ANTIMICROBIAL POTENTIALS OF LEAF EXTRACTS OF *TRIDAX PROCUMBENS* AND TOXICITY ON MAMMALIAN CELLS

[1]Babayi Hausatu; [2]Ofide Cyril; [3]Elisha Baba; [4]S. K. Abubakar-Akanbi & [5]Rahmat Mummy Isah

[1]Department of Microbiology, School of Life Sciences, Federal University of Technology, P.M.B. 65, Minna, Niger State, Nigeria

[2]Centre for Genetic Engineering and Biotechnology, Federal University of Technology, P.M.B. 65, Minna, Niger State, Nigeria

[3]Clinic, National Institute for Pharmaceutical Research and Development, Idi-Abuja, Nigeria

[4]Department of Morbid Anatomy, Federal Medical Centre, Bida, Niger State, Nigeria

[5]Department of Microbiology, Ibrahim Badamasi Babangida University, PMB 11 Lapai, Niger State, Nigeria

E-mail: acadbabayi@futminna.edu.ng **Phone:** 08033700406.

Abstract

Tridax procumbens leaves were extracted to obtain hexane, ethyl acetate, methanol and aqueous extracts. An in vitro antimicrobial assay was conducted against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella Typhi, Shigella flexneri, Klebsiella pneumoniae and Candida albicans. The safety of the extract was assessed by acute and sub-chronic toxicity tests to determine the toxic dose and to examine the impact of its long term administration. The aqueous extract inhibited the growth of S. Typhi and S. aureus with minimum inhibitory concentration (MIC) of 5 mg/mL and minimum bactericidal concentration (MBC) value of 10 mg/mL. The hexane extract inhibited the growth of S. Typhi, S. aureus, and C. albicans at 10 mg/mL with MIC of 5 mg/mL and an MBC value of 10 mg/mL. Acute toxicity studies showed an LD_{50} value that was greater than 5000 mg/kgbw. Sub-chronic studies revealed significant alterations in the haematological indices of animals exposed to 3000 mg/kgbw extract concentration. These results indicated that the oral administration of T. procumbens leaves is non-toxic at low doses and safe for use as a therapeutic for managing clinical conditions associated with Salmonella Typhi and Staphylococcus aureus. However, long-term administration at elevated doses may have adverse effect on renal and hepatic tissues.

Keywords: Tridax Procumbens, Toxicity, Mammalian Cells, Staphylococcus aureus, Escherichia coli.

Introduction

Man has always been inundated with the ever-present innumerable number of microorganisms of the disease producing type that are always trying to develop resistance (Culyba & VanTyne, 2021). Presently there are rising global concerns of microbial resistance to antibiotics arising from their misuse, abuse, indiscriminate and irrational usage (Vivas *et al*., 2019). The steady increase over the years of the prevalence and incidence of infections caused by multi-drug resistant (MDR) pathogenic microorganisms is obviously reflected by the increasing number of familiar acronyms used to describe the causative agent and sometimes the infection generally (Garoy *et al*., 2021).The available antibiotic agents which are getting fewer as a result of multi drug resistance (Cerceo *et al.*, 2016), are costly, associated with noxious side effects (such as hypersensitivity, allergic reactions, immunosuppression, to mention but a few) and treatment failure (a major contributory factor to high cost) (Kharchoufa *et al*., 2020).It has been estimated that by 2050, there will be no effective antibiotic left to combat infections if no new drug is discovered (Rolain *et al*., 2016).

The demerits associated with the usage of synthetic antimicrobial agents highlighted above has called for the search for new potent economical alternatives (Ashraf *et al*., 2021) from other sources such as plants with minimal to near absence of toxic effects (Knöss, 2017).As a result of coevolution of plants with pathogens through eons of time, they have developed an array of structural, chemical protection pathways, and protein-based defenses designed for protection against biotic and abiotic stressors. Man also in turn benefited from these protection mechanisms when he ingested these plants. One of such plants known to be associated with various pharmacological and antimicrobial potential is *Tridax*

procumbens. *T. procumbens* (*Asteraceae*) is one of the most important plants commonly found in sub-tropical countries. Its common English names are Coat Buttons, Tridax Daisy. The Igbo people of south eastern Nigeria call it *Mbuli*. Traditionally it is used for the treatment of bronchial catarrh, dysentery, malaria, stomach ache, diarrhoea, high blood pressure and to check haemorrhage from cuts, bruises and wounds and to prevent falling of hair. Contrary to the belief that natural medicine has no ill effects; several people have been hospitalized for consuming plants of unknown properties. Hence this study was carried out to provide new information on the antimicrobial activity of the leaf extract of *T. procumbens* and determine its phytochemical constituents in a bid to verify and validate its efficacy.

Materials and Methods

Collection and Identification of Plant

Fresh and healthy leaves of the plant were collected from River Basin Nursery, Minna, Niger State and were identified as *T. procumbens* at the Department of Biological Sciences, Federal University of Technology, Minna by two independent ethnobotanists. Voucher specimen was deposited at the Herbarium Unit of the Department.

Microbial Isolates

The test isolates used were *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staohylococcus aureus*, *Escherichia coli*, *Shigella* species, *Salmonella* Typhi and *Candida albicans* obtained from the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja. The method described by NIPRD (2004, 2006) were employed in standardizing the fungal and bacterial isolates. Briefly, 5 ml of sterile nutrient broth were dispensed into test tubes and a loopful of each test isolate was inoculated into the broth. Incubation was done at 37℃ for 24 hours. Exactly 0.2 mL of overnight culture of each organism was dispensed into 20 mL of sterile nutrient broth and incubated for 5 hours at 37℃ to standardize the culture to 10^6 cfu/mL.

Laboratory animals

Forty-eight (48) albino rats were collected from the animal house of the Biochemistry Department, Federal University of Technology, Minna. They were housed in plastic cages bedded with dry clean wood shavings. They were maintained at room temperature of 28±2℃ and observed under 12 hour light/dark cycle in a well-ventilated room for one week before the commencement of the experiment. The animals were fed with standard NIPRD formulated feed and water *ad libitum*. The experimental rooms were cleaned and disinfected regularly. Soiled wood shavings were replaced when due. The feed containers and animal cages were cleaned regularly. The animals were housed and cared for in accordance with good laboratory practice (GLP) regulations of World Health Organisation (1998) and the principles of animal care (National Institute of Environmental and Health Sciences (NIEHS), 1985) were followed throughout this study.

Extract Preparation

The method recommended by NIPRD (2009) was employed for the extraction of leaves of *T. procumbens*. The leaves were briskly plucked from the stem and air dried at ambient temperature (28±2℃) for three (3) weeks. The dried leaves were crushed and pulverized into powder using mortar and pestle. Fifty grams (50 g) of the powdered sample was extracted in 200 ml of sterile distilled water by refluxing for six (6) hours. The mixture was filtered with a Whatman filter paper No.1 and evaporated to dryness using a rotary evaporator at 50℃. Hexane, methanol and ethyl acetate were also used for extraction of the plant sample as described above. The extracts were collected in sterile and airtight sample bottles and stored at 4℃ until required for use.

Phytochemical Screening

The phytochemical studies of the extracts of *T. procumbens* were performed qualitatively to determine the presence of secondary metabolites such as alkaloids, glycosides, flavonoids, phenols, tannins, saponnins, sterols and terpenes according to the method of Harborne (1973).

Antimicrobial Assay of the Extracts of *T. procumbens*

Agar dilution method of Collins *et al*. (1995) was used to test for the antibacterial activity of aqueous extract. The efficacy of the extract was compared with the MIC of ampiclox (Beechan) for the test organisms. One gram (1.0 g) of the extract was reconstituted in 5 ml sterile distilled water to obtain a final concentration of 20 mg/mL. The extract was vortexed for homogeneity. One millilitre of the reconstituted extract was added to Petri dish containing 19 mL of sterile molten nutrient agar (Oxoid) to obtain a final concentration of 10 mg/mL. The plates were prepared in duplicates and allowed to set at ambient temperature (28±2℃).

A loopful each of the standardized test organisms was streaked on the solidified medium incorporated with extract. The plates were incubated for 24 h at 37℃. Control plates comprised medium without inoculum represented as the medium sterility control (MSC) plate, medium with extract tagged as the extract sterility control (ESC), and inoculum with medium designated as organism viability control (OVC) plates were prepared in parallel. The activities of hexane, methanolic and ethyl acetate extracts were similarly determined. NIPRD (2006) protocol was employed for the determination of antifungal activity of the medicinal plant extracts. The experiment was done in duplicate. The extract efficacy was compared with the MIC of fluconazole (Diflucan) for the fungus.

Minimum Inhibitory Concentration

The MIC was evaluated using the two fold serial broth dilution method of Chattopadhyay *et al*. (2001). Concentration range of 20 mg/mL, 10 mg/mL, 5.0 mg/mL and 2.5 mg/mL of the stock solution were obtained using the method above. A loopful of the standardized organisms was introduced into each of the concentration obtained above. Incubation was done at 37℃ for 18-24 h. The least concentration with no visible growth was taken as the MIC of the extract. Minimum fungistatic concentration of the extract for the fungus was also determined.

Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

The MBC and MFC were determined using the agar dilution method described by Lajubutu *et al*. (1995). A loopful of the MIC samples where no visible growth occurred were subcultured into fresh sterile nutrient agar and incubated at 37℃ for 18-24 h. Absence of growth on plate was interpreted as bacteriocidal while presence of growth was interpreted as bacteriostatic.

Acute toxicity studies (LD_{50}) of aqueous extract of *T. procumbens*

The method of Aniagu *et al.* (2005) was employed in the acute toxicity studies (LD_{50}) of aqueous extract of *Tridax procumbens*. Aqueous extract was selected as against hexane extract which inhibited more organisms. Twelve (12) rats were divided into six (6) groups of two each and treated with various doses of the aqueous leaf extract of *T. procumbens* corresponding to 10 mg/kgbw, 100 mg/kgbw and 1000 mg/kgbw on the first day; 1600 mg/kgbw and 2900 mg/kgbw on the second day and 5000 mg/kgbw on the third day were administered orally in the three (3) day study group. Another group of two rats served as control and this received 1 ml of distilled water.

They were all placed under observation immediately for thirty (30) minutes, four (4) hours, twenty four (24) hours, and seventy two (72) hours for any behavioural changes: Bizarre behaviour such as self-mutilating and walking backward, stereotype behaviour such as excessive grooming and repetitive circling, neurological changes and then mortality. All observations of wellness parameters were systematically recorded with individual records being maintained for each animal. Observations included changes in skin and fur, eyes and mucous membranes, gait, posture, grip/grasp strength, and response to handling. Attention was given for observations of tremors, convulsions, presence of clonic or tonic seizures, Occurrence of secretions/excretions or other evidence of autonomic activity such as lacrimation, piloerection, pupil size change, unusual respiratory pattern, salivation, diarrhoea, lethargy, sleep, coma and mortality.

Sub-chronic toxicity studies of aqueous extract of *T. procumbens*

The method of Aniagu *et al*. (2005) and Salawu *et al*. (2009) was employed in the sub chronic toxicity study of aqueous leaf extract of *T. procumbens* for 28 days in albino rats. Thirty-six rats were divided into six (6) groups of six (6) each. Three (3) groups were administered, orally 1000, 2000 and 3000

mg/kgbw of the plant extract respectively, the next two (2) groups were administered orally 16.7 mg/kgbw of immunostimulant drug, Jobelyn formula and 0.167 mg/kgbw of immunosuppressant drug, dexamethasone respectively, daily for twenty-eight (28) days. The last was administered 0.4 ml normal saline serving as the control. The six (6) groups were observed for any sign of toxicity and mortality for the study period.

On the 28th day, the rats were sacrificed under chloroform anaesthesia. The anesthetized rats were restrained in dorsal recumbency and the abdominal wall was incised just caudal to the xiphoid cartilage. The xiphoid cartilage was grasped using sterile forceps, and the rib cage was retracted cranially to visualize the heart. A (21- SWG) gauge needle affixed to a 5-ml syringe was used to collect the blood from the left ventricle (oxygenated blood) and was transferred into plain capped bottles containing ethylenediaminetetraacetic acid (EDTA) for estimation of differential cells and packed cell volume (PCV), haemoglobin concentration (HB), red blood cell count (RBC), platelet, white blood cell count (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) using micro haematocrit and the haemocytometer methods (Schalm *et al.,* 1975).

Histopathology Studies

The methods of Vernon (2005) and Iqbal *et al.* (2016) were employed for the histopathology investigation of *T. procumbens* on the tissues. At the end of the experiment, the rats were weighed and anaesthesized in a chloroform jar. The organs were removed after dissecting each rat through an incision on the abdomen. The brain, heart, lungs, liver, kidney, spleen, intestines and stomach were removed and examined macroscopically for any structural, physiological or physical changes such as congestion, consolidation, necrosis, hyperaemia and hypertrophy and were graded according to none (1), mild (2), moderate (3) or severe (4). After each weight had been taken, the tissue samples were fixed by immersion in 10% phosphate-buffered formalin for 24 h, dehydrated through a series of graded concentrations of alcohol, cleared in xylene, infiltrated and embedded in molten paraffin wax.

Tissue blocks were sectioned at 5µ using a microtome, deparaffinised, fixed on grease-free glass slides and stained with haematoxylin and eosin (H&E) for light microscopic examination. Organ pathological changes were scored in the rats according to the method of Fadina *et al*. (1999). Photomicrographs of some of the lesions from the sections were taken using an Olympus light microscope attached with a 20 megapixel Kodak digital camera at different magnifications.

Statistical analysis

All numeric data were expressed as the mean ± standard error of mean (SEM). Statistical analysis was determined using Statistical package for social sciences version 19.0 software. Significant difference between means was accessed by student t-test and ANOVA at 95% level of significance.

Results

The aqueous leaf extract obtained was dark brown and sticky. The percentage (%) yield of the leaf extracts were 19.42%, 25.17%, 23.06% and 28.3% for the hexane, ethyl acetate, methanol and aqueous extracts respectively. Preliminary and confirmatory phytochemical investigation of the crude leaf extracts of *T. procumbens* revealed the presence of one or more of the following phytochemical compounds: saponins, terpenes, alkaloids, proteins, amino acids, cardiac glycosides as shown in Table 1. Similarly, tannins, flavonoids and free anthraquinones were not detected in the crude extracts tested in the present study.

Table 1: Phytochemical constituents of leaf extracts of *Tridax procumbens*

Phytochemical constituents	Extracts			
	Hexane	**Ethyl acetate**	**Methanolic**	**Aqueous**
Saponins	−	−	+	+
Tannins	−	−	−	−
Terpenes	+	+	+	+
Flavonoids	−	−	−	−
Proteins	+	+	+	+
Free anthraquinones	−	−	−	−
Alkaloids	+	+	+	+
Amino acids	+	+	+	−
Cardiac glycosides	−	+	+	−
Glycosides	−	−	+	+
Steroids	+	+	+	−

+: Present −: Absent

In vitro antimicrobial assay showed that aqueous leaf extract of the plant inhibited the growth of *S* .Typhi and *S. aureus*. *Salmonella* Typhi, *S. aureus* and *C. albicans* were susceptible to the hexane extract at 10 mg/mL, *K. pneumoniae, P. aeruginosa* and *Shigella* sp. were resistant to hexane extract at 10 mg/mL (Table 2).

Table 2: Antimicrobial activity of the aqueous leaf extract of *T. procumbens*

Test organisms	Extracts (mg/mL)			
	Hexane	**Ethyl acetate**	**Methanolic**	**Aqueous**
S. aureus	+	−	−	+
S. Typhi	+	−	−	+
Shigella species	−	−	−	−
P. aeruginosa	−	−	−	−
E. coli	−	−	−	−
K. pneumoniae	−	−	−	−
C. albicans	−	+	−	−

+: Activity, −: No activity, mg/mL: gram per millilitre

The MIC of the aqueous extract for *S.* Typhi was 2.5 mg/mL and 5 mg/mL for *S. aureus*. MBC of the extract was 5 mg/mL for *S.* Typhi and 10 mg/mL against the *S. aureus* as shown in Table 3. The MIC of the hexane extract for *S.* Typhi, *S. aureus* and *C. albicans* were 2.5 mg/mL, 5 mg/mL and 2.5 mg/mL respectively, while the MBC were 5 mg/mL, 10 mg/mL and 10 mg/mL respectively (Table 4).

Table 3: The MIC and MBC of aqueous leaf extract of *Tridax procumbens*

Test organisms	Concentration of extract (mg/mL)					
	10	**5.0**	**2.5**	**1.25**	**MIC**	**MBC**
S. Typhi	+	+	+	−	2.5	5.0
S. aureus	+	+	−	−	5.0	10.0
C. albicans	−	−	−	−		

+: Activity, −: No activity, MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration, mg/mL: Milligram per millilitre.

Table 4: The MIC and MBC of hexane extract of *Tridax procumbens*

Test organisms	Concentration of extract (mg/mL)					
	10	**5.0**	**2.5**	**1.25**	**MIC**	**MBC**
S. Typhi	+	+	+	−	2.5	5.0
S. aureus	+	+	−	−	5.0	10.0
C. albicans	+	+	+	−	2.5	5.0

+: Activity, −: No activity, MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration, mg/mL: Milligram per millilitre

Acute toxicity evaluation of aqueous leaf extract of *T. procumbens* revealed an oral LD_{50} value greater than 5000 mg/kgbw as shown in Table 5. Neither mortality nor abnormal clinical signs were observed in the rats at 10, 100, 1000, 1600, 2900 and 5000 mg/kgbw after 72 h post oral treatment.

Table 5: Acute toxicity of oral administration of aqueous extract of *T. procumbens* in rats

Groups	Dose (mg/kgbw)	No. of Demised/ survived animals
Control	0.4 ml of distilled water	0/3
1	10	0/3
2	100	0/3
3	1000	0/3
4	1600	0/3
5	2900	0/3
6	5000	0/3

The mean daily feed and fluid intake reduced significantly ($P<0.05$) across all the groups but the decline was more pronounced in the 3000 mg/kgbw group and dexamethasone group compared to the control. The reduction in feed and fluid consumption by the administration of 1000, 2000 and 3000 mg/kgbw was shown in Tables 6 and 7 did not affect the animals physically. The result as shown in Table 6 revealed the group that received 1000, 2000 mg/kgbw and Jobelyn formula showed increase in cumulative body weight which was not significantly ($p>0.05$) different compared with the control. Conversely, the groups administered 3000 mg/kgbw and Dexamethasone showed reduction in body weight significantly lower ($p<0.05$) than the control.

Table 6: Effect of aqueous leaf extract of *Tridax procumbens* on daily feed intake of rats

Treatments (mg/kgbw)	Feed intake (g)			
	1st week	**2nd Week**	**3rd Week**	**4th Week**
Control	102.86 ± 3.09^{a}	131.14 ± 6.36^{a}	163.14 ± 1.55^{a}	179.42 ± 3.39^{a}
1000	108.28 ± 2.00^{a}	103.14 ± 0.98^{b}	97.86 ± 1.30^{b}	87.42 ± 2.03^{b}
2000	108.29 ± 0.61^{a}	106.29 ± 2.51^{b}	99.43 ± 1.58^{b}	80.86 ± 3.54^{b}
3000	88.57 ± 3.57^{b}	82 ± 5.27^{c}	82 ± 2.83^{b}	61.43 ± 4.96^{c}
Jobelyn	100.29 ± 3.29^{a}	127.86 ± 6.14^{a}	161.85 ± 1.53^{a}	179 ± 2.86^{a}
Dexamethasone	88 ± 3.27^{b}	82.57 ± 1.72^{a}	80.00 ± 5.29^{b}	58.57 ± 4.98^{c}

Control = 0.4 ml normal saline. Values are means ± S.E.M for n = 6. *: Mean data on column carrying the same superscript do not differ significantly from each other ($p>0.05$).

Table 7: Effect of aqueous leaf extract of *Tridax procumbens* on daily fluid intake of rats

Treatments (mg/kgbw)	Fluid intake (ml)			
	1st week	**2nd Week**	**3rd Week**	**4th Week**
Control	217.71 ± 7.52^{a}	294.71 ± 4.37^{a}	331.86 ± 3.13^{a}	371 ± 5.10^{a}
1000	218.571 ± 3.86^{a}	211 ± 1.63^{b}	203.71 ± 0.96^{b}	193.85 ± 1.21^{b}
2000	220.14 ± 6.66^{a}	211.43 ± 5.86^{b}	207.14 ± 1.99^{b}	167.14 ± 6.51^{b}
3000	208.57 ± 4.43^{a}	169.28 ± 4.27^{c}	151.14 ± 8.47^{c}	98.57 ± 5.36^{c}
Jobelyn	215.71 ± 6.28^{a}	287.42 ± 3.48^{a}	330.29 ± 3.28^{a}	360.29 ± 7.26^{a}
Dexamethasone	194.57 ± 4.58^{a}	162.14 ± 7.53^{c}	152.57 ± 5.74^{c}	90.43 ± 6.48^{c}

Control = 0.4 ml normal saline. Values are means ± S.E.M for n = 6. *: Mean data on column carrying the same superscript do not differ significantly from each other ($p>0.05$)

Table 8: Effect of leaf extract of *Tridax procumbens* on the body weight of rats

Treatments (mg/kgbw)	Weight (g)				
	0 Week	1st week	2nd Week	3rd Week	4th Week
1000	136.87±3.27[a]	141.25±3.40[a]	145.33±2.51[a]	149.33±2.42[a]	164.12±3.45[a]
2000	127.40±2.87[a]	136.95±2.40[a]	147.17±2.68[a]	157.55±3.21[a]	174.67±2.32[a]
3000	129.40±3.83[a]	128.73±3.90[b]	127.22±4.43[b]	120.00±2.93[b]	119.25±3.95[b]
Control	137.00±4.48[a]	146.78±4.23[a]	166.98±4.35[a]	169.56±4.78[a]	197.28±4.92[a]
Jobelyn	124.15±3.30[a]	140.93±3.31[a]	157.32±1.90[a]	158.43±2.37[a]	170.39±1.50[a]
Dexamethasone	136.80±2.75[a]	137.60±2.50[b]	130.34±3.44[b]	124.07±3.26[b]	121.80±3.34[b]

Control = 0.4 ml normal saline. Values are presented as Means ±SEM for n=6 determinations. Values for each of the group of rat weights with different superscripts are significantly different ($p<0.05$) relative to the starting weight at the 1st week of treatment.

The results of the relative organ weight of rats following oral administration of aqueous leaf extract of *T. procumbens* are shown in Table 9. There was significant increase ($p<0.05$) in the relative weight of brain, liver and intestine of the group administered with 3000 mg/kgbw when compared with the control.

Table 9: Effect of the plant extract on the relative organ weights (g) of rats

Organs	Treatment (mg/kgbw)					
	1000	2000	3000	Control	JOB	DEX
Brain	0.68±0.10[a]	0.79±0.07[a]	1.16±0.40[b]	0.67±0.05[a]	0.83±0.06[a]	0.84±0.12[a]
Heart	0.33±0.02[a]	0.31±0.03[a]	0.38±0.04[a]	0.28±0.04[a]	0.38±0.04[a]	0.28±0.02[a]
Spleen	0.51±0.03[a]	0.56±0.06[a]	0.63±0.09[a]	0.30±0.04[a]	0.36±0.05[a]	0.41±0.07[a]
Kidney	0.99±0.08[a]	0.85±0.05[a]	0.97±0.13[a]	0.51±0.05[a]	0.71±0.09[a]	0.65±0.05[a]
Lungs	1.44±0.33[b]	1.59±0.21[b]	1.44±0.22[b]	0.88±0.14[a]	1.32±0.13[a]	0.89±0.07[a]
Liver	3.56±0.27[b]	3.62±0.21[b]	4.44±0.82[c]	2.87±0.35[a]	3.57±0.47[b]	3.59±0.48[b]
Stomach	1.20±0.16[a]	1.44±0.08[a]	1.47±0.22[a]	1.31±0.04[a]	1.45±0.07[a]	1.80±0.13[a]
Intestines	7.71±0.78[b]	7.82±0.40[b]	9.44±0.87[c]	6.48±0.81[a]	9.01±1.52[b]	7.84±1.05[b]

Values are presented as Means±SEM for n=6 replicates. Values for each of the organs with different superscripts are significantly different ($p<0.05$) relative to the control. Control = normal saline (0.4ml). JOB=Jobelyn, DEX=Dexamethasone

Table 10 shows the differential count indices following the oral administration of the aqueous leaf extract of *T. procumbens.* No significant changes were observed in the basophils and the eosinophil counts when compared with the control. However, the neutrophils and monocyte counts reduced significantly ($p>0.05$) when compared with the control group. The lymphocyte count was significantly increased in all the groups ($p<0.05$) when compared with the control group.

Table 10: Effect of the leaf extract of *T. procumbens* on differential counts of albino rats

Differential leucocytes (10^3/mcL)	Treatment (mg/kgbw)					
	1000	2000	3000	Control	JOB	DEX
Basophils	0.20±0.20[a]	0.17±0.17[a]	0.25±0.25[a]	0.25±0.25[a]	0.00±0.00[a]	0.20±0.20[a]
Neutrophils	44.20±3.64[a]	43.00±2.78[a]	42.50±2.53[a]	50.75±4.34[a]	44.25±4.96[a]	44.00±1.90[a]
Lymphocytes	48.80±2.82[b]	50.17±2.37[b]	49.25±2.46[b]	39.75±3.66[a]	49.00±4.34[b]	40.40±1.89[a]
Eosinophils	2.80±0.58[b]	3.00±0.68[b]	2.50±0.50[ab]	3.25±0.48[b]	3.00±0.58[b]	2.20±0.37[a]
Monocytes	4.00±0.63[a]	4.50±0.56[a]	6.00±0.91[b]	6.00±1.47[b]	3.75±0.48[a]	6.20±1.16[b]

Values are Mean± SEM for n=6 determinations. Values with the same superscript along the row are not significantly different at p>0.05. Control= normal saline (0.4ml). JOB=Jobelyn, DEX=Dexamethasone

Results obtained in the study revealed a reduction in haemoglobin concentration of rats administered 2000 mg/kgbw, 3000 mg/kgbw and Jobelyn which did not differ significantly from the control ($p<0.05$) (Table 11). Results from the present study showed a significant ($p<0.05$) decrease in the PCV, MCV and MCH levels of the group exposed to 3000 mg/kgbw of extract. The 1000 mg/kgbw group had a significantly ($p<0.05$) increased WBC count when compared to the control group. Results obtained in the study revealed increase in the platelets and RBC counts which was not significant. The groups that were administered 1000, 2000 3000 mg/kgbw and dexamethasone showed increase in mean RBC count which did not differ significantly from the control.

Table 11: Effect of aqueous leaf extract of *T. procumbens* on haematograms of albino rats

Haematological Parameters	Treatment (mg/kgbw)					
	1000	**2000**	**3000**	**Control**	**JOB**	**DEX**
Hb (g/dl)	12.90±0.81^{b}	12.33±0.87^{a}	11.45±1.09^{a}	13.15±0.35^{b}	11.15±1.00^{a}	13.98±0.37^{b}
PCV (%)	40.40±1.44^{b}	37.17±2.70^{b}	34.00±3.08^{a}	39.50±2.08^{b}	34.00±2.70^{a}	42.60±1.29^{b}
RBC (10^6/uL)	3.74±0.25^{b}	3.52±0.23^{b}	4.30±0.35^{b}	3.98±0.13^{b}	3.20±023^{a}	3.60±0.20^{b}
WBC (10^9/L)	5.24±0.33^{b}	4.18±0.21^{a}	3.98±0.13^{a}	3.93±0.11^{a}	4.40±0.24^{b}	4.26±0.26^{a}
Platelet (10^9/L)	318±2.95^{a}	327±5.18^{a}	333±4.44^{a}	330±5.00^{a}	335.67±2.52^{a}	324±3.24^{a}
MCHC (g/dl)	32.80±0.20^{a}	32.87±0.18^{a}	33.63±0.38^{a}	31.83±1.24^{a}	31.73±1.32^{a}	32.02±1.17^{a}
MCH (pg)	36.00±2.45^{b}	34.50±4.02^{b}	27.50±4.84^{a}	33.00±2.08^{b}	35.00±1.78^{b}	39.00±1.76^{b}
MCV (fL)	99.75±3.82^{ab}	92.20±4.67^{b}	71±5.32^{a}	105±2.09^{ab}	103.33±1.51^{b}	123.4±2.13^{b}

Values are Means± SEM for n=6. Values with the same superscript along the row are not significantly different at p>0.05. Control = normal saline (0.4ml). JOB=Jobelyn, DEX=Dexamethasone

The organ pathology scores of the rats following oral administration of aqueous leaf extract of *T. procumbens* are presented in Table 12. No treatment-related gross pathological changes were found in the brain, heart, spleen, stomach and intestines of the rats in all the treatment groups. The liver, kidney, and lungs of the rats in all the treatment groups showed variable histological changes compared with the morphologically intact control group.
Histological investigations revealed mild hepatic, renal congestion, consolidation, glomerulomatous inflammation, mild renal tubulointerstitial necrosis at extract dose above 2000 mg/kgbw.

Table 12: Effects of aqueous leaf extract of *T. procumbens* on histology of rats

Organ	Control	1000 mg/kgbw	2000 mg/kgbw	3000 mg/kgbw	JOB	DEX
Brain	None	None	None	None	None	None
Heart	None	None	None	None	None	None
Lung	None	None	Congestion[1]	Congestion[1]	None	None
Liver	None	Congestion[1]	Congestion[2]	Consolidation[2]	Congestion[1]	Congestion[2]
Kidney	None	None	None	Necrosis[1]	None	None
Spleen	None	None	None	None	None	None
Intestines	None	None	None	None	None	None
Stomach	None	None	None	None	None	None

1= Mild, 2= Moderate, mg/ml: milligram per millilitre

Fig. 1b is a photomicrograph of a section treated with 3000 mg/kgbw of the plant extract showing lung tissues with reduced and collapsed alveolar air spaces and formation of fibromyloid masses abundantly infiltrated by mixed inflammatory cells with a predominance of macrophages and fibroblast. Fig. 2b is a section of the intestine of the group treated with Jobelyn, showing prominent mesenteric lymphoid follicle composed of lymphoid cells in diffuse sheet. There is scant interstitium and vascular channels. Fig. 3b is a photomicrograph of a section showing the hepatic tissue of the group treated with dexamethasone containing focal nodules of many oval like structure suggestive of worm infestation.

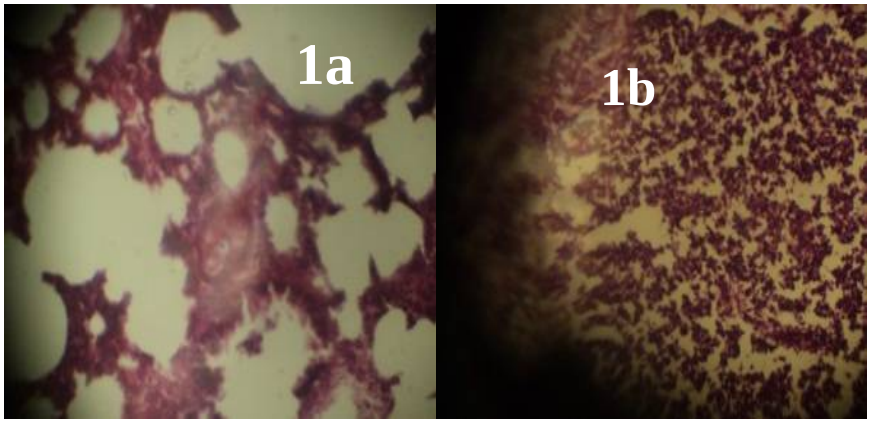

Fig. 1a: Photomicrograph of the control tissue.

Fig. 1b: Photomicrograph of pulmonary tissue pulmonary treated with 3000 mg/kgbw of the plant extract showing an infiltration by inflamatory cells

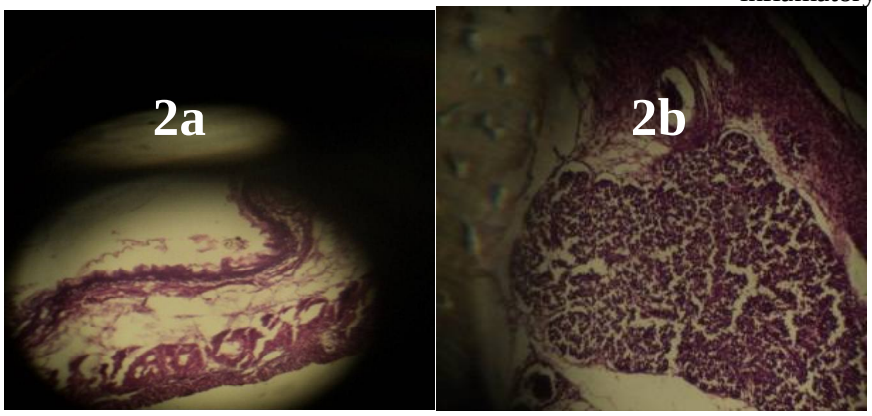

Fig. 2a: Cross-section of the the control group

Fig 2b: Cross-section of the intestine treated with intestine of Jobelyn drug showing prominent mesenteric lymphoid follicle composed of lymphoid cells in diffuse sheet.

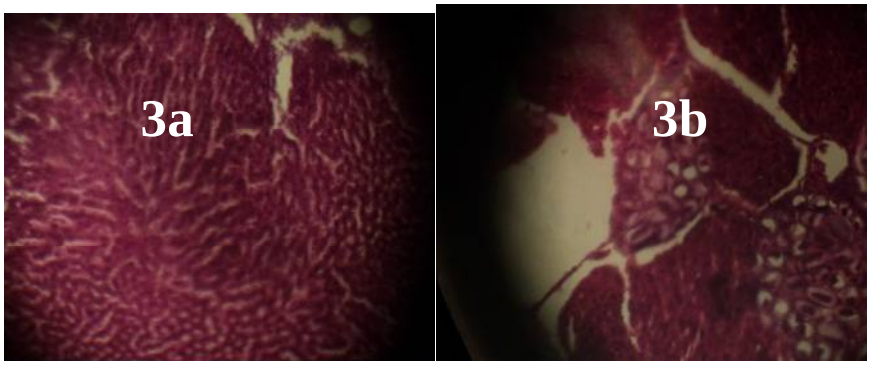

Fig. 3a: Cross-section of the hepatic tissue control group showing hepatocytes radially arranged.

Fig. 3b: Photomicrograph of the liver of the group treated with dexamethasone containing focal nodules of many oval like structure suggestive of worm infestation.

Discussion

Preliminary phytochemical investigation of the crude leaf extracts of *Tridax procumbens* revealed the presence of one or more of the following phytochemical compounds saponins, terpenes, alkaloids, proteins, amino acids, cardiac glycosides, steriods and glycosides. The crude extracts have been reported by other researchers (Yadawa & Saurabh, 1998; Ali *et al*., 2001) to contain these phytochemical compounds.

The result obtained in the present study is consistent with the findings of Rizvi *et al.* (2011) who reported that hexane extract was found to have a more potent inhibitory effect in a study carried out to evaluate the antibacterial potential in the aerial parts of wild *Tridax procumbens*. In addition, the report of the study also agrees with the report of Okiei *et al*. (2011), who observed that hexane fraction components Hex a and Hex b were both active against *Staphylococcus aureus* and *Candida albicans* in a study carried out to comparatively investigate the antimicrobial activity of components of different polarities from the leaves of *Nauclea latifolia*. The potency of the aqueous and hexane extracts observed in the present study could be attributed to the presence of bioactive phytochemical compounds and their differential sensitivity could be ascribed to their morphological differences or adduced to their chemical composition (Ogbonnia *et al.,* 2008).

The result suggests that oral application of the leaf extract may not produce toxic effects at doses lower than 5000 mg/kgbw and so may be considered acutely safe. This observation in the present study is in agreement with the findings of Bulus *et al*. (2011) who reported similar LD_{50} value. Suppression of appetite in these animals may have activated compensatory physiological responses to cope with the stress of hunger and thirst (Babayi *et al.,* 2007) which may have led to reduction in feed and fluid consumption of the animals administered with 1000, 2000 and 3000 mg/kgbw of the extract.

The weight loss observed can be considered an advantage rather than a drawback, by improving overall cardiac health and lowering the risk of the incidence of coronary diseases, diabetes mellitus, dyslipidemia hypertension, obesity and physical inactivity (Reisin *et al*., 1978; Shah, 1991; Krauss *et al*., 2000; Trussell *et al*., 2005; Bantle *et al*., 2006). The result obtained in the present study corroborates the findings of Ikewuchi *et al*. (2010). Increased organ weight (either absolute or relative) observed in the brain, liver and intestine of the group administered with 3000 mg/kgbw could be indicative of organ toxicity (Simmons *et al*., 1995).

Changes in eosinophils, basophil and monocytes indicate a response of the immune system to allergic reactions, while variations in lymphocytes and neutrophil counts may be pointer to the presence of an antigen or an infection. Eosinophils have been recognized for their role as professional antigen

presenting cells in protecting the body against parasites, certain helminths and allergies. The observed elevated lymphocyte counts of the test groups and the group treated with Jobelyn formula suggests a more effective antibody production which corroborates the reports of Tiwari *et al*. (2004) and Oladunmoye (2006) and is a further confirmation that *T. procumbens* extract might have immunostimulatory properties.

Haemoglobin (Hb) is the iron-containing oxygen-transport metalloprotein found in the red blood cells. PCV is the percentage of RBCs circulating in the blood. MCV defines the size of the red blood cells while MCH quantifies the amount of haemoglobin per red blood cell (Bunn, 2011). The low PCV levels suggest that the extract had adverse effect on the erythron, implying reduced oxygen intake by haemoglobin and transport to tissues. The result obtained in this study is consistent with the findings of previous studies (Olakunle & Abatan, 2008; Gupta *et al.,* 2011; Taheri *et al.,* 2013). However, the result differed from the finding of Agbai *et al.* (2015) who observed a significant decrease in WBC counts.

It has been reported that the safety assessment of biologically active compounds in experimental animals are very crucial in accurately predicting toxicity in humans (Zhao *et al*., 1995). Liver and kidney are important organs of metabolism, detoxification, storage and excretion of xenobiotics and their metabolites and especially vulnerable to damage (IARC, 1995). Small intestine and heart just like any other organ can also be affected by toxic effect of chemicals. It is noteworthy that the cytological changes observed in the study which varied in spectrum of severity from mild to severe was consistent with the results of the post mortem pathological scores evidently and most strikingly observed in the kidney and liver of the animals as the extract dose were increased. These findings corroborate the report of Abubakar *et al.* (2012) who observed similar histological changes even at lower extract concentration.

The cytological changes that were observed in the lungs of the group treated with 3000 mg/kgbw of the extract as shown in the photomicrograph may have been due to mode of administration of the extract or may be due to congenital abnormalities. The presence of lymphocytes in the intestines of the group treated with jobelyn is a confirmation of the immunostimulatory property of the drug. The presence of ova in liver of the group treated with dexamethasone showed and confirmed that dexamethasone, a steroid suppresses the immune system.

Conclusion

The present study has proven that the extracts of *T. procumbens* have antimicrobial potential confirming the use of the plant traditionally in the treatment of enteric and wound infections thus supporting their application as an herbal remedy. Further studies are needed to evaluate the effect of the plant extract on plasma biochemical parameters.

Conflicts of Interest

The authors declare that there are no conflicts of interest related to this article.

References

Abubakar, A., Ogbadoyi, E. O.,Okogun, J. I., Gbodi, T. I. & Tifin, U. F. (2012).Acute and Sub Chronic Toxicity of *Tridaxprocumbens*In Experimental Animals. IOSR *Journal of Environmental Science, Toxicology and Food Technology,* 1(6), 19-27.

Agbai, E. O., Mounmbegna, P. P. E., Njoku, C. J., Nwanegwo, C. O., Awemu, G. A.&Iwuji, S. C. (2015). Effect of *Annonamuricata* Seed Extract on Blood Glucose, Total and Differential White Cell Count after Repeated Exposure to Clozapine. *Research in Neuroscience,*4(1), 10-15.

Ali, M., Rawinder, E. & Ramachamdram, R. (2001). A new flavonoid from the aerial parts of *Tridaxprocumbens*. *Fitoterapia,* 3 (1), 313-315.

Aniagu, S. O., Nwinyl, F. C., Akumka, D. D., Ajoku, G. A., Dzarma, S., Izebe, K. S., Ditse, M., Nwaneri, P. E. C., Wambebe, C. & Gamaniel, K. (2005). Toxicity Studies in Rats Fed Nature Cure Bitters. *African Journal of Biotechnology*, 4(1), 72-78.

Ashraf, V. K., Kalaichelvan, V. K. & Venkatachalam, V. V. (2021). Acute and Subacute Toxicity Assessment of Ethyl Acetate Extracts from Aerial Parts of *Clerodendrumthomsoniae*Balf in Rodents. *Biointerface Research in Applied Chemistry*,11(6), 13952 – 13961.

Babayi, H. M.,Ijah, U. J. J., Abalaka, J.A., Okogun, J. I., Salawu, O.A., Akumka, D.& Inyang, U.S . (2007). Effects of Oral Administration of Aqueous Whole Extracts of *Cassythafilliforms* on Hematograms and Plasma Biochemical Parameters in Rats. *Journal of Medical Toxicology*, 3(4),146-157.

Bantle, J. P., Wylie-Rosett, J., Albright, A. L., Apovian, C. M., Clark, N. G., Franz, M. J. & Wheeler, M. L. (2006). Nutrition Recommendations and Interventions for Diabetes-2006: A position statement of the American Diabetes Association. *Diabetes Care*,9, 2140-2157.

Bulus, T., Atawodi, S. E. & Mamman, M. (2011). Acute Toxicity Effect of the Aqueous Extract of *Terminaliaavicennioides*on White Albino Rats. *Science World Journal*,6,2.

Bunn, H. F. (2011). Approach to the anaemia. In: Goldman, L., Schaffer, A. I. (eds). *Cecil Medicine. 24th ed*. Philadelphia, Pa: Saunders Elsevier, pp. 161.

Cerceo, E., Deitelzweig, S. B.,Sherman, B. M. & Amin, A. N. (2016). Multidrug-resistant Gram-negative bacterial infections in the hospital Setting: overview, implications for clinical practice, and emerging treatment options. *Microbial Drug Resistance*,22,412–431.

Chattopadhyay, D., Maiti, K., Kundu, A.P., Chakraborty, M. S., Bhadra, R, Mandal, S. C.& Manda, A. B. (2001). Antimicrobial activity of *Alstoniamacrophylla*: a folklore of bay island. *Journal of Ethnopharmacology*,77, 49–55.

Collins, C. H., Lynes, P. M. & Grange, J. M. (1995). *Microbiological Methods*. (7th ed..) Butterwort-Heinemann Ltd., Britain, pp.175-190.

Culyba, M. J. & Van Tyne, D. (2021). Bacterial evolution during human infection: Adapt and live or adapt and die. *PLoSPathog*,17(9), e1009872.

Garoy, E. Y., Gebreab, Y. B., Achila, O. O., Tecklebrhan, N., Tsegai, H. M. & Hamida, M. E. (2021). Magnitude of multidrug resistance among bacterial isolates from surgical site infections in two national referral hospitals in Asmara, Eritrea. *International Journal of Microbiology*,66(9)1-12.

Gupta, A., Sehgal, V. & Mehan,S. (2011). Hyperlipidemia an updated review. International *Journal of Biopharmaceutical and Toxicological Research*,1(1), 81-89.

Harborne, J. B. (1973). *Phytochemical Methods*. London,: Chapman and Hall, Ltd...

Ikewuchi, C. J., Ikewuchi, C. C. & Onwuka, C. F. (2010). Effect of Aqueous Extract of *Tridaxprocumbens*Linn on Plasma Electrolytes of Salt-Loaded Rats. *Pakistan Journal of Nutrition*, 9(2), 103-105..

International Agency for Research on Cancer, IARC (1995). Peroxisome proliferation and its role in carcinogenesis. Views and expert opinions of an IARC working group, Lyon, France, IARC. *IARC Technical Report*, No 24, 1995.

Iqbal, I., Afzal, M., Aftab, M. N., Manzoor, F., Kaleem, A. & Kaleem, A. (2016). Hepatotoxicity of Cassia fistula extracts in experimental chicks and assessment of clinical parameters. *Kuwait Journal of Science,* 43(3), 135-141.

Kharchoufa, L., Bouhrim, M., Bencheikh, N., El-Assri, S. & Elachouri, M. (2020). Acute and Subacute Toxicity Studies of the Aqueous Extract from *HaloxylonscopariumPomel* (*Hammadascoparia* (Pomel) by Oral Administration in Rodents.*BioMed Research International*,2020,4020647.

Knöss, W. (2017). Toxicity of Herbal Medicines: From Past to Present to Future. In: *Toxicology of Herbal Products*. Pelkonen, O., Duez, P., Vuorela, P.M. and Vuorela, H. Eds. Springer International Publishing: Cham, pp. 1-9.

Krauss, R.M., Eckel, R.H., Howard, B.,Appel, L.J., Daniels, S.R.,Deckelbaum,R. J. & Bazzarre, T.L. (2000). AHA Dietary Guidelines: Revision 2000: A Statement for Healthcare Professionals From the Nutrition Committee of theAmerican Heart Association. *Circulation,*102,2284-2299.

Lajubutu, B. A., Pinney, R. J., Robert, M. F., Odelola, H. A. & Oso, B. A. (1995). Antimicrobial activity of diosquinone and plumbagin from *Diospyrosmespiliformis* (Hostch) (Ebenaceae). *Phytotherapy Research,* 9, 346-350.

National Institute for Pharmaceutical Research and Development, NIPRD (2004). *Extraction of Medicinal Plants.* Idu, Abuja, Nigeria.

National Institute for Pharmaceutical Research and Development, NIPRD (2006). *Evaluation of the Anti-tubercular Activities of Medicinal Plants' Extract against Mycobacterium bovis* (BCG). Idu, Abuja, Nigeria.

National Institute for Pharmaceutical Research and Development, NIPRD (2009). *Extraction protocols of Medicinal Plants.* Idu, Abuja, Nigeria.

National Institute on Health Publication (1985). Respect for life, National Institute of Environmental Health Sciences-NIEHS.

Ogbonnia, S. O., Enwuru, N. U., Onyemerem, E. U., Oyedele, G. A. & Enwuru, C. A. (2008). Phytochemical evaluation and antibacterial profile of *Treculia Africana* Tecne bark extract on gastrointestinal bacterial pathogen. *African Journal of Biotechnology,* 7(10), 1385-1389.

Oladunmoye, M. K. (2006). Immunomodulatory effects of ethanolic extract of *T. procumbens*onswiss Albino ratsorogastrically dosed with *Pseudomonas aeruginosa* (NCIB 950). *International Journal of Tropical Medicine,*1(4), 152 -155.

Olukunle, O. J. & Abatan, O. M. (2008). The Effects of Aqueous Leaf Extract of *TridaxProcumbens* on the Haematological and Serum Biochemical Parameters of Wistar Albino Rats. *International Journal of Agricultural Sciences, Sciences, Environment and Technology,*7, 2-5.

Reisin, E., Able, R., & Modan, M. (1978). Effect of weight loss without salt restriction in the reduction of blood pressure in overweight hypertensive patients. *Northern England Journal of Medicine*, 298, 1-6.

Rizvi, S. M. D., Zeeshan, M., Khan, S., Biswas, D., Al-Sagair, O. A.& Jamal, M. A. (2011). Evaluation and distribution of antibacterial potential in the aerial parts of wild *Tridaxprocumbens. Journal of Chemical and Pharmaceutical Research,*3(2), 80-87.

Rolain, J., Abat, C., Jimeno, M., Fournier, P. & Raoult. D. (2016). Do we need new antibiotics? *Clinical Microbiology and Infection,*22,408–415.

Salawu, O. A., Chindo, B. A., Tijani, A. Y., Obidike, I. C., Salawu, T. A. & Akingbasote, A. J. (2009). Acute and Sub-acute Toxicological Evaluation of *Crossopteryxfebrifuga* in Rats. *African Journal of Pharmacy and Phamacology,*3(12), 621-626.

Schalm, O. W., Jain, N. C., &Carrol, E. J. (1975). Veterinary haematology. 3rd edn. Philadelphia, Lea and Febiger, pp. 807.

Shah, M. G. K. (1991). Lipoprotein disorders in noninsulin-dependent diabetes mellitus. *International Journal of Diabetes in Developing Countries,* 11,25-29.

Simmons, J. E., Yang, R. S. H. & Berman, E. (1995). Evaluation of nephrotoxicity of complex mixtures containing organics and metals: Advantages and disadvantages of the use of real world complex mixtures. *EnvironmentalHealth Perspectives,*103, 67-71.

Taheri, S., Solati, A., Moradi, P., Tavassoly, A. & Yadi, J. (2013). Toxic effects of *Nerium oleander* aqueous leaf extract on haematological parameters and histopathological changes of the lungs and heart in rabbits. *Comparative Clinical Pathology,* 22(6), 1189-1193.

Tiwari, U., Rastogi, B., Singh, P., Saraef, D. K. & Vays, S. P. (2004). Immunomodulatory effects of aqueous extract of *Tridaxprocumbens* in experimental animals. *Journal of Ethnopharmacology,*92,113-119.

Trussell, K.C., Hinnen, D., Gray, P., Drake-Nisly,S. A., Bratcher, K. M., Ramsey, H. & Early, J. (2005). Case Study: Weight Loss Leads to Cost Savings and Improvement in Metabolic Syndrome. *Diabetes Spectrum,* 18, 77-79.

Vernon, S. E. (2005). Continuous throughput rapid tissue processing revolutionizes histopathology workflow. *Laboratory Medicine,*36,300-302.

Vivas, R., Barbosa, A.N.T., Dolabela, S. S. & Jain, S. (2019). Multidrug-Resistant Bacteria and Alternative Methods to Control Them: An Overview. *Microbial Drug Resistance,*25(6), 890 – 908.

World Health Organisation, WHO (1998). Handbook: Principles of Good Laboratory Practice. *http://www.who.int/tdr/publications.*

Yadawa, R. N. & Saurabh, K. (1998). A new flavones glycoside: 5,7,4–trihydraxy–6,3–dimethaseyfalavone 5-0 alpha –L- rhamnopyramoside from the leaves of *Tridaxprocumbens*Linn. *Journal of Asian Natural Products Research,* 1(2), 147-152.

Zhao, G. X., Gu, Z. M., Zeng, L., Chao, J. F., Wood, K. U., Kozlowski, J. K. & Mclaughlin, J. L. (1995). The absolute configuration of trilobacin and trilobin, a novel highly potent acetogenin from the stem bark of *Asiminatriloba* (Annonaceae). *Tetrahedron,*51, 7149-7160.