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GC/MS ANALYSIS, ANTISALMONELLAL POTENTIAL OF METHANOL LEAF EXTRACTS OF *TRISTEMMA MAURITIANUM* AND EFFECTS ON HEMATOLOGICAL PARAMETERS ON WISTAR RATS INFECTED WITH *SALMONELLA* TYPHI

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ABSTRACT

Typhoid fever remains a marked problem of public health in the world especially Africa and Asia. This work aimed to evaluate the antisalmonellal potential of extracts and fractions from *Tristemmamauritianum*. The microdilution technique was used for MIC and MBC determination whereas GC/MS was used to identify some components of the methanol extract. This extract was tested *in vivo*against salmonella-induced typhoid in rats. Among crude extracts, the methanol extract showed the better MICs ranging from 96 to 192 µg/ml. *In vivo*, the activity of the methanol extract was comparable to the one of oxytetracycline since both at the tested doses were able to heal infected rats. The hematological parameters showed an increase of WBC and the decrease of RBC and differentials in non treated groups'. From GC/MS 11 compounds were identified using NIST library. The results of this work provide the baseline information for the possible usage of *Tristemmamauritianum* to treat typhoid fever and others salmonellosis.

Keywords: *Tristemmamauritianum*, antisalmonellal, typhoid fever, hematological parameters, GC/MS

INTRODUCTION

Typhoid and paratyphoid fevers are serious systemic infections, with nearly 22 million cases and 216 500 deaths annually estimated in 2004, primarily in Asia and Africa.[1,2] They remain a marked problem of public health in sub-saharan Africa and many parts of Asia where it is still endemic due to precarioushygienic conditions.[3,2] Safe water, adequate sanitation, appropriate personal and food hygiene, and typhoid vaccination are the most effective strategies for typhoid prevention and control. In 2008, World Health Organization (WHO) recommended the use of available typhoid vaccines for controlling and strengthening the surveillance of typhoid but typhoid is still a marked problem in

developing countries.[2] Typhoid fever is caused by *Salmonella*Typhi whereas paratyphoid fivers are caused by *Salmonella*Paratyphi A and B. Other bacteria of *Salmonella* genus (*S.* Typhimurium, *S.* Dublin, *S.*Enteriditis) are causative agents of gastroenteritis. The treatment of typhoid and paratyphoid fevers is based on common antibiotics (including the third generation quinolones) against which many causative agents have rapidly gained resistance. [4,5,6] Hence, there is a need of new substances for typhoid therapy.

Plants have long time been used as the primary source for human treatment, and according to WHO's report, about 80% of the world population rely on plants and derived products for their treatment[7]. Medicinal plants in general are known for their diversity and richness in bioactives metabolites with enormous pharmacological potentials. Plants of Cameroonian flora possess many pharmacological activities including antimicrobial activity. [8,9,10] Plant extracts can then be the alternative for the treatment of many infectious diseases including typhoid fevers. In addition, many plant extracts have been reported for their antisalmonellal activity and many compounds have recently, been isolated from many Cameroonian medicinal plants. [11,12,13,14,15,16,17] Medicinal plants can then be the alternative for the treatment of many infectious diseases including typhoid and paratyphoid fevers. Among these plants, *Tristemmamauritianum*is used in the western part of the country.

Tristemmamauritianum is an herb or shrub of 1.25 m high, in marshy and moist places from Senegal to west Cameroon and Equatorial Guinea, extending to Congo (Brazzaville) and upper Shari.[18] The fleshy fruits are eaten. In west Cameroon, this plant is used in association with others, to treat typhoid fever, haemorrhoid and reproductive problems. In addition, it is used to treat sore throat in Uganda, Epilepsy in Mali. [19,20] Hence, in the continuation of searching the plants with antisamonellal potential, this work aimed to evaluate *in vitro* and *in vivo* the antisamonellal activity of extracts from leaves of *Tristemmamauritianum*.

MATERIALS AND METHODS

Plant material: The leaves of *Tristemmamauritianum* were collected in Fongo-Tongo, West region, Cameroon, in October 2014. The authentification was carried out at the Cameroon National Herbarium in Yaounde in comparison to the specimen registered under the reference 5895 SRF/Cam. After dryness, the leaves were ground for extraction.

Chemicals for antibacterial assays: Ciprofloxacin (Sigma-Aldrich, St Quentin Fallavier, France), oxytetracycline (Pantex, Hollande) were used as reference antibiotics whereas p-iodonitrotetrazolium chloride (INT) from Sigma Aldrich was used as bacterial growth indicator.

Test bacteria and culture media: The antimicrobial activities of different substances were tested on four bacteria isolates namely *Salmonella*Typhi, *Salmonella*Paratyphi A, *Salmonella*Paratyphi B and *Salmonella* Typhimurium obtained from the Medical Bacteriology Laboratory of the *Centre Pasteur*, Yaoundé, Cameroon. *Salmonella*Typhi ATCC6539 obtained from American Type Culture Collection was used as reference strain. Three culture media were used. Mueller Hinton Agar (MHA) was used for the activation of isolates whereas Mueller Hinton Broth (MHB) was used for *in vitro* antibacterial test and the Salmonella-Shigella Agar (SSA) was used to count colonies during the *in vivo* antibacterial assay.

Experimental animals: In this study, 48 Wistar albino rats of both sexes (24 males and 24 females), 8-9 weeks old weighing 140-175 g were used. These animals were bred in the animal house of the University of Dschang and received food and water *ad libitum*. The studies were conducted according to the ethical guidelines of Committee for Control and Supervision of Experiments on Animals (Registration no. 173/CPCSEA, dated 28 January, 2000, Government of India, on the use of animals for scientific research).

Preparation of extracts and fractions: The powder from leaves of *Tristemmamauritianum* (1000 g) was macerated at room temperature for 48h in the methanol (MeOH) and filtered using Wattman paper N° 1. The solvent was evaporated at 45 °C under reduction pressure using the rotary evaporator (R-200) to obtain the crude extract. Part of MeOH extract was suspended into the mixture water/MeOH (90:10) and partitioned successively in the n-hexane and ethyl acetate using column partitioner. These yielded the hexane, ethyl acetate and residual fractions. For the aqueous extract, 200 g of powder were macerated in 2 l of distilled water during 48 h. The mixture were filtered using Wattman paper N° 1 and the filtrate was concentrated in drying oven at 45 °C to obtain of aqueous crude extract.

GC/MS analysis: GC-MS analysis of methanol extract of *T. mauritianum* leaves was performed using TurboMass GC System, fitted with an Elite-5 capillary column (30 m, 0.25 mm inner diameter,

0.25 µm film thickness; maximum temperature, 350 °C), coupled to a Perkin Elmer Clarus 600C MS. Helium was used as gas carrier at a constant flow rate of 1.0 ml/min. The injection, transfer line and ion source temperatures were 280 °C. The ionizing energy was 70 eV. The oven temperature was programmed from 70 °C (hold for 2 min) to 280 °C (hold for 10 min) at a rate of 5 °C/min. The crude extract was solubilised with chloroform and filtered with syringe filter (Corning, 0.45 µm). A volume of 1 µl of the crude extract was injected with a split ratio 1:20. The data were obtained by collecting the mass spectra within the scan range 50-550 m/z. The identification of chemical compounds in the extract was based on GC retention time; the mass spectra matched those of standards available at NIST library.

***In vitro*antisalmonellal assay:** Minimal Inhibitory Concentrations and Minimal Bactericidal Concentrations (MICs and MBCs) of the plant extracts, fractions and reference antibiotics were determined by microdilution method using rapid INT colorimetric assay. [21] Briefly, the samples were first dissolved in 10% Dimethyl-sulfoxide (DMSO)/Mueller Hinton Broth (MHB). The microplates were prepared by dispensing into each well 100 µl of Mueller Hinton Broth (MHB) to which the tested substances were added to the first line of microplates. They were serially diluted two fold (in a 96-well microplate). One hundred microliters of inoculum (1.5×10^6 CFU/ml) prepared in MHB were then added. The plates were covered with a sterile plate sealer and then agitated with a shaker to mix the contents of the wells and incubated at 37 °C for 18 h. The final concentration of DMSO was 2.5%, and did not affect the bacterial growth. Wells containing MHB, 100 µl of inoculum, and DMSO at a final concentration of 2.5% served as the negative control. The MIC of each sample was detected after 18 h of incubation at 37 °C following addition of 40 µl INT (0.2 mg/ml) and incubation at 37 °C for 30 min. Viable bacteria reduced the yellow dye into a pink. The MIC was defined as the lowest sample concentration that prevented this change of coloration. The MBC of the sample was determined by sub-culturing 50 µl of the suspensions from the wells which did not show any growth after incubation during MIC assays in 150 µl of fresh broth, and re-incubated at 37 °C for 48 h before revelation. The MBC was defined as the lowest concentration of sample which completely inhibited the growth of bacteria. Each assay was performed in triplicate.

***In vivo*antisalmonellal assay:** Salmonellosis was induced using the method developed previously with some modifications. [22] Briefly, the rats received 1 ml (for the rat of 100 g) of *Salmonella* Typhi ATCC6539 suspension ($1.5\ 10^8$ UFC/ml). After 3 days of observation to confirm effectiveness of infection, each group consisted of 8 rats (4 males and 4 females) was treated as follow:

Group 0 animals served as the neutral control and were not infected and not treated throughout the experiment.

Group 1 animals served as the negative control and were not treated after infection.

Groups 2, 3 and 4 animals were treated with the methanol extract of *T. mauritianum* at the doses of 20, 40 and 80 mg/kg body weight respectively based on MIC, 2MIC and 4MIC.

Group 5 animals served as positive control and were treated with the reference antibiotic (oxytetracycline) at the dose of 20 mg/kg body weight as recommended by the manufactured.

All the animals were given free access to food and water. The blood of each animal was collected each 48 hours during the experimental period and inoculated in the prepared SSA on Petri dishes. [23,24] The inoculated dishes were incubated at 37 °C for 24 h. To evaluate efficacy of treatment, the emerged colonies were counted and converted to the number of colonies/ml of blood.

Collection of blood: At the end of the treatment period, the animals were anesthetized with the intraperitoneal injection of diazepam/ketamine mixture prior to dissection. Blood samples were collected by cardiac puncture into tubes containing EDTA. The blood was used to evaluate hematological parameters using the hematological analyzer following the instruction of the manufacturer.

Statistical analysis: Results were expressed as mean value ± standard deviation (S.D). Within groups, comparisons were performed by the analysis of variance using ANOVA test. Significant difference between control and experimental groups were assessed by Waller Duncan-test. Statistical analyses were performed using SPSS for Window software version16.0.

RESULTS

GC/MS analysis: The relative retention time of methanol extract of *T. mauritianum* and mass spectra of different components were compared with those of standard from the NIST library. As shown in Table 1, 10 compounds were identified. This extract is characterized by the presence of esters and phenolic compounds like 2,4-di-*tert*-butylphenol, 2-((octyloxy)carbonyl)benzoic acid and sitosterol.

***In vitro* antibacterial potential:** The Table 2 presents the results of MICs and MBCs of the plant extracts against bacteria of *Salmonella* genus. All the tested extracts presented inhibitory potential against the tested bacteria with MIC values varying from 96 to 1536 µg/ml. Among extracts, methanol extract showed the best activity with two MICs at 96 µg/ml while the ethyl acetate fraction was the most active fraction, since it inhibits four bacteria growth with the MIC less than 100 µg/ml.

***In vivo*antisalmonellal potential of methanolic extract:** The Figure 2 presents the number of colonies in rats (females and males respectively) during the *invivo* experimentation. In general, this figure shows curves with three phases: the first phase where the number of colonies did not significantly vary, the second phase, where the number of bacteria increases and the third phase where the number of bacteria decreases, and finally disappeared in the blood of the treated groups. At the fourth day of treatment, colonies of Salmonella were absent in the blood of both male and female rats receiving plant extract at the doses 80 and 40 mg/kg and for those receiving the reference drug (Figure 2). This disappearance of colonies in the blood occurs on the sixth day of treatment for rats receiving plants extract at the dose 20 mg/kg. This phase correspond to the action of plant extract or reference drugs in the treatment of rats. On infected and untreated rats (negative control), the number of bacteria did not decrease and remained significantly ($p < 0.05$) high at the end of experimentation when compared with neutral control and treated groups.

Effect of the treatment on the haematological parameters of rats: The red blood cells and differentials are presented in the Table 3 below. From this table, the level of RBC, Hb, MCH, MCHC and Hematocrite (Ht) significantly ($p < 0.05$) decreased with the infection whereas the administration of the extract or reference antibiotic reestablished the normal levels compared to the neutral control groups. The Table 4 presents the level of WBC and differentials. This table shows that the infection significantly ($p < 0.05$) increased the level of WBC and differentials. The level of platelets (Pt) also increased in females rats whereas the MPV did not significantly change. The administration of extract and reference drug reduced these parameters near to those of the control group (NINT) in both males and females.

DISCUSSION

The extracts of *T. mauritianum* showed variable antibacterial activities against the tested Salmonellae serovars. The methanol extract showed MICs of 96 µg/ml on *S*. Typhi and *S*. Paratyphi B. According to the litterature,[9] this extract possess a significant activity against ST and SPB whereas its ethyl acetate fraction possesses significant activity against four bacteria. The inhibitory potential of ethyl acetate fraction revealed that the active compounds may be more concentrated in it and may be of intermediate polarity. All the extracts of *T. mauritianum* are bactericids, since in all the cases the ratio MBC/MIC is less or equal to 4.[25]

The methanol extract of *T. mauritianum* was tested against *in vivo* model of salmonellosis induced by *Salmonella*Typhi. The infected rats were generally weak and their stools were soft and sometime liquid. In addition, the abundance of *Salmonella* colonies in Petri dishes after the blood culture confirmed the establishment of the salmonellosis. The administration of plant extract significantly ($p < 0.05$) reduced the number of colonies in the blood of rats in a dose-dependent manner and improved the general conditions of treated rats. The non-reduction of Salmonella colonies in untreated rats and the reduction of those colonies in treated rats (with extract and antibiotic) are the evidence that the activity of the methanol extract of *T. mauritianum* is maintained *in vivo*. The observed differences in the healing of animals after treatment can be due to the individual reaction to the infection and treatment but generally, the plant extract help rats to recover their health.

Among the compounds identified by GC/MS, some like 2-((2-ethylhexyloxy) carbonyl) benzoic acid; 2,4-di-*tert*-butylphenol and sitosterol had been reported for their antibacterial activities including antisalmonellal activity.[26,27,28,29,30,31] The observed activity may be due to the synergistic effect of those compounds which, based on their structure, inhibit various metabolic partway of bacteria.

The analysis of haematological parameters can be used to show the deleterious effects due to the intake of chemical substances as well as plant extracts on the blood constituents of animals. In this study, some RBC parameters (Hb, MCH, MCV, MCHC, RCDW) were measured to investigate the positive effects of the administration of *T. mauritianum* on haematological parameters of rats infected by *Salmonella*. The level of RBC, Hb, MCH, MCHC was significantly ($p < 0.05$) reduced in the infected

rats which did not receive treatment. The alteration of these parameters is known to induce the aenemic condition.[32] The level of RBC and its related indices in treated groups was high but not significantly ($p \geq 0.05$) different to those of the control group (NINT). This gives an indication that the methanol extract of this plant may contain some compounds that can stimulate the formation or secretion of erythropoietin in the stem cells of the animals. Erythropoietin is a glycoprotein hormone which stimulates stem cells in the bone marrow to produce RBC.[33] The stimulation of this hormone enhances rapid synthesis of RBC which is supported by the improved level of MCH and MCHC. These parameters are used to evaluate the oxygen carrying capacity of the blood and the concentration of the hemoglobin. The mechanisms by which plants extract improved the level of RBC and its related indices were not studied in this work. But, could be attributed to the capacity of compounds founded to reduce the lipid peroxidation of RBC through his antioxidant capacity.[34,29]

The White Blood Cells (WBC) and differentials are the immune cells which lead to the suppression of foreign organisms or molecules. They are used in non-specific immunity whereas lymphocytes are involved in specific immunity. The level of those cells depends on the health status of the host organism. These parameters increased significantly in case of host attack by the foreign organisms such as bacteria, fungi.[35,36] In this study, bacterial cells in the blood of treated rats were rare and too high in untreated rats at the end of the experimentation and may be responsible of the high level of WBC and differentials of untreated rats. This may be due to the stimulation of immune system by the presence of bacteria in the blood. The same observation had being made by another author.[22] Plant extracts can react in the living organism either by stimulating the immune system or by inhibiting the growth of microorganisms.[15] There was no proliferation of immune cells in rats receiving the extract of *T. mauritianum*as well as the positive control rats compared to the neutral control. This may be due to the inhibition of bacteria growth by the tested substances leading to the lowering of the mobilization of WBCs by the immune system. Platelets aggregation is the mechanism used by organisms to reduce blood loss and repaired vascular injury. Platelets known as thrombocytes help to mediate blood clotting when injury occurs. The level of platelets was high in untreated rats whereas the MPV did not change significantly. This observation is similar to another the result available in the literature.[37]

CONCLUSION

The results of this study show that *Tristemmamauritianum* possesses an interesting *in vitro* and *in vivo*antisalmonellal potential. The methanol extract may also improve the erythropoietin system. For the better utilization of these results, further investigations are needed such as toxicological studies.

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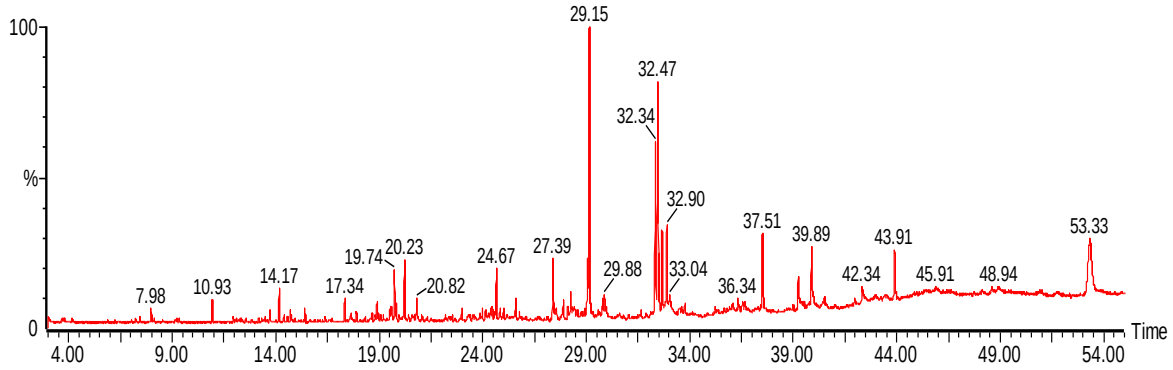
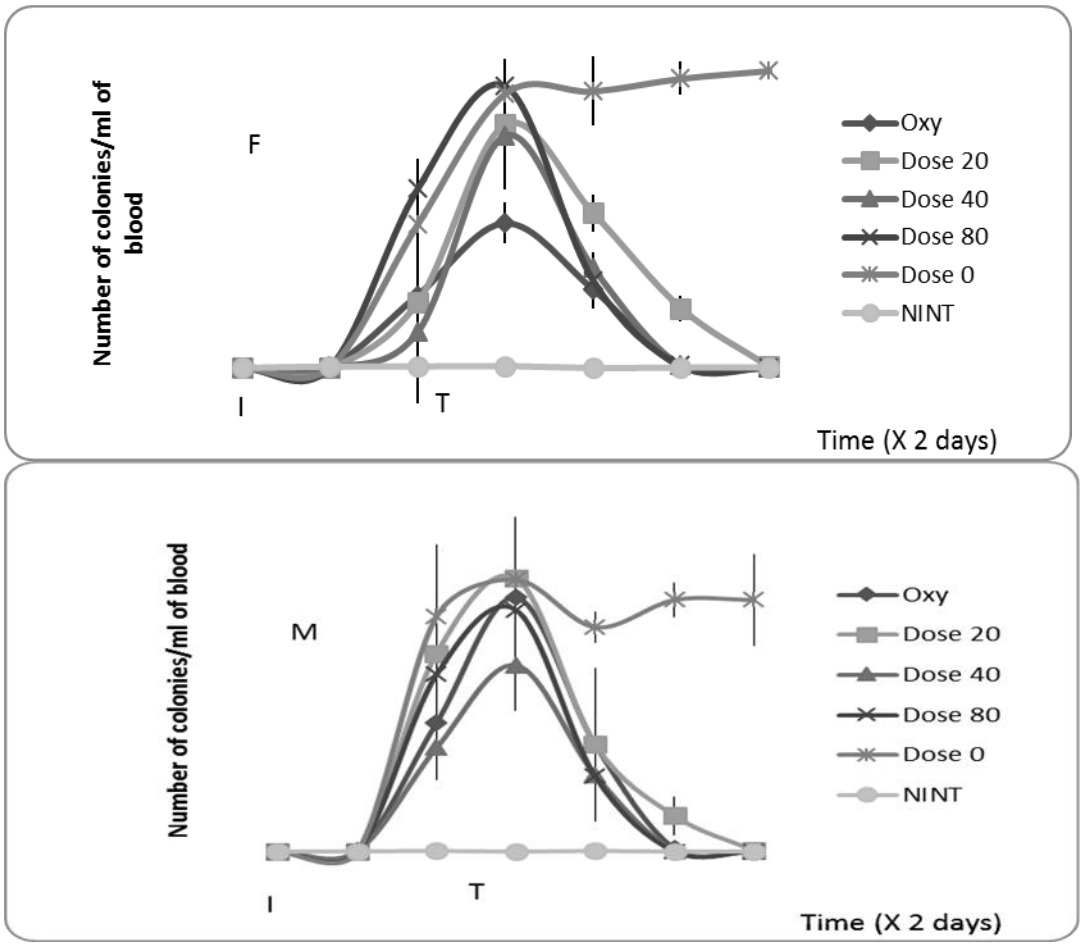


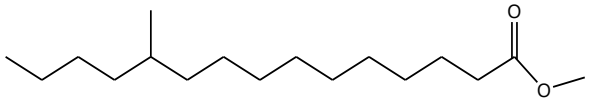
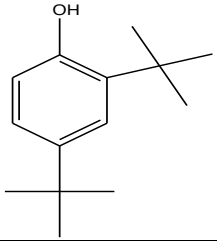
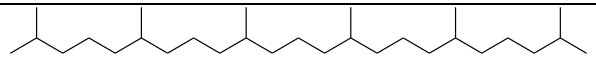
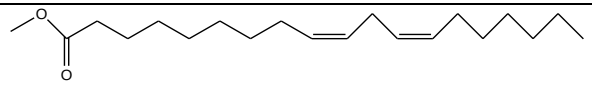
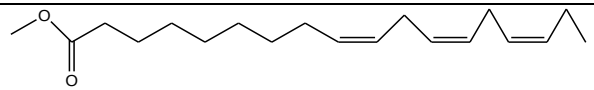
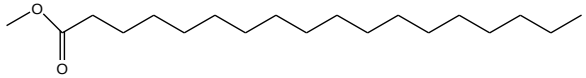
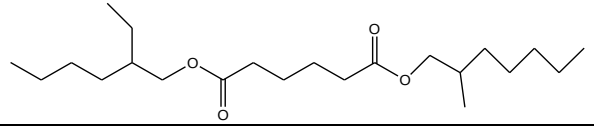
Figure 1: GC/MS chromatogram of methanol extract of *Tristemmamauritianum*.

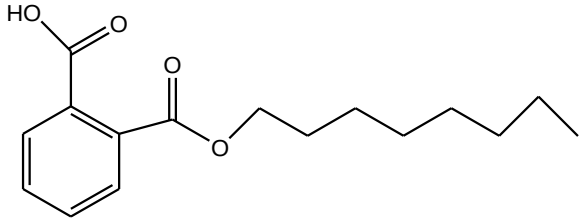
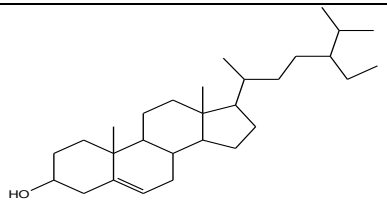


I: infection, T: Commencement of treatment, NINT: non infected rats, Oxy: Rats receiving the reference antibiotic (oxytetracycline), Dose 20, 40, 80: rats receiving the plant extract respectively at the doses 20, 40 and 80 mg/kg body weight, Dose 0: infected and untreated rats.

Figure 2: Number of colonies of *Salmonella* in females (F) and males (M) respectively during the treatment.

Table 1: Identification of compounds by GC/MS

N°	RT	Structure	Name	Formula	MW
1	19.15		methyl 11-methylpentadecanoate	$C_{17}H_{34}O_2$	270.26
2	20.23		2,4-di-*tert*-butylphenol	$C_{14}H_{22}O$	206.17
3	24.73		2,6,10,14,18,22,26,30-octamethylhentriacontane	$C_{39}H_{80}$	548.63
4	29.15		2,6,10,14,18,22-hexamethyltricosane	$C_{29}H_{60}$	408.47
5	32.34		(9Z,12Z)-methyl nonadeca-9,12-dienoate	$C_{20}H_{36}O_2$	308.27
6	32.47		(9Z,12Z,15Z)-methyl octadeca-9,12,15-trienoate	$C_{19}H_{32}O_2$	292.24
7	32.90		Methyl stearate	$C_{19}H_{38}O_2$	298.29
8	37.51		2-ethylhexyl 2-methylheptyl adipate	$C_{22}H_{42}O_4$	370.31

9	39.89		2-((octyloxy)carbonyl)benzoic acid	$C_{16}H_{22}O_4$	278.15
10	53.33		17-(5-ethyl-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-10,13-dimethyl-1H-cyclopenta[a]phenanthren-3-ol Or Sitosterol	$C_{29}H_{50}O$	414,39

RT: retention time, MW: molecular weight

Table 2: Minimum Inhibitory and Bactericidal Concentrations (MIC, MBC and MBC/MIC) of plant extracts and reference antibiotics.

Tristemmamauritianum	**Colours**	**Parameters (µg/ml)**	**Bacteria**				
			SPA	STM	SPB	ST	ST ATCC6539
		MIC	192	192	96	96	192
Methanol extract	Black	MBC	1536	768	96	384	768
		MBC/MIC	8	4	1	4	2
		MIC	384	768	384	192	384
Hexane fraction	Black	MBC	1536	768	384	384	384
		MBC/MIC	4	1	1	2	1
Ethyl acetate fraction		MIC	96	192	48	48	96
	Brown	MBC	96	384	384	192	192
		MBC/MIC	1	2	8	8	2
Residual fraction		MIC	192	768	768	1536	768
	Black	MBC	192	-	-	-	1536
		MBC/MIC	1	/	/	/	2
		MIC	768	384	768	768	1536
Aqueous extract	Brown	MBC	1536	768	768	768	1536
		MBC/MIC	2	2	1	1	1
		MIC	0.1875	0.375	0.1875	0.375	0.375
Ciprofloxacine	White	MBC	0.375	0.75	0.1875	0.375	0.375
		MBC/MIC	2	2	1	1	1
		MIC	2	8	4	4	8
Oxytetracycline	Yellow	MBC	16	32	16	32	32
		MBC/MIC	8	4	4	8	4

MIC: Minimal Inhibitory Concentration, MBC: Minimal Bactericidal Concentration,
SPA: *Salmonella*Paratyphi A, SPB: *Salmonella*Paratyphi B, STM: *Salmonella* Typhimurium, ST: *Salmonella*Typhi.

Table 3: Effect of treatment on red blood cells and the differentials in rats

	Parameters						
Rats Groups	**RBC (X 10^6/µl)**	**Hb (g/dl)**	**MCH (pg)**	**MCV (fl)**	**RCDW (%)**	**MCHC (g/dl)**	**Hematocrite (%)**
Females							
Oxy	5.60 ± 0.323^{bc}	12.53 ± 0.231^{ab}	22.40 ± 1.044^{c}	67.43 ± 10.538^{ab}	18.60 ± 1.212^{bcd}	33.63 ± 3.420^{cd}	38.80 ± 2.762^{bc}
Dose 20	5.28 ± 0.482^{b}	12.10 ± 0.346^{ab}	23.83 ± 1.419^{c}	67.93 ± 4.826^{ab}	16.67 ± 1.893^{abc}	35.17 ± 0.473^{d}	38.10 ± 1.153^{abc}
Dose 40	6.22 ± 0.249^{c}	12.33 ± 1.266^{ab}	19.73 ± 1.320^{ab}	60.03 ± 2.570^{a}	17.70 ± 0.265^{abcd}	33.07 ± 3.177^{cd}	37.23 ± 0.874^{ab}
Dose 80	5.73 ± 0.443^{bc}	12.90 ± 0.608^{b}	22.50 ± 0.700^{c}	64.47 ± 3.350^{ab}	16.43 ± 0.153^{ab}	35.03 ± 1.801^{d}	38.83 ± 1.150^{bc}
Dose 0	4.89 ± 0.687^{a}	11.07 ± 0.666^{a}	17.93 ± 0.737^{a}	72.07 ± 3.253^{ab}	15.53 ± 0.929^{a}	27.90 ± 0.781^{ab}	34.33 ± 1.168^{a}
NINT	5.42 ± 0.365^{bc}	12.20 ± 1.709^{ab}	22.17 ± 1.650^{c}	69.70 ± 2.784^{ab}	19.20 ± 1.153^{cd}	32.10 ± 3.651^{abcd}	37.47 ± 1.060^{abc}
Males							
Oxy	5.49 ± 0.050^{bc}	12.47 ± 0.709^{ab}	22.63 ± 1.266^{c}	75.33 ± 4.965^{b}	18.27 ± 0.850^{bcd}	30.13 ± 0.503^{abc}	41.33 ± 2.743^{c}
Dose 20	5.45 ± 0.215^{bc}	12.00 ± 0.400^{ab}	21.97 ± 1.415^{bc}	64.73 ± 3.467^{ab}	17.20 ± 2.007^{abcd}	34.03 ± 0.723^{cd}	37.57 ± 1.405^{abc}
Dose 40	5.76 ± 0.569^{bc}	12.63 ± 0.987^{ab}	21.93 ± 1.504^{bc}	68.83 ± 5.550^{ab}	16.43 ± 2.003^{ab}	$32.17 \pm 4.366ab^{cd}$	39.53 ± 4.067^{bc}
Dose 80	5.59 ± 0.462^{bc}	12.70 ± 0.265^{ab}	22.73 ± 1.553^{c}	70.17 ± 7.617^{ab}	16.27 ± 1.877^{ab}	32.60 ± 1.473^{bcd}	38.93 ± 1.601^{bc}
Dose 0	4.10 ± 0.402^{a}	10.83 ± 0.611^{a}	18.10 ± 0.458^{a}	66.00 ± 3.351^{ab}	19.40 ± 0.346^{d}	27.37 ± 0.666^{a}	34.63 ± 1.106^{a}
NINT	5.57 ± 0.300^{bc}	11.93 ± 1.206^{ab}	22.77 ± 0.208^{c}	68.03 ± 5.563^{ab}	16.57 ± 0.451^{abc}	33.67 ± 2.875^{cd}	38.97 ± 0.643^{bc}

Along each column and same sex, values with the same letter superscripts are not significantly different. Waller-Duncan ($p<0.05$).
RBC: Red Blood Cells, Hb: Haemoglobin, MCH: Mean Concentration of Haemoglobin, MCV: Mean Corpuscular Volume, MCHC: Mean Corpuscular Haemoglobin Concentration, RCDW: Red Cells Distributed Width, Oxy: oxytetracycline, NINT: Non infected groups.

Table 4: Effect of treatment on white blood cells and differentials in rats

	Parameters					
Rats Groups	**WBC (X 10^3/µl)**	**Lymphocytes (%)**	**Monocytes (%)**	**Granulocytes (%)**	**Platelets (X 10^3/µl)**	**MPV (fl)**
Females						
Oxy	4.73 ± 1.33^{ab}	81.90 ± 7.590^{ab}	5.40 ± 0.40^{a}	5.70 ± 0.458^{a}	196.00 ± 16.64^{ab}	8.20 ± 0.436^{a}
Dose 20	6.60 ± 3.20^{abc}	80.93 ± 1.222^{ab}	5.67 ± 0.47^{a}	9.67 ± 1.266^{cde}	208.00 ± 65.50^{abcd}	8.13 ± 0.404^{a}
Dose 40	5.47 ± 2.023^{abc}	84.40 ± 2.095^{ab}	4.87 ± 0.83^{a}	9.73 ± 1.012^{de}	203.00 ± 27.05^{abc}	8.30 ± 0.265^{a}
Dose 80	5.27 ± 1.943^{abc}	85.10 ± 2.000^{ab}	5.03 ± 1.63^{a}	6.87 ± 1.320^{ab}	180.67 ± 28.29^{a}	7.87 ± 0.252^{a}
Dose 0	8.73 ± 0.321^{c}	89.67 ± 0.351^{b}	8.53 ± 0.74^{c}	11.80 ± 0.346^{ef}	490.00 ± 68.69^{f}	8.57 ± 1.242^{a}
NINT	3.13 ± 0.751^{a}	86.87 ± 1.320^{ab}	6.07 ± 1.11^{ab}	5.97 ± 0.153^{ab}	261.00 ± 8.54^{cde}	7.67 ± 0.416^{a}
Males						
Oxy	3.87 ± 1.069^{a}	87.27 ± 0.751^{ab}	4.77 ± 1.76^{a}	6.97 ± 1.677^{ab}	279.00 ± 42.72^{cde}	7.57 ± 0.416^{a}
Dose 20	3.27 ± 1.793^{a}	84.87 ± 3.745^{ab}	4.43 ± 1.68^{a}	8.03 ± 2.183^{bcd}	218.33 ± 43.46^{bcd}	8.03 ± 0.666^{a}
Dose 40	4.73 ± 2.937^{ab}	80.43 ± 5.450^{ab}	6.20 ± 1.51^{b}	9.70 ± 0.361^{cde}	236.00 ± 29.00^{bcd}	7.77 ± 0.503^{a}
Dose 80	3.53 ± 0.289^{a}	82.33 ± 7.116^{ab}	5.43 ± 1.26^{a}	7.57 ± 1.464^{bcd}	331.67 ± 37.84^{e}	7.37 ± 0.208^{a}
Dose 0	7.63 ± 0.666^{bc}	88.57 ± 0.603^{ab}	8.17 ± 0.91^{bc}	12.27 ± 0.503^{f}	282.67 ± 20.55^{de}	7.67 ± 0.153^{a}
NINT	3.87 ± 1.504^{a}	79.83 ± 6.673^{a}	5.43 ± 1.06^{a}	7.40 ± 0.889^{bc}	226.33 ± 29.77^{abcd}	8.43 ± 0.208^{a}

Along each column and same sex, values with the same letter superscripts are not significantly different. Waller-Duncan ($p<0.05$).
WBC: White Blood Cells, MPV: Mean Platelets Volume, Oxy: oxytetracycline, NINT: non infected groups

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