ELISA based method was used for the assay(Zhangand An, 2007). Test kit was purchased from Elascience, and the procedure as stated by the manufacturer followed strictly.

2.16.2 INTERLEUKIN I BETA (IL-Iβ) ACTIVITY

An ELISA based method was employed for the assay(Zhangand An, 2007). This kit was purchased from Elabscience and the instructions stated on kit manual adhered to strictly.

2.17 SERUM TOTAL PROTEIN DETERMINATION: Methods as stated on test kits were used. Commercial kit was obtained from Randox Laboratories limited UK and the standard protocols given were adhered to strictly. Three test tubes were set up labeled test, blank and standard. 1.0ml of the total protein reagent was introduced into the test tube labeled test and standard. The same volume of distilled water was introduced into test tube labeled blank 20 µl of the test sample (serum) was introduced into the test tube labeled test. The mixture were incubated at 20-25^{o}C for 30 minutes and absorbance of the test and standard read on the spectrophotometer after zeroing with the blank at 560nm. Total protein content of sample was obtained using the formula below

Total protein =absorbance of the test x concentration of standard

Where the concentration of standard =5.95mg/dl

2.18 HEMATOLOGICAL PARAMETERS

Whole blood was used to check of haemoglobin level, packed cell volume, platelets count, red blood cell and white blood cell differentials including Neutrophil, Eosinophil, Basophil, Lymphocyte and monocyte. Also the erythrocyte and leucocyte level were checked. An analyzer was used for the analysis.

2.18 MORPHOLOGICAL AND HISTOLOGICAL EVALUATION

This was assayed according to the method described by Dakar *et al*., (2015). The stomach was dissected out and weighed and was subsequently transferred to 4% formalin solution for fixation and later processed for histopathological studies following the standard procedure. The microtome sections were cut processed and stained with haematoxylin. The section cut obtained was scanned to Trinocular Carlzeiss microscope (Germany) under different magnifications. Changes in the cytoarchitecture were noticed.

3.0 RESULTS

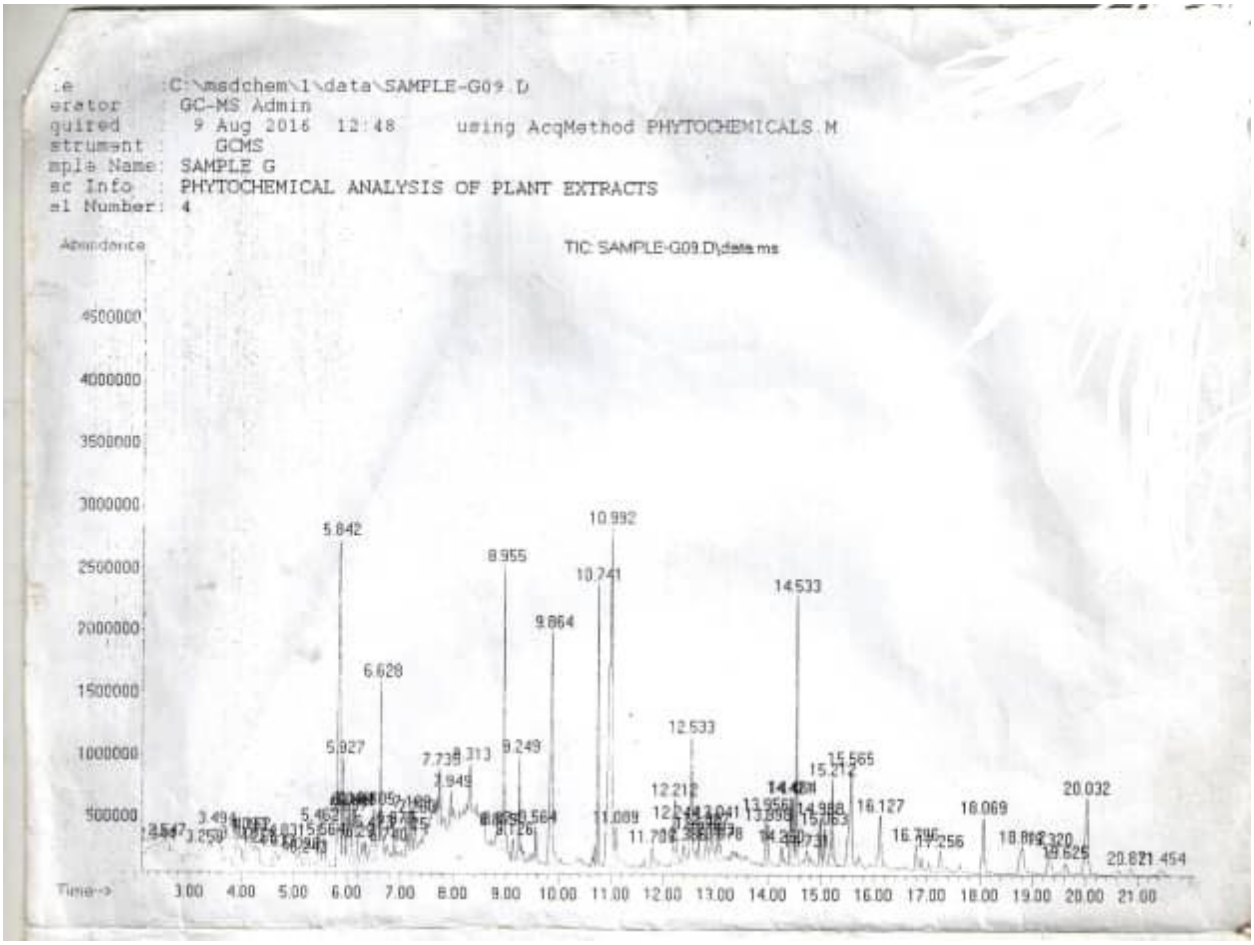

Fig 3.1 Image representation of GCMS spectrum

Table 3.1: GCMS result of leaf extract of chloroform *Chromolaena odorata*

S/No	RT	compound name	MW	FORMULAR	AREA %
1	2.344	Erythritol	122	$C_4H_{10}O_4$	0.82
2	2.547	Tetrahydro-4H-pyran-4-ol		$C_5H_{10}O_2$	0.41
3	3.258	1-Pentene	70	C_5H_{10}	0.62
4	3.494	1,3,5-Hexatriene,3-methyl-,(E)-	94	C_7H_{10}	2.45
5	4	Catechol	110	$C_6H_6O_2$	1.2
6	4.093	Cycloheptatrienylium, iodide	218	C_7H_7I	0.93
7	4.162	Isopropenyl bromide	120	C_3H_5Br	0.41
8	4.285	2-Propanone,dimethylhydrazone	100	$C_5H_{12}N_2$	0.68
9	4.633	Hydroquinone	110	$C_6H_6O_2$	0.65
10	4.831	2-Isopropoxyethylamine	103	$C_5H_{13}NO$	0.5
11	5.098	DL-Cystine	240	$C_6H_{12}N_2O_4$	0.45
12	5.243	Metaraminol	167	$C_9H_{13}NO_2$	0.43
13	5.462	α-cubebene	204	$C_{15}H_{24}$	1.09
14	5.564	2-Octyne	110	C_8H_{14}	0.85
15	5.842	Calarene	204	$C_{15}H_{24}$	7.64
16	5.927	β-Cubebene	204	$C_{15}H_{24}$	2.06
17	5.997	1,5-Heptadien-3-yne	92	C_7H_8	0.97
18	6.045	α-Farnesene	204	$C_{15}H_{24}$	1.18
19	6.104	Methyl 4,7,10,13,16,19-docosahexaenoate	342	$C_{23}H_{34}O_2$	1.13
20	6.146	Humulene	204	$C_{15}H_{24}$	1.35
21	6.291	1,2-Propaanediol,3-chloro	110	$C_3H_7ClO_2$	0.57
22	6.473	Spiro[5.5]undec-2-ene,3,7,7-trimethyl-11-methylene-,(-)-	204	$C_{15}H_{24}$	0.75
23	6.505	diazoadamantane	162	$C_{10}H_{14}N_2$	1
24	6.628	Benzene, 1, 2-diethyl-	134	$C_{10}H_{14}$	3.39
25	6.74	Arginine	174	$C_6H_{14}O_2$	0.34
26	6.879	7-Octen-2-ol, 2-methyl-6-methylene-	154	$C_{10}H_{18}O$	0.37
27	7.189	Ethanamine, 2-phenoxy-	137	$C_8H_{11}NO$	0.76
28	7.28	Taurultam	136	$C_3H_8N_2O_2S$	0.62
29	7.73	Oxetane, 2, 2, 4-trimethyl-	100	$C_6H_{12}O$	0.82
30	7.949	3- Aminocrotonitrile	82	$C_4H_6N_2$	0.78
31	8.313	1-[-]-4-Hydroxy-1-methylproline	145	$C_6H_{11}NO_3$	0.86
32	8.815	1-Nitro-2-acetamido-1,2-dideoxy-d-glucitol	252	$C_8H_{16}N_2O_7$	0.34
33	8.874	1, 2, 3, 4-Butanetetrol,[S-(R,R)]-	122	$C_4H_{10}O_4$	0.54
34	8.955	1,9-Nonanediol,dimethanesulfonate	316	$C_{11}H_{24}O_6S_2$	3.93
35	9.126	1,2-Benzenediol, 4-[2-(methylamino)ethyl]-	167	$C_9H_{13}NO_2$	0.42

36	9.249	2-Cyclohxen-1-one, 4,4-dimethyl-	124	$C_8H_{12}O$	1.35
37	9.564	Methyl 2-O-mthyl-β-D-xylopyranoside	178	$C_7H_{14}O_5$	0.51
38	9.864	n-Hexadecanoic acid	256	$C_6H_{10}O_3$	5.9
39	10.741	Oxiran,decyl	184	$C_{12}H_{24}O$	3.29
40	10.992	9, 12, 15-Octadecatienoic acid,(Z,Z,Z)-	278	$C_{18}H_{30}O_2$	11.18
41	11.789	Adipamide	144	$C_6H_{10}O_3$	0.66
42	12.212	Bicyclo[3. 1. 1]heptane, 2,6,6-trimethyl-,1R-(1α, 2α, 5α)]-	138	$C_6H_{12}N_2O_2$	1.62
43	12.244	Bicyclo[2. 2. 1]heptan-2-one,4, 7, 7-trimethyl-,semicarbazone	209	$C_{10}H_{18}$	0.74
44	12.388	Acetic acid, 2-(1-methyl-2-oxohydrazino)-,N'-[€-(2-hydroxyphenyl)methylidene] hydrazide, N-oxide	252	$C_{11}H_{19}N_3O$	0.58
45	12.533	1H-3a, 7-methanazulene, octahydro-1, 4, 9, 9-tetramethyl-	206	$C_{10}H_{12}N_4O_4$	2.85
46	12.672	Tricyclo[4. 3. 1. 1(3,8)]undecane-1-carboxylic acid	194	$C_{15}H_{26}$	0.49
47	12.827	Benzenemethanol, alpha-[(methylamino)methyl]-	151	$C_{12}H_{18}O_2$	0.75
48	12.923	Phenylephrine	167	$C_9H_{13}NO$	0.98
49	13.041	Cyclohexanol,1R-4-trans-acetamido-2,3-trans-epoxy-	171	$C_9H_{13}NO_2$	0.71
50	13.956	1-Eicosanol	298	$C_8H_{13}NO_3$	0.84
51	13.998	5-Hydroxy-4',7-dimethoxyflavanone	300	$C_{17}H_{16}O_5$	0.62
52	14.26	Pyridine, 2,3-dimethyl-	107	C_7H_9N	0.46
53	14.421	4',5-Dihydroxy-7-methoxyflavanone	286	$C_{16}H_{14}O_5$	1.15
54	14.464	Tricyclo[4. 3. 1.0(3,8)]decan-10-ol	152	$C_{10}H_{16}O$	1.16
55	14.533	Squalene	410	$C_{30}H_{50}$	3.69
56	14.731	benzaldehyde,3-hydroxy-, oxime	137	$C_7H_7NO_2$	0.4
57	14.988	phenol, 2,6-dimethyl-4-nitro-	167	$C_8H_9NO_3$	0.89
58	15.212	Ethanone, 1-(2-hydroxy-5-methylphenyl)-	166	$C_9H_{10}O_3$	1.89
59	15.565	Leucopterin	195	$C_6H_5N_5O_3$	3.02
60	16.127	benzaldehyde,3-hydroxy-5-nitro-	167	$C_7H_5NO_4$	1.5
61	16.796	Vitamin E	430	$C_{29}H_{50}O_2$	0.62
62	17.256	sarcosine, N-(3-cyclopentylprpionyl)-,tetradecyl ester	409	$C_{25}H_{47}NO_3$	0.65
63	18.069	2-Pentadecyn-1-ol	224	$C_{15}H_{28}O$	1.55
64	18.812	Estran-3-one, 17-(acetyloxy)-2-methyl-,(2α, 5α, 17β)	332	$C_{21}H_{32}O_3$	1.41
65	19.32	2,4-Dimethylamphetamine	163	$C_{11}H_{17}N$	0.99
66	20.032	Cyclohexane, 1-ethenyl-1-methyl 2, 4-bis(1-methylethenyl)-, [1S-(1α, 2α, 4β)]-	204	$C_{15}H_{24}$	2.72
67	20.871	Acetamide, N-(aminocarbonyl)-2-chloro-	136	$C_3H_5ClN_2O_2$	0.34

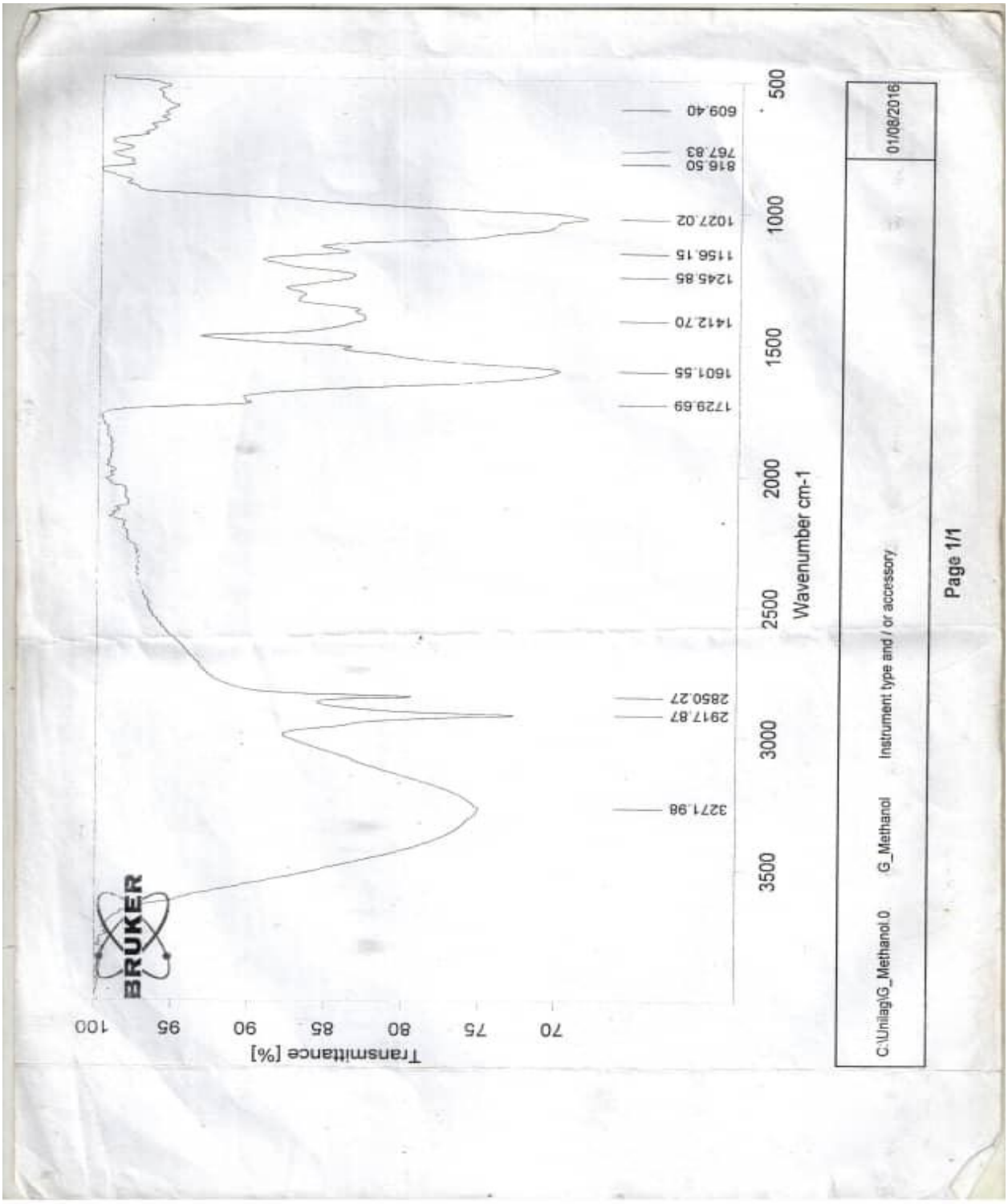

Fig 3.2 Image representation of FT-IR spectrum

Table 3.2: FTIR result of leaf extract of *Chromolaena odorata*

S/No	WAVE NUMBER (cm^{-1})	FUNCTIONAL GROUP
1	609.4	C-H deformation (Alkynes)
2	767.83	O-H bend (Alchohols and Phenols)
3	816.5	C-H(tri substituted Alkenes, vinyl group)
4	1027.02	C-X (fluoroAlkanes, alkyl halides)
5	1156.15	C-O (Aromatic ethers)
6	1245.85	C-H (Arenes)
7	1412.7	P-C (Aromatic organophosphorus compounds
8	1601.55	C=C (Aromatics, with benzene ring)
9	1729.69	C=O (Saturated aldehydes)
10	2850.27	CH_3, CH_2 and CH (Alkanes)

Table 3.3 The acute toxicity result of the chloroform extract Of *Chromolaenaodorata* leaves

Group	Dose mg/kg	D/T	toxicity signs
1	10	0/3	no toxic effect observed
2	100	0/3	no toxic effect observed
3	1000	0/3	Scratching of the mouth
4	2000	o/1	Scratching of the mouth
5	4000	0/1	Scratching of the mouth
6	5000	0/1	Calmness for a while followed by rigourous movements

These results indicate that the leaf extract of Chromolaena odorata exhibited low toxicity at lower doses (10 mg/kg and 100 mg/kg) as no toxic effects were observed. However, at higher doses (1000 mg/kg and above), some mild toxic effects were seen, such as scratching of the mouth in groups 3, 4, and 5, and in group 6, a brief period of calmness followed by rigorous movements was observed. It is important to note that mortality (D/T: 1/1) was only observed in one rat in group 6 at the highest dose of 5000 mg/kg. Overall, the test results suggest that the leaf extract has a generally low toxicity profile, making it relatively safe for administration at appropriate dosages.

Table 3.3 above shows the acute toxicity LD_{50} test on leaf extract of *Chromolaena odorata.* No mortality was observed even at the highest dose of 5000mg/kg.

Table 3.4. The effect of *Chromolaena odorata leaf extract* on gastric-mucosa of aspirin induced ulcer rats.

Parameters	Normal Control Group 1	Negative Control Group 2	20 mg/kg omeprazole Group 3	Extract 250 mg/kg Group 4	Extract 500 mg/kg Group 5	Extract 750 mg/kg Group 6
TUS	0	9.60 ± 0.58^{a}	3.54 ± 0.58^{b}	5.33 ± 0.58^{b}	3.67 ± 0.58^{b}	1.67 ± 0.58^{c}
UA	0	6.33 ± 0.58^{a}	3.67 ± 0.58^{b}	4.33 ± 0.58^{b}	3.33 ± 0.58^{b}	1.67 ± 0.58^{c}
UP	0	70.33 ± 2.52^{a}	25.00 ± 5.00^{b}	41.33 ± 3.51^{c}	35.67 ± 4.04^{c}	17.67 ± 3.79^{d}
UI	0	8.63 ± 0.32^{a}	3.03 ± 0.64^{b}	5.10 ± 0.26^{c}	4.27 ± 0.47^{c}	2.10 ± 0.46^{d}
% Inhibition	100	0	64.64	40.81	50.43	73.54

Keys: values are mean±SD for N=5. The values with different lowercase letters (a, b, c, d) within the same row indicate significant differences between groups ($p < 0.05$).TUS=Total Ulcer Score UA=Average number of ulcer per animal UP= Percentage of animal with ulcer UI=Ulcer Index

The table shows the effects of the extract at different doses and the standard drug (omeprazole) on ulcer-related parameters. The extract exhibited dose-dependent inhibition of ulcers, with higher doses showing greater inhibition. At the highest dose of 750 mg/kg, the extract demonstrated the highest percentage inhibition (73.54%). The standard drug, omeprazole, also showed significant inhibition of ulcers (64.64%) compared to the Negative Control Group.

Table 3.5. The effect of *Chromolaena odorata* leaf extract on mucosal endogenous aggressive factors in gastric juice of aspirin induced ulcer rats

Group	Treatment	pH	Total acidity	Free acidity	Pepsin activity
1	2ml of distilled water	2.74 ± 0.53^{a}	8.00 ± 0.73^{a}	3.60 ± 0.26^{a}	81.24 ± 1.35^{a}
2	1000mg/kg of aspirin	2.34 ± 0.06^{a}	11.35 ± 0.09^{b}	6.26 ± 0.10^{b}	106.03 ± 1.68^{b}
3	20mg/kg of omeprazole	3.40 ± 0.31^{b}	9.37 ± 1.00^{c}	3.86 ± 0.25^{a}	81.25 ± 3.24^{a}
4	250mg/kg of leaf extract	2.62 ± 0.04^{a}	11.19 ± 0.09^{b}	5.93 ± 0.25^{c}	104.08 ± 1.64^{b}
5	500mg/kg of leaf extract	2.77 ± 0.06^{a}	10.72 ± 0.07^{bd}	5.47 ± 0.07^{d}	101.64 ± 0.95^{c}
6	750mg/kg of leaf extract	2.46 ± 0.03^{a}	10.16 ± 1.27^{d}	5.83 ± 0.08^{c}	96.13 ± 1.61^{d}

Keys: values are mean±SD for N=5. Values in the same column bearing the same letter of alphabets are not significantly different from each other ($p\leq0.05$). (1) Normal control (2). Negative control.

The data indicates that different treatments have varying effects on the parameters measured. In the Negative Control group (Group 2), induced with 1000mg/kg of aspirin, there is a significant decrease in pH and increase in total acidity, free acidity, and pepsin activity compared to the Normal Control (Group 1). However, treatment with omeprazole (Group 3) and leaf extract at different doses (Groups 4, 5, and 6) shows a trend of restoring these parameters closer to the Normal Control levels.

Table 3.6. The activity of *Chromolaena odorata* leaf extract on cyclo-oxygenase 1 enzyme concentration and prostaglandin endoperoxide production in stomach tissues of aspirin induced ulcer rats.

Group	Treatment	Cox- 1(ng/ml)	PGE(pg/ml)
1	2ml of distilled water	28.90 ± 2.04^{a}	347.71 ± 14.05^{a}
2	1000mg/kg of aspirin	2.82 ± 0.21^{b}	288.09 ± 14.05^{b}
3	20mg/kg of omeprazole	23.03 ± 1.15^{c}	325.79 ± 9.76^{b}
4	250mg/kg of leaf extract	29.87 ± 1.24^{a}	303.16 ± 48.43^{b}
5	500mg/kg of leaf extract	27.30 ± 3.37^{a}	355.08 ± 9.44^{a}
6	750mg/kg of leaf extract	30.86 ± 3.79^{a}	335.98 ± 14.84^{ab}

Keys: values are mean±SD for N=5. Values in the same column bearing the same letter of alphabets are not significantly different from each other ($p \leq 0.05$). (1) Normal control (2). Negative control. COX-1: cyclooxygenase 1. PGE_2: prostaglandin endoperoxide

The data indicates that different treatments have varying effects on the COX-1 enzyme concentration and PGE2 production in stomach tissues. In the Negative Control group (Group 2), induced with 1000mg/kg of aspirin, there is a significant decrease in COX-1 levels and PGE2 production compared to the Normal Control (Group 1). Treatment with omeprazole (Group 3) shows an intermediate effect, but leaf extract at different doses (Groups 4, 5, and 6) demonstrates varied effects on COX-1 and PGE2 levels.

Table 3.7: Effect of *Chromolaena odorata* leaf extract on phase 1 antioxidant enzymes in serum of aspirin induced ulcer rats

Group	Treatment	Myeloperoxidase(U/ml)	SOD(U/ml)	MDA(µmol/ml)
1	2ml of distilled water	238.92 ± 12.98^{a}	50.53 ± 0.77^{a}	2.40 ± 0.10^{a}
2	1000mg/kg of aspirin	271.75 ± 15.12^{b}	27.07 ± 1.12^{b}	5.13 ± 0.12^{b}
3	20mg/kg of omeprazole	249.79 ± 7.30^{a}	45.18 ± 2.35^{c}	2.94 ± 0.06^{a}
4	250mg/kg of leaf extract	263.27 ± 14.22^{b}	28.50 ± 0.58^{b}	5.41 ± 0.46^{b}
5	500mg/kg of leaf extract	262.87 ± 6.62^{b}	35.63 ± 2.93^{d}	4.81 ± 0.71^{b}
6	750mg/kg of leaf extract	261.18 ± 14.30^{b}	33.00 ± 1.00^{d}	4.57 ± 0.14^{b}

Keys: values are mean±SD for N=5. Values in the same column bearing the same letter of alphabets are not significantly different from each other ($p \leq 0.05$). (1) Normal control (2). Negative control. SOD: Superoxide dismutase. MDA: Malondealdehyde.

Table 3.7 shows an increase in myeloperoxidase activity for group 2 when compared with group 1 showing an NSAID induced stress condition. The standard drug reduced significantly myeloperoxidase levels as that observed for the group 1. The extract did not affect any change in myeloperoxidase levels at different doses when compared to group 2. SOD levels decreased for group 2 when compared to group 1 establishing an NSAID induced stress condition. The standard drug and extract at different doses of 250, 500, 750mg/kg increased SOD levels significantly when compared to group 2 models. A manifold increase was observed in malondealdehyde for group 2 when compared to group 1, establishing an NSAID induced stress condition. The standard drug reduced malondealdehyde levels significantly when compared to group 2. The extract reduced malondealdehyde production at doses of 250, 500 and 750mg/kg in a dose dependent manner but this effect was not statistically significant at $p \leq 0.05$.

Table 3.8: Effect of *Chromolaena odorata* leaf extract on inducible nitric oxide synthase concentration in stomach tissues of aspirin induced ulcer rats

Group	Treatment	INOS(ng/ml)
1	2ml of distilled water	29.51 ± 1.76^{a}
2	1000mg/kg of aspirin	23.56 ± 1.28^{b}
3	20mg/kg of omeprazole	27.2 ± 1.87^{a}
4	250mg/kg of leaf extract	26.88 ± 2.64^{a}
5	500mg/kg of leaf extract	25.20 ± 1.89^{b}
6	750mg/kg of leaf extract	26.07 ± 3.05^{a}

Keys: values are mean±SD for N=5. Values in the same column bearing the same letter of alphabets are not significantly different from each other ($p\leq0.05$). (1) Normal control (2). Negative control. INOS : inducible nitric oxide synthase

Table 3.8 demonstrates the effect of Chromolaena odorata leaf extract on the concentration of Inducible Nitric Oxide Synthase (INOS) in stomach tissues of rats with aspirin-induced ulcers. Group 2, which received 1000mg/kg of aspirin, exhibited a significant decrease in INOS concentration compared to Group 1 (2ml of distilled water). The standard drug, omeprazole (Group 3), increased the INOS level compared to Group 2.

At doses of 250mg/kg, 500mg/kg, and 750mg/kg of the leaf extract (Groups 4, 5, and 6, respectively), INOS concentration significantly increased compared to Group 2 and was restored to levels comparable to Group 1 ($p\leq0.05$).

These results suggest that the leaf extract of Chromolaena odorata may have a potential regulatory effect on INOS concentration in stomach tissues, which could be beneficial in the context of aspirin-induced ulcers. Further investigation is warranted to elucidate the underlying mechanisms and explore the extract's therapeutic potential for managing gastric ulcer conditions. The data presented in Table 3.8 are statistically sound and support the conclusions drawn from the study.

Table 3.9: Effect of *Chromolaena odorata* leaf extracton phase 2 antioxidant enzyme expression in stomach tissues of aspirin induced ulcer rats

Group	Treatment	Glutathione(ηmol/mg)	glutathione s transferase(U/mg)	thioredoxin reductase(U/mg)	quinone oxidoreductase(U/mg)
1	2ml of distilled water	32.08±1.38[a]	26.70±0.75[a]	2.33±0.15[a]	350.34±29.79[a]
2	1000mg/kg of aspirin	29.65±1.81[a]	23.21±1.56[b]	2.82±0.17[b]	251.60±6.85[b]
3	20mg/kg of omeprazole	31.30±0.53[a]	24.59±1.21[a]	2.77±0.1[b]	261.18±29.04[b]
4	250mg/kg of leaf extract	29.87±1.10[a]	24.37±1.05[a]	2.50±0.15[a]	305.66±15.04[c]
5	500mg/kg of leaf extract	30.13±0.98[a]	24.93±2.75[a]	2.49±0.13[a]	317.41±13.89[ac]
6	750mg/kg of leaf extract	30.03±1.45[a]	23.92±1.50[a]	2.32±0.16[a]	316.24±24.33[ac]

Keys: values are mean±SD for N=5. Values in the same column bearing the same letter of alphabets are not significantly different from each other ($p \leq 0.05$). (1) Normal control (2). Negative control.

Table 3.9 presents the effect of Chromolaena odorata leaf extract on the expression of phase 2 antioxidant enzymes in stomach tissues of rats with aspirin-induced ulcers. The measured parameters include Glutathione, Glutathione S Transferase (GST), Thioredoxin Reductase, and Quinone Oxidoreductase.

Group 2, treated with 1000mg/kg of aspirin, exhibited decreased levels of Glutathione, GST, and Thioredoxin Reductase compared to the normal control (Group 1). However, the standard drug, omeprazole (Group 3), did not show significant restoration of these enzymes.

Groups 4, 5, and 6, treated with 250mg/kg, 500mg/kg, and 750mg/kg of the leaf extract, respectively, demonstrated comparable levels of Glutathione and GST to the normal control, indicating a potential protective effect of the extract on these enzymes. Additionally, the expression of Thioredoxin Reductase and Quinone Oxidoreductase in these groups was significantly higher than in the negative control (Group 2).

These results suggest that Chromolaena odorata leaf extract may exert beneficial effects on phase 2 antioxidant enzymes in aspirin-induced ulcer rats. The data presented in Table 3.9 are statistically reliable and provide valuable insights into the potential antioxidant properties of the leaf extract. Further research is warranted to explore the underlying mechanisms and potential therapeutic applications of these findings in managing gastric ulcer conditions.

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Group	Treatment	MMP 1(ng/ml)
1	2ml of distilled water	2.46±0.17[a]
2	1000mg/kg of aspirin	5.01±0.31[b]
3	20mg/kg of omeprazole	3.71±0.23[c]
4	250mg/kg of leaf extract	3.22±0.36[c]
5	500mg/kg of leaf extract	3.35±0.30[c]
6	750mg/kg of leaf extract	3.30±0.17[c]

Table 3.10: Effect of*Chromolaena odorata* leaf extract on matrix metalloproteinase 1 concentration in stomach tissues of aspirin induced ulcer rats

Keys: values are mean±SD for N=5. Values in the same column bearing the same letter of alphabets are not significantly different from each other (p≤0.05). (1) Normal control (2). Negative control. MMP 1: matrix metalloproteinase 1.

Groups 3, 4, 5, and 6, treated with 20mg/kg of omeprazole and various doses of the leaf extract (250mg/kg, 500mg/kg, and 750mg/kg, respectively), demonstrated reduced MMP 1 concentrations compared to the aspirin-treated group (Group 2). Notably, the MMP 1 levels in these treatment groups were closer to that of the normal control, indicating potential protective effects of omeprazole and the leaf extract on stomach tissues.

These findings suggest that Chromolaena odorata leaf extract may have a beneficial impact on MMP 1 levels in aspirin-induced ulcer rats, potentially contributing to the management of gastric ulcer conditions. The results presented in Table 3.10 provide valuable insights into the potential therapeutic applications of the leaf extract in mitigating tissue damage and promoting tissue repair. Further research is warranted to elucidate the underlying mechanisms and validate the efficacy of the leaf extract as a potential treatment option for gastric ulcer management Table

3.11: Effect of *Chromolaena odorata* leaf extract on pro inflammatory cytokine concentration, in stomach tissues of aspirin induced ulcer rats.

Group	Treatment	Tumor Necrosis Factor α(pg/ml)	interleukin 1 β(pg/ml)
1	2ml of distilled water	92.53±6.09[a]	29.49±2.70[a]
2	1000mg/kg of aspirin	133.33±4.17[b]	46.37±2.71[b]
3	20mg/kg of omeprazole	121.46±3.74[c]	34.20±1.76[a]
4	250mg/kg of leaf extract	130.13±5.15[b]	32.04±4.81[a]
5	500mg/kg of leaf extract	123.80±5.77[b]	24.89±3.45[a]
6	750mg/kg of leaf extract	128.65±5.1[b]	21.98±9.71[a]

Keys: values are mean±SD for N=5. Values in the same column bearing the same letter of alphabets are not significantly different from each other (p≤0.05). (1) Normal control (2). Negative control.

Table 3.10 presents the effect of Chromolaena odorata leaf extract on matrix metalloproteinase 1 (MMP 1) concentration in stomach tissues of rats with aspirin-induced ulcers. MMP 1 is an enzyme involved in tissue remodeling and repair processes.

Group 2, treated with 1000mg/kg of aspirin, showed a significant increase in MMP 1 concentration compared to the control group (Group 1) receiving distilled water. This elevated MMP 1 level indicates tissue damage and degradation caused by aspirin-induced ulcers.

Table 3.11 displays the effect of Chromolaena odorata leaf extract on the concentration of pro-inflammatory cytokines, Tumor Necrosis Factor α (TNF-α), and Interleukin 1 β (IL-1β) in stomach tissues of rats with aspirin-induced ulcers.

Group 2, treated with 1000mg/kg of aspirin, exhibited a significant increase in TNF-α and IL-1β concentrations compared to the control group (Group 1) receiving distilled water. This elevation indicates a pro-inflammatory response due to aspirin-induced ulceration.

Groups 3, 4, 5, and 6, treated with 20mg/kg of omeprazole and various doses of the leaf extract (250mg/kg, 500mg/kg, and 750mg/kg, respectively), demonstrated reduced TNF-α and IL-1β concentrations compared to the aspirin-treated group (Group 2). Notably, the levels in these treatment groups were closer to that of the normal control (Group 1), indicating potential anti-inflammatory effects of omeprazole and the leaf extract.

These findings suggest that Chromolaena odorata leaf extract may have a beneficial impact on pro-inflammatory cytokine levels in aspirin-induced ulcer rats, potentially contributing to the mitigation of inflammatory responses and ulcer healing. The results presented in Table 3.11 provide valuable insights into the potential therapeutic applications of the leaf extract in managing gastric ulcer conditions by regulating pro-inflammatory cytokine levels. Further research is warranted to elucidate the underlying mechanisms and validate the efficacy of the leaf extract as a potential treatment option for gastric ulcers with an inflammatory component.

Table 3.12: Effect of *Chromolaena odorata* leaf extract on serum total protein in aspirin induced ulcer rats

Group	Treatment	total protein(mg/ml)

1	2ml of distilled water	8.13±0.74[a]
2	1000mg/kg of aspirin	7.00±0.74[b]
3	20mg/kg of omeprazole	6.87±0.03[b]
4	250mg/kg of leaf extract	6.98±0.35[b]
5	500mg/kg of leaf extract	6.81±0.16[b]
6	750mg/kg of leaf extract	7.15±0.55[b]

Keys: values are mean±SD for N=5. Values in the same column bearing the same letter of alphabets are not significantly different from each other ($p \leq 0.05$). (1) Normal control (2). Negative control.

Table 3.12 showed that group 2 recorded a reduction in serum total protein when compared to group 1. The standard drug and leaf extract at different doses of 250, 500 and 750mg/kg had no significant effect at increasing serum total protein levels when groups 3, 4, 5 and 6 were compared to group 2 at $P \leq 0.05$.

GRP	Treatment	RBC(x10^{12}/L)	PCV(%)	Hb(g/dl)	MCV(fl)	MCH(pg)	MCHC(g/dl)	Platelet(x10^{9}/L)
1	2ml of distilled water	9.73±0-71[a]	63.97±0.74[a]	16.57±1.42[a]	66.08±0.09[a]	17.10±0.12[a]	261.67±2.21[a]	690.33±13.93[a]
2	1000mg/kg of aspirin	9.37±0.03[a]	58.27±0.22[b]	15.59±0.17[a]	62.00±0.00[b]	16.80±0.07[a][b]	269.00±0.71[b]	651.00±3.94[a]
3	20mg/kg of omeprazole	7.42±0.01[b]	49.13±0.11[c]	12.43±0.11[b]	66.52±0.76[a]	16.60±0.19[b]	254.33±1.11[c]	526.33±4.03[b]
4	250mg/kg of leaf extract	9.71±0.12[a]	64.80±0.86[a]	17.00±0.78[a][c]	66.75±0.05[a]	17.10±0.19[a]	263.33±1.78[a]	574.00±47.63[b][d]
5	500mg/kg of leaf extract	8.53±0.14[c]	53.10±0.40[d]	13.37±0.47[b]	60.45±0.25[c]	15.47±0.22[c]	257.33±2.68[c][d]	647.00±32.19[a][d]
6	750mg/kg of leaf	8.60±0.02[c]	55.30±0.61	15.23±0.66[a]	63.23±0.76	16.77±0.04[a]	260.94±5.04[a]	598.33±15.53[b]

Table 3.13: Effect of *Chromolaena odorata* leaf extract on red blood cells and platelet parameters in aspirin induced ulcer rats

extract	e	d	d	b	d	d

Keys: values are mean±SD for N=5. Values in the same column bearing the same letter of alphabets are not significantly different from each other (p≤0.05). (1), Normal control (2), Negative control. RBC: red blood concentration. PCV: packed cell volume. Hb: hemoglobin count. MCV: mean cell volume. MCH: mean cell hemoglobin. MCHC: mean cell hemoglobin concentration.

Table 3.13 shows that the NSAID had no significant effect (P≤0.05) on the RBC, Hb, MCH and platelet when group 2 is compared with group 1. There was significant reduction in PCV and MCV but MCHC significantly increased (P≤0.05). the standard drug reduced significantly RBC, PCV, Hb, MCH, MCHC and platelet for group 3 when compared to group 2 (P≤0.05). There was no significant effect on RBC, PCV, Hb, MCV, MCH, and MCHC for groups treated with 250 mg/kg of the leaf extract. At doses of 500 and 750mg/kg of the extract, RBC, PCV, Hb, MCV, MCH, and MCHC and platelet were reduced significantly for groups 5 and 6 when compared to group 2. This effect was dose dependent (P≤0.05).

Table 3.14: Effect of *Chromolaena odorata* leaf extract on white blood cell differentials in aspirin induced ulcer rats.

GRP	Treatment	WBC(x10^9/L)	Neutrophil(%)	Lymphocyte(%)	Monocyte(%)	Eosinophil(%)	Basophil(%)
1	2ml of distilled water	8.70±0.55^a	56.33±2.72^a	34.67±2.27^a	6.00±1.00^a	4.00±1.00^a	1.00±0.00^a
2	1000mg/kg of aspirin	5.03±0.89^b	36.67±1.08^b	57.00±2.00^b	5.00±0.71^a	2.00±0.00^b	1.00±0.00^a
3	20mg/kg of omeprazole	10.17±0.74^c	48.33±2.69^c	44.67±2.58^c	3.67±0.41^b	4.00±0.00^a	1.00±0.00^a
4	250mg/kg of leaf extract	7.10±0.37^d	41.00±0.71^d	51.33±0.47^d	5.00±0.00^a	2.67±0.41^b	1.00±0.00^a
5	500mg/kg of leaf extract	3.03±0.45^e	40.67±1.18^d	52.00±1.87^d	5.00±0.71^a	2.67±0.41^b	0.33±0.41^b
6	750mg/kg of leaf extract	8.40±0.71^a	41.00±0.00^d	53.33±1.49^d	5.00±0.00^a	2.67±0.61^b	0.67±0.41^a

Keys: values are mean±SD for N=5. Values in the same column bearing the same letter of alphabets are not significantly different from each other (p≤0.05). (1), Normal control (2), Negative control. WBC: white blood count.

Table 3.14 shows that the NSAID reduced significantly WBC, neutrophil, lymphocyte and eosinophil for group 2 when compared to group 1 but had no significant effect on monocyte and basophil when compred to group 1 at P≤0.05. The standard drug increased

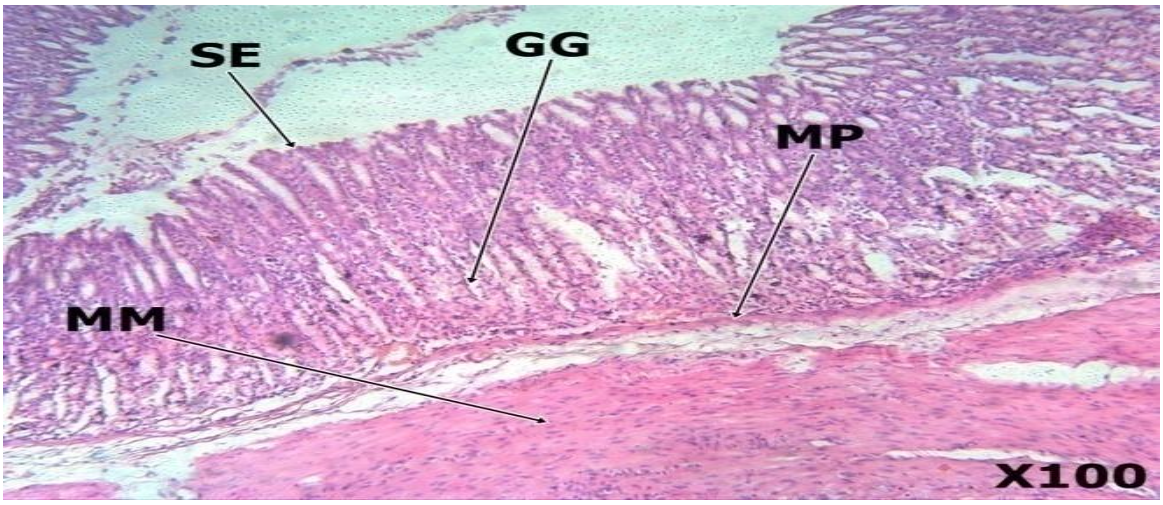
significantly WBC, neutrophil and eosinophil for group 3 when compared to group 2. At the lowest and highest dose 250 and 750mg/kg of the extract, there was increased WBC,neutrophil, and eosinophil. The leaf extract at 500mg/kg reduced significantly at $P \leq 0.05$, WBC, and basophil when compred to group 2. The different doses of the leaf extract showed same significant effect at increasing neutrophil and eosinophil as well as reducing lymphocyte, when compared with group 2.

Plate 1. Photomicrograph of stomach tissue of group 1 rat model.

keys: GG.gastric glands, MM.muscularismucosa, MP.muscularis propria, SE.surface epithelium

Plate1, above shows 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.

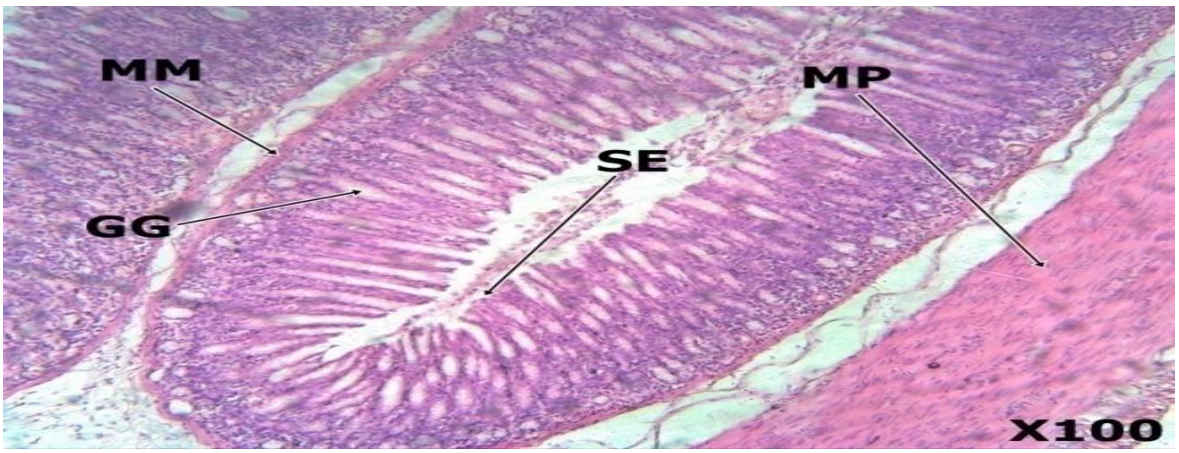
Plate 2. photomicrograph of stomach tissue of group 2 rat model.



keys: GG.gastric glands, MM.muscularismucosa, MP.muscularis propria, SE.surface epithelium

Plate 2 above show a more pronounced infiltration of stroma by inflammatory cells.

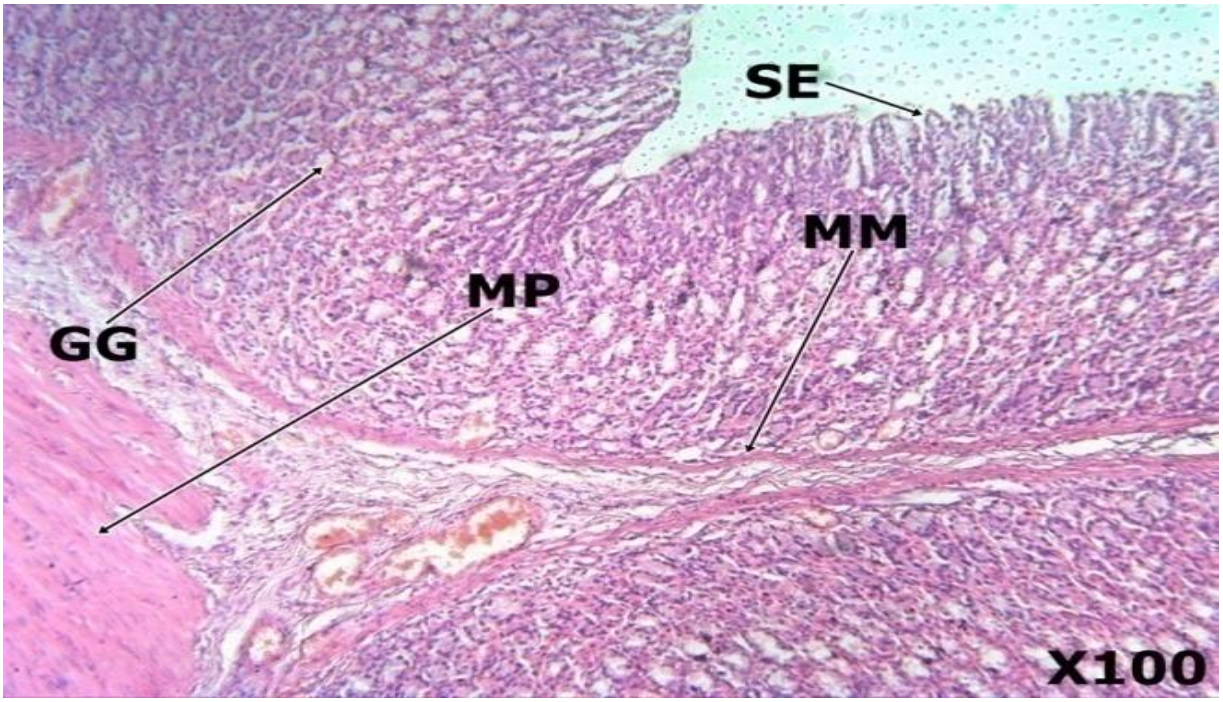
Plate 3. Photomicrograph of stomach tissue of group 3 rat model.



keys: GG.gastric glands, MM.muscularismucosa, MP.muscularis propria, SE.surface epithelium.

Plate 3 above show no significant epithelial restoration when group 3 was compared to group 2.

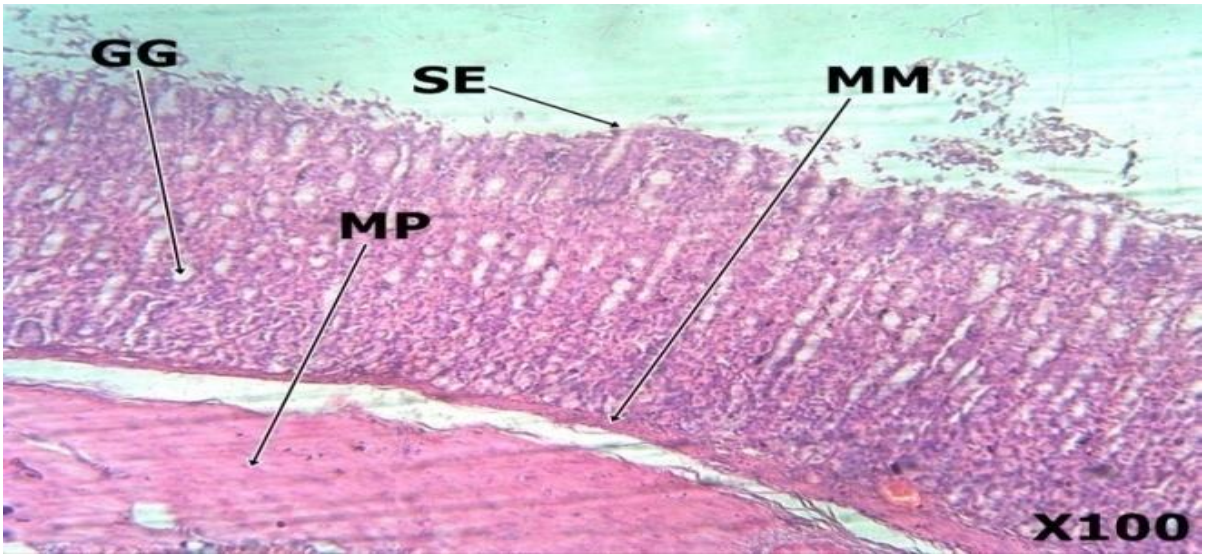
Plate 4 Photomicrograph of stomach tissue of group 4 rat model.



keys GG.gastric glands, MM.muscularismucosa, MP.muscularis propria, SE.surface epithelium

Plate 4 above shows there is mild mucosal surface epithelial restoration when group 4 was compared to group 2. The intervening stroma is thinly fibrocollagenous and is infiltrated by moderate population of mononuclear inflammatory cells, predominantly lymphocytes.

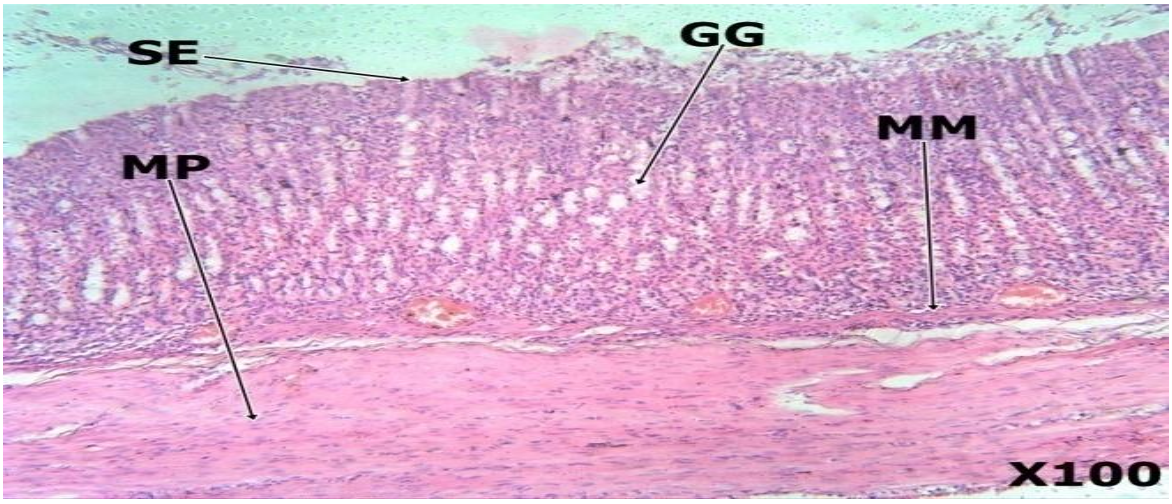
Plate 5. Photomicrograph of stomach tissue of group 5 rat model.



keys: GG.gastric glands, MM.muscularismucosa, MP.muscularis propria, SE.surface epithelium

Plate 5, shows there is marked mucosal surface epithelial restoration when group 5 was compared to group 2. The intervening stroma is thinly fibrocollagenous and is infiltrated by scant mononuclear inflammatory cells, predominantly lymphocytes.

Plate 6. Photomicrograph of stomach tissue of group 6 rat model.



keys: GG.gastric glands, MM.muscularismucosa, MP.muscularis propria, SE.surface epithelium

Plate 6 Compared to G2 there is marked mucosal surface epithelial restoration. The intervening stroma is thinly fibrocollagenous and is infiltrated by scant mononuclear inflammatory cells, predominantly lymphocytes.

Fig 3.3: Image of stomach for Group 1 rats

Fig 3.4: Image of stomach for Group 2 rats

Fig 3.5: Image of stomach for Group 3 rats

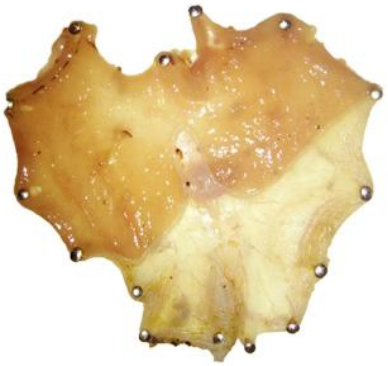


Fig 3.6: Image of stomach for Group 4 rats

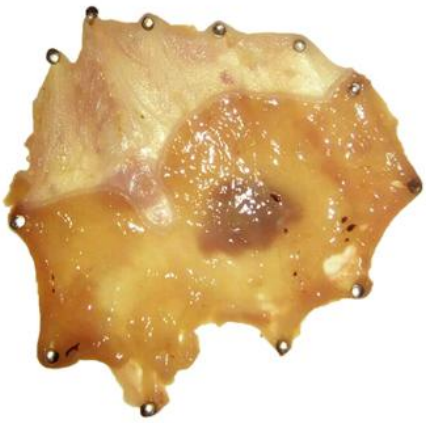


Fig 3.7: Image of stomach for Group 4 rats

Fig 3.8: Image of stomach for Group 6 rats

4.0 Discussions

The plant Chromolaena odorata has a history of use in folk medicine for treating topical wounds and is considered effective for therapeutic purposes. This study aimed to explore its potential in treating non-topical wounds, including ulcers, and to identify bioactive compounds responsible for its therapeutic effects. Gas chromatography coupled with mass spectrometry (GCMS) was used for phytochemical screening of the C. odorata leaves to detect bioactive compounds.

The GCMS analysis revealed the presence of sixty-seven compounds in the plant sample, many of which are known for their anti-inflammatory, wound healing, and cytoprotective properties. Among the compounds, 9, 12, 15-octadecatienoic acid (alpha-linolenic acid) was found to be the most abundant, exhibiting antioxidant, pesticide, antimicrobial, and lubricant properties, as reported by Isiah *et al.* (2016). This compound may contribute to the plant's anti-inflammatory properties. Overall, the study suggests that Chromolaena odorata contains several bioactive compounds that could potentially be beneficial in treating wounds, inflammation, and other health conditions. However, further research is needed to fully understand the mechanisms and therapeutic applications of these compounds (Isiah et al., 2016).

In a study conducted by Ren and Chung (2007), it was proposed that the anti-inflammatory effect of 9, 12, 15-octadecatienoic acid (Z, Z, Z) is attributed to its ability to inhibit the production of nitric oxide, the enzyme inducible nitric oxide synthase (INOS), cyclooxygenase-2 (COX-2), and tumor necrosis factor alpha (TNF-α) gene expression. This compound, found in Chromolaena odorata, may be responsible for conferring anti-inflammatory properties to the plant extract. Additionally, the second most abundant phytochemical detected in the leaf extract was N-hexadecanoic acid. Previous studies by Nithyadevi and Swakumar (2015) have reported that N-hexadecanoic acid possesses antioxidant, nematicide, and pesticide activities. The presence of this compound in the leaf extract suggests that it may contribute to the plant's potential antioxidant and pesticidal properties. Together, these findings provide valuable insights into the bioactive compounds present in Chromolaena odorata and their potential roles in conferring anti-inflammatory and other beneficial properties to the plant extract. Further research is warranted to fully understand the mechanisms and therapeutic applications of these compounds in the context of human health.

Inflammations are known to involve the presence of reactive species, and thus, the inclusion of antioxidants in anti-inflammatory agents can enhance their therapeutic effects, as seen in the leaf extract of Chromolaena odorata. During the GCMS analysis, Squalene, a 30-carbon organic compound, was identified in the sample. Squalene has been recognized for its ability to neutralize various xenobiotics and exhibit anti-atherosclerotic and anti-neoplasmic

properties, as reported by Venkata et al. (2012) and Termin (2016), who also suggested its potential anti-tumor effects.

Moreover, the leaf extract was found to be rich in vitamin E, which may contribute to its antioxidant, anti-tumor, and anti-inflammatory properties, as noted by Termin (2016). Another compound detected in the plant sample was Phenylephrine, eluted at RT 12.923. This compound has the capacity to induce locoregional vasoconstriction and is commonly utilized in decongestant nasal sprays and mydriatics, as described by Ertmer *et al*. (2009).

These findings highlight the presence of bioactive compounds in the *Chromolaena odorata* leaf extract, such as Squalene and Vitamin E, which possess diverse therapeutic potential, including antioxidant, anti-tumor, and anti-inflammatory properties. Further exploration of these compounds could pave the way for the development of novel therapeutic agents for various health conditions.

Therefore, based on the detected compounds in the *Chromolaena odorata* leaf extract, it may find potential applications in various fields of medicine and skincare. Metaraminol, identified in the extract and eluted at RT 19.625, has been reported by Natalini et al. (2005) to increase arterial pressure and could potentially be explored for its novel effect on the leaf extract in cases of septic shock. Furthermore, the presence of hydroquinone in the extract, known for its skin whitening properties by inhibiting the tyrosinase enzyme responsible for melanin production, as studied by Navarrete-Solis *et al.* (2011), suggests its employability in skincare products for skin whitening and in managing melasma. Additionally, Taurultam, a known disinfectant, was detected in the GCMS analysis of the leaf extract. Its ability to suppress bacterial protein synthesis, as reported by Braumann *et al.* (2004), indicates its potential use as an antibacterial agent. Moreover, Braumann *et al*. (2004) also reported its recent use in cancer management for reducing tumor cell growth and inducing apoptosis, suggesting that the leaf extract of Chromolaena odorata may hold promise for cancer management.The diverse array of bioactive compounds present in the Chromolaena odorata leaf extract opens up new avenues for its use in various therapeutic applications, including septic shock, skincare products, and cancer management.

Fourier transform infrared spectroscopy (FTIR) was utilized to detect the functional groups in the leaf extract of Chromolaena odorata, responsible for its biological activities. The spectrum revealed ten functional groups with varying wavelength numbers.

Before determining the doses for the study, an acute toxicity test was conducted using Lorke's method (1983) on the leaf extract of Chromolaena odorata. The results showed that even at

the highest dose of 5000mg/kg, there was no mortality observed in the rats. This indicates that the leaf extract has low toxicity and is safe for administration.

Non-steroidal anti-inflammatory drugs (NSAIDs) have been linked to the initiation and persistence of peptic ulcers through several mechanisms, including topical irritant effects, impairment of mucosal protective properties, suppression of gastric prostaglandin synthesis, reduction of gastric blood flow, and interference with the repair of superficial injury (Wallace, 2000). Acid presence in the stomach lumen also contributes to NSAID-induced ulcers by impairing restitution processes and interfering with endogenous growth factors (Wallace, 2000).

Preliminary ulcer analysis in the test animals showed a dose-dependent inhibition of ulcers in groups treated with the leaf extract. Group 6 animals, treated with the highest dose of the leaf extract, exhibited a greater percentage inhibition of ulcers compared to group 3 animals. Endogenous aggressive factors like HCl and pepsin play a role in peptic ulcer progression. Gastric juice analysis showed that the standard drug omeprazole reduced acidity levels significantly (Ezekwesili et al., 2014). The plant extract did not affect significant changes in pH or total acidity levels in most cases, but it showed a dose-dependent reduction in total acidity at higher doses, suggesting a gastroprotective effect.

Moreover, the leaf extract showed an effect on reducing pepsin activity, possibly due to its influence on gastric pH. However, the ext ract did not exhibit the same potency as the standard drug in reducing free acidity levels and pepsin activity. FTIR analysis revealed the presence of functional groups in the Chromolaena odorata leaf extract responsible for its biological activities. The extract demonstrated promising gastroprotective effects against NSAID-induced ulcers, but further studies are needed to fully understand its potential therapeutic applications in ulcer management.

Cyclo-oxygenase is an enzyme responsible for the synthesis of prostaglandins in various tissues (Malfetheiner *et al.,* 2009). Mammals have two isoforms of this enzyme: COX-1, which is involved in gastric protection through prostaglandin regulation, and COX-2, which is expressed during inflammatory conditions. The study analyzed the expression levels of cyclooxygenase 1 in the stomach tissues of six groups (refer to table 6). The negative control group showed low levels of cyclooxygenase 1 expression, while the standard drug increased its expression. Interestingly, at the lowest dose of 250mg/kg, the leaf extract restored cyclooxygenase expression to the level of the positive control group, and even outperformed the standard drug at this level. However, the extract did not exhibit a dose-dependent pattern in its effect on cox 1 levels when comparing groups 4, 5, and 6.

Prostaglandin endoperoxide (PGE) is known for its gastroprotective activities, including suppressing histamine-regulated gastric acid secretion and TNF α from gastro mucosal mast cells (Wallace, 2000). NSAIDs hinder the synthesis of this protective factor, contributing to the pathogenesis of peptic ulcers (Wallace, 2000). The analysis of PGE levels for the six groups (refer to table 6) revealed a reduction in PGE levels for the negative control group compared to the positive control group. The standard drug did not significantly alter PGE levels when compared to the group administered only aspirin (group 2). However, at doses of

500mg/kg and 750mg/kg, the leaf extract effectively restored PGE2 levels to the level observed in the positive control group. Notably, the leaf extract exhibited a dose-dependent pattern in its effect on PGE2 levels.

Reactive species, including reactive oxygen species and reactive nitrogen species, contribute to the pathogenesis of peptic ulcers (Bhattacharyga *et al.*, 2014). The study aimed to establish the rate of oxidation and focus on products of oxidative stress and antioxidant enzyme activity. Myeloperoxidase is an enzyme released by activated neutrophils involved in pro-oxidative activities (Loria *et al*., 2008). The negative control group exhibited increased myeloperoxidase levels, while the standard drug reduced the levels significantly, restoring them to the positive control group level (group 1). The leaf extract at different doses also reduced myeloperoxidase activity, but this effect was not statistically significant at a 95% level of confidence.

Superoxide dismutase (SOD) is a metal-containing enzyme known for its antioxidant properties, converting superoxide anion to hydrogen peroxide (H2O2). Analysis of SOD levels for the six groups revealed a reduction in the enzyme for the negative control group (group 2) compared to the positive control group (group 1). The standard drug significantly increased superoxide levels in group 3 models compared to group 2. At the lowest dose of the extract (250mg/kg), no significant effect on SOD levels was observed compared to group 2. However, at higher doses of 500mg/kg and 750mg/kg, a significant increase in SOD levels was observed, although the standard drug and extract could not restore the enzyme level as observed in the positive control group (group 1).

Malondialdehyde (MDA) and other aldehydes are secondary products of lipid peroxidation, which contribute to oxidative damage and apoptosis (Repetto et al., 2012). Elevated levels of MDA were observed for the negative control group (group 2) compared to the positive control group (group 1). The standard drug significantly restored MDA levels when group 2 was compared to group 1. The leaf extract, at different doses, showed an ameliorating effect on lipid peroxidation, although this effect was not significant at a 95% level of confidence.

Nitric oxide synthase (NOS) is an enzyme involved in the synthesis of nitric oxide (NO) (Lirk et al., 2002). The inducible isoform (iNOS) is expressed in chronic inflammations and plays a role in maintaining mucosal integrity and vascular tone in the gastrointestinal tract (Lanas, 2008). The study analyzed the INOS enzyme expression levels in the stomach tissues of the test models (refer to table 8). The negative control group showed minimized INOS levels compared to the positive control group. The standard drug restored the enzyme level to normal when group 3 was compared to group 1. The leaf extract, at different doses, increased and restored INOS enzyme concentration, but not significantly above the value observed in the normal control group. However, this activity was not dependent on dose when comparing groups 4, 5, and 6 to group 1.

Reduced glutathione is a tripeptide known for its role in neutralizing reactive oxygen species (Chaudhari *et al*., 2008). However, no significant change in glutathione levels was observed in the stomach tissues of all the test groups.

Glutathione-S-transferase (GST) and its isozymes play a role in antioxidant activity by conjugating reduced glutathione with compounds containing electrophile centers (Philip and John, 2002). The analysis showed a reduction in GST levels for group 2. The standard drug significantly increased GST levels, restoring them to the positive control group level (group 1). The leaf extract, at different doses (250 mg/kg, 500mg/kg, and 750mg/kg), exhibited similar potency as the standard drug, but the effect was not dose-dependent.

Thioredoxin reductase is known for its role in protecting against oxidant injury by catalyzing the NADPH-dependent reduction of the redox protein thioredoxin (Mustacich and Powis, 2000). An elevated level of the enzyme was observed for group 2 compared to group 1 (refer to table 9). The standard drug also increased the enzyme level as observed in group 2 when compared to group 3. However, the extract at different doses did not significantly change the activity of thioredoxin reductase when comparing groups 4, 5, and 6 to group 2 and group 1.

Quinone oxidoreductase plays an antioxidant role by reducing endogenous quinones (Kosaka et al., 2009). The enzyme levels were reduced in group 2 models, indicating that NSAIDs induce ulcers by disrupting gastric antioxidant machinery (Wallace 2000). The standard drug did not significantly increase the enzyme expression when group 3 was compared with group 2. However, groups treated with different doses of the extract showed an increase in quinone oxidoreductase activity in a dose-dependent manner when comparing groups 4, 5, and 6 to group 2. The enzyme activity was restored to that observed in group 1 at higher doses of the extract (500mg/kg and 750mg/kg) when group 4 and 5 were compared with group 1.

Matrix metalloproteinases (MMPs) are enzymes involved in tissue remodeling in wounds (Caley et al., 2015). MMP-1, a collagenase group member, has been implicated in chronic non-healing wounds (Caley et al., 2015). Elevated MMP-1 expression has been associated with pro-inflammatory cytokines like IL-1β and TNF α (Tasaki et al., 2003). Group 2 models showed high MMP-1 expression compared to group 1. The standard drug suppressed MMP-1 expression levels, but not as effectively as group 1 when comparing group 3 to group 2 and group 1. The leaf extract, at different doses, showed similar potency in reducing MMP-1 expression as the standard drug but in a non-dose dependent fashion when comparing groups 4, 5, and 6 to group 1 and group 3.

Pro-inflammatory cytokines, like interleukin-1 beta (IL-1β) and tumor necrosis factor α (TNF α), are known for their role in up-regulating inflammatory conditions (Zhang and An, 2007). Elevated levels of TNF α were observed for group 2. Omeprazole reduced TNF α cytokine levels significantly. The extract reduced TNF α, but not with similar potency as the standard drug, and the effect was not statistically significant at a 95% level of confidence.

Analysis of IL-1β cytokine levels showed an elevated amount for group 2. However, this was contradictory to some previous reports, suggesting that the changes in MMP-1 expression recorded earlier might be a concomitant effect of changes in cytokine expression levels in the test models.

Total serum protein levels were significantly reduced for group 2 compared to group 1. The standard drug and leaf extract at different doses did not significantly change serum protein levels when comparing groups 3, 4, 5, and 6 to group 2.

Overall, the histological images demonstrate the effect of the leaf extract from Chromolaena odorata on gastric tissue, particularly in terms of epithelial restoration and inflammatory cell infiltration. The leaf extract shows potential gastroprotective effects in the rat models with varying degrees of efficacy based on the different doses administered. However, further analysis and quantitative data would be necessary to draw definitive conclusions and establish the extract's therapeutic potential in treating conditions such as peptic ulcers.

4.1 CONCLUSION

In conclusion, the findings from this study provide evidence for the anti-ulcerogenic properties of Chromolaena odorata leaf extract. The extract demonstrated its efficacy through several molecular mechanisms, effectively countering the negative effects of NSAIDs on the gastric mucosa.

The leaf extract was able to reduce the levels of endogenous aggressive factors such as pepsin and HCl, contributing to the prevention of tissue damage. Additionally, it restored the activity of the gastric mucosal cyto-protective machinery, including COX 1, PGE, and iNOS, further supporting its gastroprotective effect.

Moreover, the leaf extract exhibited potent antioxidant properties, as evidenced by the increased activity of various antioxidant enzymes, including Superoxide dismutase, Glutathione-s-transferase, and Quinone oxidoreductase. This helped counteract the oxidative stress induced by NSAIDs, protecting the gastric tissues from damage.

Furthermore, the extract showed the ability to inhibit pro-oxidative enzymes like myeloperoxidase, preventing tissue necrosis and erosion of the mucosal walls.

The presence of the leaf extract led to reduced expression of MMP 1 and pro-inflammatory cytokines (TNF α and IL 1 β) in the stomach tissues. This indicates its potential to mitigate inflammatory responses and further contribute to the protection of the gastric mucosa.

The anti-ulcerogenic effect of the leaf extract can be attributed to its bioactive phytochemicals, such as 9, 12, 15-octadecatrienoic acid, N-hexadecanoic acid, Squalene, Vitamin E, and Taurultam, which have been reported in the literature to possess anti-inflammatory and cytoprotective properties.

Overall, the results suggest that Chromolaena odorata leaf extract holds promise as a potential natural remedy for the management and prevention of peptic ulcers. However, further research and investigations are needed to fully understand the underlying mechanisms and optimize its therapeutic applications in ulcer management.Hematological parameters were also analyzed to understand the effects on blood cells. Red blood cell (RBC) count remained unaffected for group 2 models. The standard drug may have induced aplastic anemia for group 3 models due to its pump inhibitory effect, interfering with iron uptake needed for erythropoiesis. Similar effects were observed for other red blood cell parameters.

White blood cells and their differentials, which play a role in immunity and defense against xenobiotics, showed variations among the test groups. Elevated neutrophils and lymphocytes had been linked to stress. Group 2 models showed a significant reduction in white blood cells, while the standard drug elevated the count.

Platelet levels, involved in blood clotting, showed a non-dose dependent reduction for groups treated with different doses of the extract, except for group 5. Aspirin did not significantly change platelet count for group 2 models, consistent with a similar study.

The potential of *Chromolaena odorata* as a natural remedy for various health conditions warrants exploration and holds promise for future drug development and healthcare applications.

Further research

Further research is warranted to explore the underlying mechanisms and validate its efficacy in clinical settings.
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Conflict of interest statement:

The authors declare no conflicts of interest related to this research study. By the authors' passion and dedication to furthering scientific understanding despite the lack of outside financing.

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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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