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Antinociceptive effect of aqueous leaves extract of *Fagara tessmannii* on acetic acid-induced pain in Wistar albino rats

Emmanuel Dikioye Peters [1 *], Goodness Chizi Nyeche [2] and Promise Chukwuka Ali [3]

[1] *Department of Medical Biochemistry, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria.*
[2] *Department of Biochemistry, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria.*
[3] *Department of Biochemistry, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria.*

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Abstract

The study was designed to investigate the analgesic effect of the aqueous leaf extract of *Fagara tessmannii* engl using the acetic acid-induced pain model in Wistar rats. Twenty rats were divided into five groups of four rats each. Group 1 received distilled water while groups 2-5 received 0.2 ml/kg BW of 0.6% intraperitoneal injection of acetic acid 30 mins after treatment with extracts and reference drug and writhing counted. Rats were sacrificed and blood was collected for biochemical and heamatological analyses. Also, Gas chromatography and phytochemical characterization of alkaloids in the aqueous leaf extract were done. The result revealed a significant decrease ($p \leq 0.05$) in the mean number of writhing in group 4 and non-significant decrease ($p \geq 0.05$) in groups 3 and 5 when compared to control group. Percentage inhibition of nociception in groups 3, 4, and 5 were 2%, 41.7%, and 10.5%, respectively. Non-significant differences ($p \geq 0.05$) in WBC, lymphocyte, neutrophil, eosinophil, and basophil counts in groups 5, 4, and 3 were observed when compared to normal and negative control groups. Monocyte count and ESR significantly decreased ($p \leq 0.05$) when negative was compared to normal control. CRP, IL-6, and PTG-S1 concentrations significantly increased ($p \leq 0.05$) when the negative control group was compared to the normal control. Groups 5 and 4 for CRP and 3 and 4 for IL-6 concentrations were significantly reduced ($p \leq 0.05$) while PTG-S1 was none significantly reduced in a dose-dependent manner when compared to the negative control. Some abundant alkaloids identified were acetic acid (12.3442%), benzoic acid (11.7984%), phenol, 4-methoxy-, acetate (6.8281), and phenol (4.3901%). Aqueous leaf extract of *Fagara tessmannii* Engl exhibited antinociceptive potential through the action of bioactive constituents.

Keywords: *Fagara tessmannii* antinociceptive; Biochemical; Heamatological; Inhibition

1. Introduction

The practice of herbal medicine dates back to the very earliest period of known human history [1]. There is evidence of herbs that have been used in the treatment of diseases and for revitalizing the body system in almost all ancient civilizations. The science of life has provided a rational basis for the treatment of various ailments. Pain, inflammation, and fever are common complications in human beings. [2] Defined pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. Pain is a protective mechanism and occurs whenever any tissue is damaged which causes the individual to react to the pain stimulus [3]. Chronic pain not only affects physical activity but may also impact the psychosocial health of the patient leading to a lowering of quality of life [4].

* Corresponding author: Peters Dikioye Emmanuel
Department of Medical Biochemistry, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria.

Various nonsteroidal anti-inflammatory drugs have been used in the treatment of pain and inflammation but are often associated with many side effects. However, there are medicinal plants with anti-inflammatory, analgesic, and pyretic properties with low or no side effects which are being used in traditional medicine and also as complementary medicine in the civilized world the for treatment of several disease conditions. The African continent is richly endowed with diverse medicinal plants and many have been shown to be effective in the treatment of various disease conditions [5].

Fagara tessmannii (*Zanthoxylum tessmannii*) is a plant genus belonging to Rutaceae, a family that includes over 160 genera and 2000 species with more species still being identified [6]. Plant species in this genus are distributed all over the world with the majority found in Asia, America, and Africa [7]. In Africa, *Fagara tessmannii* is widely distributed in countries such as Sierra Leone, Kenya, Susan, Angola, Malawi, Zambia, and Zimbabwe. Many Zanthoxylum species are traditionally used as medicinal plants in managing various health conditions. Secondly, metabolites isolated from parts of species in this genus have demonstrated several pharmacological activities, such as; antioxidant, analgesic, anti-inflammatory activities, and modulatory effects against obesity, dementia, and diabetes [8]. Traditionally, decoctions of Zanthoxylum species, either as a single Plant-base preparation or in combination with other plants are commonly used in treating infections of various kinds especially those caused by parasites, sickle cell, and tumor, bacterial, fungal, and viral infections. The back of the stern and roots is commonly used as an analgesic, especially to treat burns, rheumatism, headache, stomachache, toothache, and pain after childbirth this informed the investigation of the analgesic potential of the aqueous extract of *Fagara tessmannii* in this study.

2. Material and methods

2.1. Plant material

Fresh leaves of *Fagara tessmannii* were collected from Dighiriga Otapha in Abua/Odua Local government area of Rivers State. The leaves were authenticated at the Department of Plant Science and Biotechnology Herbarium, University of Port Harcourt, by an expert taxonomist Dr. Ekeke Chimezie. A specimen representing the collection was deposited at the Herbarium with voucher number UPH/P/275.

2.2. Extraction of Plant material

The leaves of *Fagara tessmannii* were air-dried for two weeks, after which it was ground into a coarse powder using a grinding mill machine. The pulverized dried leaf sample was weighed and dissolved in a glass jar using deionized water (100g/L). The aqueous suspension was homogenized by stirring with a glass rod, left to stand for 24 hours, and filtered using No.1 Whatman filter paper. The filtrate (extract) in a crucible placed in a water bath was dried at a moderate temperature of 60^0C.

2.3. GC-MS quantitative characterization for alkaloids in aqueous leaf extract of *Fagara tessmannii*

Quantitative characterization for alkaloids in the aqueous leaf extract of *Fagara tessmannii* was done using GC-MS. Before analysis, the MS was auto-tuned to perfluorotributylamine (PFTBA) using already established criteria to check the abundance of *m/z* 69, 219, 502, and other instruments' optimal and sensitivity conditions. Determination of the levels of phytochemicals in the sample was carried out using GC-MS by operating MSD in Scan mode to ensure all levels of detection of the target constituents. Agilent 7820A gas chromatograph coupled to 5975C inert mass spectrometer (with triple-axis detector) with electron-impact source (Agilent Technologies) was used. The stationary phase of separation of the compounds was an HP-5 capillary column coated with 5% phenyl methyl Siloxane (30m length x 0.32mm diameter x 0.25µm film thickness) (Agilent Technologies). The carrier gas was helium used at a constant flow of 1.4871 mL/min at an initial nominal pressure of 1.4902 psi and an average velocity of 44.22 cm/sec. 1µL of the samples were injected in spitless mode at an injection temperature of 300 °C. Purge flow to spilled vent was 15 mL/min at 0.75 min with a total flow of 16.654 mL/min; gas saver mode was switched off. The oven was initially programmed at 40 °C for (1 min) and then ramped at 12 °C/min to 300 °C (10 min). Run time was 32.667 min with a 5 min solvent delay. The mass spectrometer was operated in electron-impact ionization mode at 70eV with an ion source temperature of 230 °C, quadrupole temperature of 150 °C, and transfer line temperature of 280 °C. Acquisition of ion was via Scan mode (scanning from *m/z* 45 to 550 amu at 2.0s/scan rate).

2.4. Drugs and Chemicals

0.6% acetic acid, 0.9% normal saline solution, and aspirin (SKG- Pharma Limited, Ikeja Lagos).

2.5. Experimental animals

Female Wistar albino rats of weight range 100-150g were procured from the Animal House of the Faculty of Basic Medical Sciences, University of Port Harcourt. All experimental protocols were in compliance with the Department of Biochemistry Research Ethics Committee, University of Port Harcourt, (approval number UPH/BCHREC/2022/008) on research in animals as well as internationally accepted principles for laboratory animal use and care.

2.6. Analgesic evaluation of *Fagara tessmannii*

The analgesic activity of *Fagara tessmannii* was evaluated using the acetic acid-induced writhing test. The rats were divided into five groups labeled 1-5, each group consisting of four rats. Group 1 received distilled water as normal control, and groups 3, 4, and 5 received 200 and 500 mg/kg BW of extract and reference drug respectively. Thirty minutes after, intra-peritoneal injections of 0.6% acetic acid in the abdominal region of all the rats in groups 2-5 were administered. The number of abdominal constrictions i.e., writhingreflexesx were counted for each group of rats starting from 5 minutes after the injection of acetic acid up to 30 minutes. The percentage Inhibition of extract against acetic acid was calculated using the following formula;

$$\% \text{ Inhbition } = \frac{NC\ Nt}{NC} \times 100$$

Where Nc is the number of writhing in Control and Nt is the number of writhing in test groups.

2.7. Collection and Preparation of Blood Sample

Blood in heparin sample bottles was collected through heart puncture put in a centrifuge and spun at 4^0C at 1500 rpm for 15 minutes to separate serum for biochemical analysis.

2.8. Biochemical Analysis

The concentrations of PTG S1, IL-6, and High-Sensitive C-Reactive Protein (HS-CRP) in the serum of Wistar rats were quantified by commercially available enzyme-linked immunosorbent assay (ELISA) kits at a wavelength of 450 nm according to the instructions provided by the manufacturer.

2.9. Heamatological analysis

2.9.1. Determination of erythrocyte sedimentation rate (ESR)

- 1.6 ml of each blood sample was added into different test tubes
- 0.4 ml of sodium citrate which acts as an anticoagulant was added into the test tubes according to the number of samples.
- The content of each test tube was mixed
- The mixtures were thoroughly pipetted into western green pipettes
- The pipettes were placed in the western green stand and allowed to stand for 1 hour for sedimentation.
- The results were taken and recorded accordingly, the reading was taken in mm/hr.

2.9.2. Determination of WBC differential count

The differential count is expressed as a percentage of the total number of cells counted e.g., 10% lymphocytes.

- A thin film was made on a slide with the blood of the Wistar rat, the film was air dried.
- After air drying, the film was stained using Leishman stain and left for 2 mins.
- The stained film was diluted with water and left for about 8mins.
- The stained film was rinsed in low running tap water and air dried.
- The film was examined under a microscope using 100 x magnification with oil immersion.

2.10. Determination of total white blood cell count (WBC)

A total of 0.02 ml of blood was added to 0.38 ml of diluting fluid. The Neubauer counting chamber was charged with the well, the diluted blood was applied into the chamber. The cells were allowed to settle for 3-5 mins, a microscope was used with 10sx objective lens to locate the four large corner squares, 1, 2, 3, 4. The areas of the squares were $4mm^2$. The

cells should be evenly distributed; the total number of white blood cells in the four large corner square were counted. After counting, the result gotten from the square area (1, 2, 3, 4) was multiplied by 50.

2.11. Statistical Analysis

Data were expressed as mean±SD using SPSS by window 22 USA. The values were analyzed by one-way ANOVA followed by multiple comparisons by Turkey and values were considered significantly different at $p < 0.05$ confidence level.

3. Results

3.1. Result of Analgesic activity

Result of Table 1 showing effect of aqueous leaf extract of *Fagara tessmannii* engl on acetic acid-induced pain in wistar rats revealed significant decrease ($p \leq 0.05$) in mean number of acetic acid-induced writhing in group 4 and non-significant decrease ($p \geq 0.05$) in groups 3 and 5 when compared to control group. Mean percentage inhibition of nociception in groups 3, 4 and 5 were 2%, 41.7% and 10.5%, respectively. Aqueous leaf extract of *Fagara tessmannii* engl at 400 mg/kg bw. Demonstrated greater mean percentage inhibition of nociception than reference drug indicating greater peripherally acting antinociceptive activity.

Table 1 Effect of aqueous leaf extract of *Fagara tessmannii* engl on Acetic Acid-induced pain in Wister rats

Groups	Treatments	No of writhing	% pain inhibition
1	Control (water only)	0.00±0.00	-
2	Control (0.6%acetic acid)	48.00±1.50a	-
3	200mg/kg bw *Fagara tessmannii*+0.6% acetic acid	47.00±0.00a	2
4	400mg/kg bw *Fagara tessmannii*+ 0.6% acetic acid	28.00±0.50b	41.7
5	300mg/kg bw aspirin+ 0.6% acetic acid	43.50±0.00a	10.5

Values are reported as mean ± standard error of mean (M±SEM) (n =4) Values with different superscript letters indicate statistically significant differences ($p \leq 0.05$) down the column while those with similar superscripts show non-significant differences ($p \geq 0.05$) down the column when compared with the control and between groups. (^{a} significant to control the rest significant to other groups).

3.2. Result of Hematological analysis

Result of the effect of aqueous leaf extract of *Fagara tessmannii* engl on hematological parameters of acetic acid-induced pain in Wistar rats in Table 2 revealed non-significant differences in WBC, lymphocyte, neutrophil, eosinophil and basophil counts in groups 5, 4 and 3 when compared to normal and negative control groups. Monocyte count and ESR significantly decreased when negative control was compared to normal group.

Table 2 Effect of aqueous leaf extract of *Fagara tessmannii* engl on hematological parameters of acetic acid-induced pain in Wistar rats

GROUP	TREATMENT	WBC (x10^9)	Lymphocyte (%)	Neutrophil (%)	Eosinophil (%)	Monocyte (%)	Basophil (%)	ESR (mm/hour)
1	Control (water only)	12.97±4.27 a	51.25±0.25 a	37.00±1.22 a	5.00±0.00 a	6.00±1.08 a	0.75±0.25 a	0.75±0.25 a
2	Control (0.6%acetic acid)	20.50±4.85 a	61.00±3.76 a	33.50±2.53 a	4.50±1.55 a	2.25±1.11 b	0.00±0.00	2.25±0.48 b
3	200mg/kg bw *Fagara tessmannii*+0.6% acetic acid	15.85±2.73 a	55.50±2.87 a	34.25±1.89 a	5.50±0.50 a	4.75±0.63 a	0.50±0.50 a	1.75±0.63 b
4	400mg/kg bw *Fagara tessmannii*+ 0.6% acetic acid	16.88±5.55 a	57.25±1.49 a	34.75±1.31 a	4.50±0.65 a	2.75±0.85 b	0.00±0.00	2.5±0.87 b
5	300mg/kg bw aspirin+ 0.6% acetic acid	8.10±0.83 b	62.75±4.21 a	27.75±4.56 a	4.00±0.41 a	5.25±1.70 a	0.25±0.25 a	2.00±0.58 b

Values are presented as mean ± standard error of mean (M±SEM) (n =4). Values with different superscript letters indicate statistical significant differences (p≤ 0.05) down the column while those with similar superscripts show non-significant differences (p≥ 0.05) down the column when compared with the control and between groups

Table 3 Effect of aqueous leaf extract of *Fagara tessmannii* engl biochemical parameters of acetic acid-induced pain in wistar rats

Groups	Treatments	CRP	IL - 6	PTG_S1
1	Control (water only)	0.19±0.24^{abcd}	3.32±0.39^{ab}	0.23±0.03^{abcd}
2	Control (0.6%acetic acid)	0.89±0.11^{a}	16.66±7.54^{a}	1.05±0.12^{a}
3	200mg/kg bw *Fagara tessmannii*+0.6% acetic acid	0.91±0.10^{b}	10.46±3.87	1.18±0.36^{b}
4	400mg/kg bw *Fagara tessmannii*+ 0.6% acetic acid	1.11±0.28^{c}	10.82±3.93	1.12±0.15^{c}
5	300mg/kg bw aspirin+ 0.6% acetic acid	0.88±0.48^{d}	16.66±1.75^{b}	1.43±0.24^{d}

Values are presented as mean ± standard error of mean (M±SEM) (n =4). Values with similar superscript letters indicate statistical significant differences (p≤ 0.05) down the column while those without superscripts show non-significant differences (p≥ 0.05) down the column when compared with the control and between groups.

3.3. Result of Biochemical analysis

Result from Table 3 showed that CRP, IL-6 and PTG-S1 concentrations significantly increased when negative control group is compared to normal control. Groups 5 and 4 for CRP and 3 and 4 for IL-6 concentrations were significantly reduced while PTG-S1 was non-significantly reduced in a dose dependent manner when compared to negative control. 400mg/kg bw aqueous leave *Fagara tessmannii* extract was most effective in reduction of CRP, IL-6 and PTG-S1 concentrations than 200mg/kg bw aqueous leave *Fagara tessmannii* extract and aspirin.

Table 4 Quantitative characterization of alkaloids in aqueous leaf extract of *Fagara tessmannii*

S/N	Alkaloids	RT	AREA PCT
1	Acetic acid	3.6818	12.3442
2	Butanoic acid, 2-methyl-	3.78	0.2877
3	Pyridine	4.0572	0.2338
4	beta-D-Ribopyranoside, methyl	4.1612	0.6057
5	2-Methyl-l-methylmannopyranoside	4.5308	0.0656
6	Butyraldehyde, semicarbazone	4.6809	0.3177
7	Propanamide, 2-hydroxy-N-methyl-	4.8542	0.1911
8	Propanamide, 2-hydroxy-N-methyl-	4.9639	0.2262
9	Cyclotetrasiloxane, octamethyl-	5.3451	3.3177
10	Selenium (IV) oxide	5.7089	0.0714
11	Phenol	5.888	4.3901
12	Phenol	6.1363	0.6406
13	Acetic acid, phenyl ester	6.3211	0.7846
14	Mequinol	6.864	3.4451
15	m-Guaiacol	7.2451	0.1901
16	Cyclopentasiloxane, decamethyl-	7.326	0.3819
17	5-Vinyl-pyrazole	7.4242	0.1749
18	4-Pentenal, 2-methyl-	7.713	0.2486
19	Benzoic acid	8.227	11.7984
20	Benzoic acid	8.6255	4.5912
21	Benzoic acid	9.099	1.5978
22	Benzoic acid	9.3531	0.7492
23	1-Hexen-3-yne, 2-methyl-	9.5495	0.8061
24	Hydrazine, 1-methyl-1-phenyl-	9.6881	2.0138
25	Benzoic acid	10.0462	4.748
26	Benzene, 1,1'-[1,2-ethanediylbis(oxy-2,1-ethanediyloxy)] bis-	10.5717	0.7979
27	1,3,4-Oxadiazole-2-thiol, 5-(3-pyridinyl)-	10.924	1.6909
28	(E,E,E)-2,4,6-Octatriene	11.2532	3.8124
29	Resorcinol	11.594	0.7956
30	3(2H)-Pyridazinone, 6-methyl-	11.877	2.8235
31	Resorcinol	12.2812	2.1849

32	5-Deoxypyridoxal	12.5296	1.6115
33	2-Butynedioic acid, di-2-propenyl ester	12.7317	0.6762
34	5-Deoxypyridoxal	12.853	1.8115
35	5-Hexyl-2-furaldehyde	13.188	1.4677
36	1-Cyclohexene-1-methanol	13.3035	1.7769
37	1-Cyclohexene-1-methanol	13.4825	0.7456
38	Spiro (6,6-dimethyl-2,3-diazobicyclo [3.1.0] hex-2-ene-4,1'-cyclopropane)	13.8059	2.1841
39	2,5-Cyclohexadien-1-one, 3,4,4-trimethyl-	13.9214	1.2795
40	Tetrahydrocarvone	14.1813	1.6577
41	Phenol, 4-methoxy-, acetate	14.3026	6.8281
42	Paradrine	14.8224	2.8809
43	Paradrine	15.423	0.3883
44	Paradrine	15.5732	0.5134
45	Phenol, 2,6-diamino-	16.1565	3.6704
46	Fumaric acid, 2-methoxyphenyl tridec-2-yn-1-yl ester	16.272	3.07
47	Paradrine	17.1844	0.0675
48	Propane, 1,1-dichloro-	17.3288	0.3647
49	Paradrine	17.8255	0.0416
50	2H-1,2,3-Benzotriazole-2-acetic acid	18.1316	0.0186
51	2H-1,2,3-Benzotriazole-2-acetic acid	18.2529	0.1227
52	2H-1,2,3-Benzotriazole-2-acetic acid	18.8131	0.0314
53	2H-1,2,3-Benzotriazole-2-acetic acid	18.9171	0.0801
54	2H-1,2,3-Benzotriazole-2-acetic acid	19.2867	0.0137
55	2H-1,2,3-Benzotriazole-2-acetic acid	19.4946	0.0271
56	7-Chloro-4-methoxy-3-methylquinoline	19.7545	0.0305
57	trans-3-Ethoxy-b-methyl-b-nitrostyrene	20.0144	0.0177
58	7-Methyl -2-phenyl-1H-indole	20.2511	0.0326
59	7-Chlorocinchoninic acid	20.6265	0.0381
60	Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl-	20.7189	0.0157
61	2,3-Dihydroxy-6-nitroquinoxaline	20.9269	0.0293
62	1,1,1,3,5,5,5-Heptamethyltrisiloxane	21.025	0.013
63	1-methyl-4-phenyl-5-thioxo-1,2,4-triazolidin-3-one	21.1694	0.0552
64	Silane, triethyl(2-phenylethoxy)-	21.4351	0.0678
65	Cyclotrisiloxane, hexamethyl-	21.9491	0.0525
66	7-Methyl -2-phenyl-1H-indole	22.2032	0.0663
67	2-Methyl-7-phenylindole	22.3996	0.0235
68	7-Methyl -2-phenyl-1H-indole	22.6479	0.1585
69	1,2-Bis(trimethylsilyl)benzene	22.9251	0.0511

70	1,1,1,3,5,5,5-Heptamethyltrisiloxane	22.9886	0.0216
71	1,1,1,3,5,5,5-Heptamethyltrisiloxane	23.1272	0.0278
72	7-Methyl -2-phenyl-1H-indole	23.2197	0.0273
73	2-Ethylacridine	23.4276	0.0403
74	Arsenous acid, tris(trimethylsilyl) ester	23.6586	0.045
75	1,1,1,3,5,5,5-Heptamethyltrisiloxane	23.7568	0.0992
76	Cyclotrisiloxane, hexamethyl-	24.0628	0.0602
77	7-Methyl -2-phenyl-1H-indole	24.2938	0.0222
78	7-Methyl -2-phenyl-1H-indole	24.4036	0.0696
79	1H-Indole, 5-methyl-2-phenyl-	24.7501	0.0545
80	Tris(tert-butyldimethylsilyloxy) arsane	24.8714	0.0255
81	Arsenous acid, tris(trimethylsilyl) ester	25.0678	0.0321
82	7-Methyl -2-phenyl-1H-indole	25.2814	0.0458
83	acetic acid, 2-[bis(methylthio)methylene]-1-phenylhydrazide	25.5009	0.0155
84	1,1,1,3,5,5,5-Heptamethyltrisiloxane	25.6453	0.0486
85	Tris(tert-butyldimethylsilyloxy) arsane	25.8359	0.0288
86	Cyclotrisiloxane, hexamethyl-	25.9398	0.0615
87	Tris(tert-butyldimethylsilyloxy) arsane	26.1477	0.0325
88	Arsenous acid, tris(trimethylsilyl) ester	26.4423	0.0478
89	Cyclotrisiloxane, hexamethyl-	26.5751	0.0155
90	1,1,1,3,5,5,5-Heptamethyltrisiloxane	26.7252	0.0258
91	Tris(tert-butyldimethylsilyloxy) arsane	26.9447	0.0164
92	Cyclotrisiloxane, hexamethyl-	27.0487	0.0263
93	Tris(tert-butyldimethylsilyloxy) arsane	27.2046	0.0411
94	Tris(tert-butyldimethylsilyloxy) arsane	27.4472	0.0498
95	1,2-Bis(trimethylsilyl)benzene	27.707	0.0349
96	Tris(tert-butyldimethylsilyloxy) arsane	28.4405	0.0152
97	1,4-Bis(trimethylsilyl)benzene	28.6715	0.0458
98	1,1,1,3,5,5,5-Heptamethyltrisiloxane	28.7293	0.0599
99	1,2-Bis(trimethylsilyl)benzene	28.8621	0.0463
100	Cyclotrisiloxane, hexamethyl-	29.0238	0.0706
101	Tetrasiloxane, decamethyl-	29.4108	0.0131
102	Arsenous acid, tris(trimethylsilyl) ester	29.5956	0.0331
103	Arsenous acid, tris(trimethylsilyl) ester	29.7919	0.0422
104	Arsenous acid, tris(trimethylsilyl) ester	29.9941	0.0472
105	Cyclotrisiloxane, hexamethyl-	30.2366	0.0298
106	Methyltris(trimethylsiloxy)silane	30.4619	0.0129
107	Cyclotrisiloxane, hexamethyl-	30.6467	0.0523

108	Arsenous acid, tris(trimethylsilyl) ester	31.3051	0.0282
109	Tris(tert-butyldimethylsilyloxy) arsane	31.5419	0.0324
110	Arsenous acid, tris(trimethylsilyl) ester	31.6805	0.022
111	Arsenous acid, tris(trimethylsilyl) ester	31.8249	0.0262
112	Tris(tert-butyldimethylsilyloxy) arsane	31.9692	0.0341
113	Tris(tert-butyldimethylsilyloxy) arsane	32.1598	0.0227
114	Cyclotrisiloxane, hexamethyl-	32.258	0.0173
115	Arsenous acid, tris(trimethylsilyl) ester	32.3735	0.0253

NOTE: (RT) is defined as Retention Time, (**AREA PCT)** as Area Percentage.

3.4. Result of Gas chromatography quantitative characterization of alkaloids in aqueous leaf extract of *Fagara tessmannii* engl

Gas chromatography quantitative characterization of alkaloids in aqueous leaf extract of *Fagara tessmannii* engl led to the elution of 115 alkaloids identified through searching the database for known fragmentation patterns. Some of the most abundant alkaloids identified were Acetic acid (12.3442%), Benzoic acid (11.7984%), Phenol, 4-methoxy-, acetate (6.8281), Phenol (4.3901%), (E, E, E)-2,4,6-Octatriene (3.8124%), Phenol, 2,6-diamino-(3.6704), Mequinol (3.4451%), Cyclotetrasiloxane, octamethyl- (3.3177%), Fumaric acid, 2-methoxyphenyl tridec-2-yn-1-yl ester (3.07), Paradrine (2.8809), 3(2H)-Pyridazinone, 6-methyl- (2.8235), Resorcinol (2.1849), Hydrazine 1-methyl-1-phenyl- (2.0138) (Table 4).

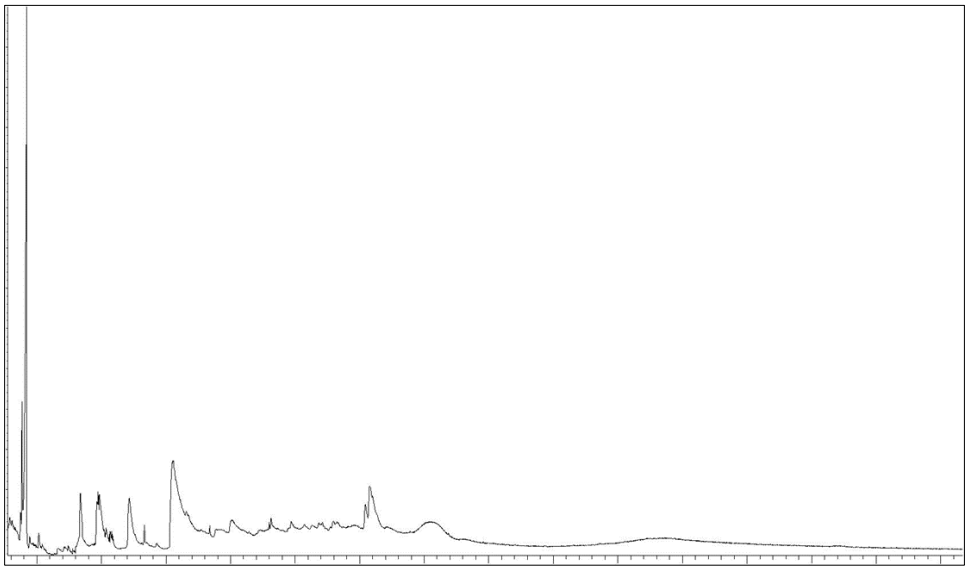

Figure 1 GC-MS chromatograph of alkaloids constituents of aqueous extract of *Fagara tessmannii* engl

4. Discussion

Plants have been utilized as therapeutic agents for the treatment of pain and related conditions. According to [1] the use of plants and their natural products in Nigeria as either extract or infusion is a widespread practice in the treatment and management of diseases. This study aims at scientifically evaluating the antinociceptive potential of the plant in order to justify the traditional use /claim of *Fagara tessmannii engl* in the treatment and management of pain, using the acetic acid-induced writhing test in wistar albino rats. Although this study was carried out using the aqueous leaf extract of *Fagara tessmannii*, most claims have it that the bark, stem and roots are commonly used as analgesic traditionally to treat painful conditions such as stomachache, burns, toothache and pain after childbirth.

Acetic acid- induced pain model is the most accepted pain assay for estimating peripherally acting antinociceptive agents [9]. Intraperitoneal injection of acetic acid activate phospholipase in peritoneal cavity leading to the release of arachidonic acid from the plasma membrane. Arachidonic acid is converted to PGH2 via PGG2 which is the common

precursor for the synthesis of PGs in peritoneal fluids [10] along with the release of histamine, serotonin, bradykinin, and cytokines (TNF-a, IL-1b, IL-6, and IL-8) which finally stimulate the primary afferent nociceptors entering the dorsal horn of the central nervous system [11, 12, 13, 14]. Acetic acid induces inflammatory pain by causing capillary permeability [15]. All types of pain, whether it is acute or chronic, peripheral or central, initiate from inflammation [16]. During inflammation, many pro-inflammatory mediators such as interleukin 6 (IL-6), IL-12, interferon (INF-γ), tumor necrosis factor (TNF), cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) are secreted [17, 18]. Result of Table 1 showing effect of aqueous leaf extract of *Fagara tessmannii* engl on acetic acid-induced pain in wistar rats revealed significant decrease ($p \leq 0.05$) in mean number of acetic acid-induced writhing in group 4 and non-significant decrease ($p \geq 0.05$) in groups 3 and 5 when compared to control group. Mean percentage inhibition of nociception in groups 3, 4 and 5 were 2%, 41.7% and 10.5%, respectively. Aqueous leaf extract of *Fagara tessmannii* engl at 400mg/kgbwt. Demonstrated greater mean percentage inhibition of nociception than reference drug indicating greater peripherally acting antinociceptive agent.

Inflammatory markers include C reactive protein (CRP), erythrocyte sedimentation rate, plasma viscosity, fibrinogen, ferritin, and several other acute phase proteins, though only the first three are commonly referred to as inflammatory markers. CRP is considered to be particularly useful in detecting bacterial infection [19]. The assessment of hematological parameters can be diagnostic of adverse effects of foreign compounds on the blood constituents of an animal [20]. Monocytes are agranulocytes found in the blood representing the recently formed precursors of the mononuclear phagocyte system. Chronic bacterial infections such as tuberculosis, inflammation and malignant disorders result in monocytosis while corticosteroid treatment is often associated with monocytopenia [21]. Result of the effect of aqueous leaf extract of *Fagara tessmannii* engl on hematological parameters of acetic acid-induced pain in wister rats in Table 2 revealed non-significant differences in WBC, lymphocyte, neutrophil, eosinophil and basophil counts in groups 5,4 and 3 when compared to normal and negative control groups. Monocyte count and ESR significantly decreased when negative control was compared to normal group.

Inflammatory markers include C reactive protein (CRP), erythrocyte sedimentation rate, plasma viscosity, fibrinogen, ferritin, and several other acute phase proteins, though only the first three are commonly referred to as inflammatory markers. CRP is considered to be particularly useful in detecting bacterial infection [19]. All types of pain, whether it is acute or chronic, peripheral or central, initiate from inflammation [16] (Omoigui, 2007). During inflammation, many pro-inflammatory mediators such as interleukin 6 (IL-6), IL-12, interferon (INF-γ), tumor necrosis factor (TNF), cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) are secreted [17, 18]. Result from Table 3 showed that CRP, IL-6 and PTG-S1 concentrations significantly increased when negative control group is compared to normal control. Groups 5 and 4 for CRP and 3 and 4 for IL-6 concentrations were significantly reduced while PTG-S1 non significantly reduced in a dose dependent manner when compared to negative control. 400mg/kg bw aqueous leave *Fagara tessmannii* extract was most effective in reduction of CRP, IL-6 and PTG-S1 concentrations than 200mg/kg bw aqueous leave *Fagara tessmannii* extract and aspirin.

Intraperitoneal administration of acetic acid raises the level of PGE2 and PGF2a in the peritoneal fluid and causes inflammatory pain [9]. In this study, non-significant reduction in PTG-S1 in a dose dependent manner in groups 3 and 4 when compared to negative control was observed suggesting that aqueous leave *Fagara tessmannii* extract showed antinociception through peripheral mechanism, by binding to visceral receptors sensitive to acetic acid to inhibit prostaglandin biosynthesis [22]. The test extract also lowered significantly IL-6 concentration in a dose dependent manner in extract treated groups when compared to negative control group. The IL- 6 during inflammation and infection is induced via stimulation of cells by IL-1 or TNF-α or through stimulation of TLRSs after binding of PAMPs [23]. The studies on the correlation between pro-inflammatory cytokines with pain intensity in chronic pain by [24, 25] showed a direct relationship between the concentration of pro-inflammatory interleukins, mainly IL-6, and the severity of chronic pain. CRP is an acute phase protein synthesized response to proinflammatory cytokines, in particular interleukin-6 by hepatocytes [26]. CRP belongs to the pentraxin family of proteins and it increases in concentration during injury, inflammation or tissue death [27]. The concentration of Interleukin 6 (IL-6), which is also a key mediator of the acute phase have been positively correlated with CRP [28].

Therapeutic benefits derived from plants such as antinociceptic, antipyretic and anti-inflammatory are mediated by bioactive constituents like alkaloids [29]. Several classes of alkaloid are responsible for anti-inflammatory and analgesic properties [30]. Inhibitory or regulatory effect of alkaloids on important inflammation mediators such as NF-*κ*B, COX-2, and iNOS suggesting anti-inflammatory activity has been reported [31, 32, 33, 34, 35,36 &37]. Hence it became imperative in this study to undertake quantitative characterization of alkaloids in aqueous leaf extract of *Fagara tessmannii* engl using GC-MS. Gas chromatography quantitative characterization of alkaloids in aqueous leaf extract of *Fagara tessmannii* engl led to the elution of 115 alkaloids identified through searching the database for known fragmentation patterns. Some of the most abundant alkaloids identified were acetic acid (12.3442%), benzoic acid

(11.7984%), phenol, 4-methoxy-, acetate (6.8281), phenol (4.3901%), (e,e,e)-2,4,6-octatriene (3.8124%), phenol, 2,6-diamino-(3.6704), mequinol (3.4451%), cyclotetrasiloxane, octamethyl- (3.3177%), fumaric acid, 2-methoxyphenyl tridec-2-yn-1-yl ester(3.07), paradrine (2.8809), 3(2h)-Pyridazinone, 6-methyl- (2.8235), Resorcinol (2.1849), Hydrazine 1-methyl-1-phenyl- (2.0138) (Table 4)**.**

The research work by [38] on "acetic acid alleviates the inflammatory response and liver injury in septic mice by increasing the expression of TRIM40" revealed that treatment of cecal ligation and puncture (CLP) -induced septic mice with intravenously injected acetic acid attenuated inflammation in septic mice by upregulating the expression of TRIM40 which in turn suppressed the production and secretion of cytokines, including IL-6 and TNF-α, increased the expression of IL-10 and inhibited the activity of TLR4 signal pathway. Acetic acid the most abundant constituent in vinegar prevents metabolic syndrome in rats and human [39], and exhibit anti-hypercholesterolemia, anti-hyperglycemia, anti-hypertension, anti-cancer and anti-inflammation [40,41&42].

Benzoic acid is the simplest aromatic carboxylic acid. It was approved at a dose of 0.5–1.0% in swine rearing by the European Union [43]. Benzoic acid supplementation has been reported to regulate the humoral immune response [44] when used as an additive in livestock nutrition. [45] in their research on Effects of Combined Supplementation of Macleaya cordata Extract (MCE) and Benzoic Acid on Immune Responses in Weaned Piglets, reported that Supplementing the diet of weaned piglets with 0.8 or 1.6% benzoic acid reduced the levels of inflammatory mediators TNF-a and IL-6 [46, 47] while [48] reported increased serum IL-10 concentration indicating improve humoral immunity of pigs by reducing and increasing proinflammatory and anti-inflammatory responses respectively [45]. Benzoic acid derivatives were potent inhibitors of cyclooxygenase activity hence could act as anti-nociception for the treatment of certain inflammatory disorders [49, [50]. Hence aqueous leaf extract of *Fagara tessmannii* engl could serve as natural herbal alternative to synthetic benzoic acid in diet of weaned piglets. [51] reported anti-inflammatory effect of 4-methoxy-3-(methoxymethyl) phenol isolate from the flower extract of *Calotropis gigantea* white in Carrageenan-induced hind paw edema in male albino rats. Methoxy phenol derivatives are used in formulating analgesics, biocides, antitumor, hepatoprotective, anti-inflammatory, anti-ulcer, antimicrobial, and antiviral agents [52]. Past studies on different extracts of *L. molleoides* have reported anti-inflammatory [53] and antinociceptive [54] activities. Dichloromethane extract of *L. molleoides* was reported to exhibit anti- inflammatory activity and immunostimulant activity on normal lymphocytes [55] and resorcinol 1,3-dihydroxy-5-(tridec-4',7'-dienyl)-benzene was identified as the major constituent and responsible for their biological activities. [56] in their work on isolation, characterization and confirmation of 5-alkenyl resorcinols from dichloromethane extract of L. molleoides using 1H NMR, 13C NMR spectral studies reported the presence of five resorcinols subunit in all of them with different side chains with various degrees of unsaturation.

5. Conclusion

Aqueous leaf extract of *Fagara tessmannii* engl exhibited antinociceptive potential by reducing PTG S1, IL-6, CRP levels in wistar rat probably through the action of bioactive constituents such acetic acid, benzoic acid, phenol, 4-methoxy-, acetate and resorcinol.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that there are no conflicts of interest upon writing of this manuscript.

Statement of ethical approval

All experimental protocols were in compliance with the Department of Biochemistry Research Ethics Committee, University of Port Harcourt, (approval number UPH/BCHREC/2022/008) on research in animals as well as internationally accepted principles for laboratory animal use and care.

References

[1] Bukar BB, Dayom DW, Uguru MO. The growing economic importance of medicinal plants and the need for developing countries to harness from it: A mini review. IOSR Journal of Pharmacy. 2016 May; 6(5): 42-52.

[2] Loeser JD, Melzack R. Pain: an overview. Lancet. 1999 May; 353(9164):1607-1609.

[3] Stout E, Sexton P, Meghani, Salimah. Racial differences in adherence to prescribed analgesia in cancer patients: An integrated review of quantitative research. Journal of Clinical Outcomes Management. 2017 Jan; 24(1): 39-48.

[4] Atkinson HJ, Slater MA, Patterson TL, Grant I, Garfin SR. Prevalence, onset, and risk of psychiatric disorders in men with chronic low back pain: a controlled study. Pain. 1991 May; 45(2): 111-121.

[5] Ahmed, AU. An overview of inflammation: Mechanism and consequences. Frontiers in Biology. 2011 Aug; 6(4): 274-281.

[6] Groppo M, Pirani JR. A new species of Zanthoxylum (Rutaceae) with a key to the species from Northeastern Brazil. Phytotaxa. 2017 July; 314(2):259-265.

[7] Plazas E, Casoti R, Avila Murillo M, Batista Da Costa F, Cuca LE. Metabolomic profiling of Zanthoxylum species: Identification of anti-cholinesterase alkaloids candidates. Phytochemistry. 2019 Dec;168:112128.

[8] Akakpo-Akue J, Kplé TKM Coulibaly K, Ahon GM, Fofié Y, Yapo-Crezoit A, Zarish GN, Kra AKM. Ethnobotanical study of medicinal plant used against sickle cell anemia in the eastern part of the Côte d'Ivoire. J. Anim. Plant. Sci. 2020 July; 45(1):7839–7852.

[9] Zulfiker A, Rahman MM, Hossain MK, Hamid K, Mazumder M, Rana MS. In vivo analgesic activity of ethanolic extracts of two medicinal plants: *Scoparia dulcis L.* and *Ficus racemosa Linn*. Biol. Med. 2010; 2(2):42–48.

[10] Duarte ID, Nakamura M, Ferreira SH. Participation of the sympathetic system in acetic acid-induced writhing in mice. Braz J Med Biol Res. 1988;21(2):341-3.

[11] Subedi NK, Rahman SM, Akbar MA. Analgesic and Antipyretic Activities of Methanol Extract and Its Fraction from the Root of Schoenoplectus grossus. Evid Based Complement Alternat Med. 2016; 2016:3820704.

[12] Martínez V, Thakur S, Mogil JS, Taché Y, Mayer EA. Differential effects of chemical and mechanical colonic irritation on behavioral pain response to intraperitoneal acetic acid in mice. Pain. 1999 May; 81(1-2):179-86.

[13] Rujjanawate C, Kanjanapothi D, Panthong A. Pharmacological effect and toxicity of alkaloids from Gelsemium elegans Benth. J Ethnopharmacol. 2003 Nov; 89(1):91-5.

[14] Trongsakul S, Panthong A, Kanjanapothi D, Taesotikul T. The analgesic, antipyretic and anti-inflammatory activity of Diospyros variegata Kruz. J Ethnopharmacol. 2003 Apr; 85(2-3):221-5.

[15] Ikeda Y, Ueno A, Naraba H, Oh-ishi S. Involvement of vanilloid receptor VR1 and prostanoids in the acid-induced writhing responses of mice. Life Sci. 2001 Nov 2; 69(24):2911-9

[16] Omoigui S. The biochemical origin of pain: the origin of all pain is inflammation and the inflammatory response. Part 2 of 3 - inflammatory profile of pain syndromes. Med Hypotheses. 2007;69(6):1169-78. doi: 10.1016/j.mehy.2007.06.033. Epub 2007 Aug 28. PMID: 17728071; PMCID: PMC2771434.

[17] Chiu YJ, Huang TH, Chiu CS, Lu TC, Chen YW, Peng WH, Chen CY. Analgesic and Antiinflammatory Activities of the Aqueous Extract from Plectranthus amboinicus (Lour.) Spreng. Both In Vitro and In Vivo. Evid Based Complement Alternat Med. 2012;2012:508137. doi: 10.1155/2012/508137. Epub 2011 Sep 11. PMID: 21915187; PMCID: PMC3170901.

[18] Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev. 1991 Jun;43(2):109-42.

[19] Johnson HL, Chiou CC, Cho CT. Applications of acute phase reactants in infectious diseases. J Microbiol Immunol Infect. 1999 Jun;32(2):73-82.

[20] Ashafa AO, Orekoya LO, Yakubu MT Toxicity profile of ethanolic extract of Azadirachta indica stem bark in male Wistar rats. Asian Pac J Trop Biomed 2012; 2(10):811-817.

[21] Howard MR, Hamilton PJ. Haematology. Blood cells. 2nd ed. 2002. p. 6-7.

[22] Sheikh BY, Zihad SM, Sifat N, Uddin SJ, Shilpi JA, Hamdi OA, Hossain H, Rouf R, Jahan IA. Comparative study of neuropharmacological, analgesic properties and phenolic profile of Ajwah, Safawy and Sukkari cultivars of date palm (Phoenix dactylifera). Orient Pharm Exp Med. 2016; 16(3):175-183.

[23] Kang S, Tanaka T, Narazaki M, Kishimoto T. Targeting interleukin-6 signaling in clinic. Immunity 2019; 50(4):1007–1023.

[24] Koch A, Zacharowski K, Boehm O, Stevens M, Lipfert P, von Giesen HJ, et al. Nitric oxide and pro-inflammatory cytokines correlate with pain intensity in chronic pain patients. Inflamm Res. 2007;56(1):32-7.

[25] Malhotra D, Saxena AK, Dar SA, Kumar V, Nasare N, Tripathi AK, et al. Evaluation of cytokine levels in fibromyalgia syndrome patients and its relationship to the severity of chronic pain. J Musculoskeletal Pain. 2012 June; 20(3):164-169.

[26] Shrivastava A K, Harsh VS, Arun R, Sanjeev KS. C-reactive protein, inflammation and coronary heart disease. The Egyptian Heart Journal. 2015 June; 67(2):89-97.

[27] Pepys MB, Hirschfield GM. C-reactive protein: a critical update. J Clin Invest. 2003 Jun;111(12):1805-12.

[28] Ni Choileain N, Redmond HP. Cell response to surgery. Arch Surg. 2006 Nov;141(11):1132-40.

[29] Kifayatullah, M., Rahim, H., Jan, N.U., Chishti, K.A., Ullah, I., Abbas, S. In vivo analgesic, antipyretic and anti-inflammatory activities of ethanol extract of pericampylus glaucus in experimental animals. Sains Malays. 2019 March; 48(3):629–635.

[30] Adebayo SA, Dzoyem JP, Shai LJ, Eloff JN. The anti-inflammatory and antioxidant activity of 25 plant species used traditionally to treat pain in southern African. BMC Complement Altern Med. 2015 May 27;15:159.

[31] Yang CW, Chen WL, Wu PL, Tseng HY, Lee SJ. Anti-inflammatory mechanisms of phenanthroindolizidine alkaloids. Mol Pharmacol. 2006 Mar; 69(3):749-58.

[32] Pradeep CR, Kuttan G. Piperine is a potent inhibitor of nuclear factor-kappaB (NF-kappaB), c-Fos, CREB, ATF-2 and proinflammatory cytokine gene expression in B16F-10 melanoma cells. Int Immunopharmacol. 2004 Dec; 4(14):1795-803.

[33] Matheus ME, Violante FDA, Garden SJ, Pinto AC, Fernandes PD. Isatins inhibit cyclooxygenase-2 and inducible nitric oxide synthase in a mouse macrophage cell line. European Journal of Pharmacology. 2007; 556(1-3):200–206.

[34] Zhao Z, Xiao J, Wang J, Dong W, Peng Z, An D. Anti-inflammatory effects of novel sinomenine derivatives. Int Immunopharmacol. 2015 Dec; 29(2):354-360.

[35] Chen DP, Wong CK, Leung PC, Fung KP, Lau CB, Lau CP, Li EK, Tam LS, Lam CW. Anti-inflammatory activities of Chinese herbal medicine sinomenine and Liang Miao San on tumor necrosis factor-α-activated human fibroblast-like synoviocytes in rheumatoid arthritis. J Ethnopharmacol. 2011 Sep 1;137(1):457-68.

[36] Zhou Y, Shi-Qing L, Ling Y, Bin H, Shi-Hau W, Qi Z, Shao-Qiang X, Hong-Jun M. Berberine prevents nitric oxide induced rat chondrocyte apoptosis and cartilage degeneration in a rat osteoarthritis model via AMPK and p38 MAPK signaling. Apoptosis. 2015; 20(9):1187–1199.

[37] Pacheco de Oliveira MT, de Oliveira Ramalho TR, Paiva Ferreira LK, Araújo Lima AL, Barbosa Cordeiro M, Ferreira Costa H, Rodrigues LC, Piuvezam MR. Synthesis, toxicity study and anti-inflammatory effect of MHTP, a new tetrahydroisoquinoline alkaloid. Immunopharmacol Immunotoxicol. 2015 July; 37(4):400-12.

[38] Yang H, Lang M, Dengbin A, Nianguo H, Hui L, Xunjun S, Xiaoyan P. Acetic Acid Alleviates The Inflammatory Response And Liver Injury In Septic Mice By Increasing The Expression Of Trim40. Experimental And Therapeutic Medicine. 2019 April; 17(4):2789-2798.

[39] Beh BK, Mohamad NE, Yeap SK, Ky H, Boo SY, JYH C, Tan SW, Ho WY, Sharifuddin SA, Long K and Alitheen NB: Anti-obesity and anti-inflammatory effects of synthetic acetic acid vinegar and Nipa vinegar on high-fat-diet-induced obese mice. Sci Rep. 2017 July; 7(1):6664.

[40] Yamashita H. Biological function of acetic acid-improvement in obesity and glucose tolerance by acetic acid in type 2 diabetic rats. Crit. Rev. Food Sci Nutr. 2016; 56: S171-S175.

[41] Mohamad NE, Yeap SK, Lim KL, Yusof HM, Beh BK, Tan SW, Ho WY, Sharifuddin SA, Jamaluddin A, Long K, *et al*: Antioxidant effects of pineapple vinegar in reversing of paracetamol-induced liver damage in mice. Chin Med. 2015 Feb; 10(3):1-10.

[42] Beh BK, Mohamad NE, Yeap SK, Lim KL, Wang YH, Yusof HM, Sharifuddin SA, Jamaluddin A, Long K and Alitheen NB: Polyphenolic profiles and the in vivo antioxidant effect of nipa vinegar on paracetamol induced liver damage. RSC Advance. 2016 June; 6(68):63304-63313.

[43] Authority E. Opinion of the Scientific Panel on additives and products or substances used in animal feed (FEEDAP) on the safety and the efficacy of product "BIO-COX 120G" as feed additive in accordance with Council Directive 70/524/EEC. EFSA J. 2004 July; 75:1–51.

[44] Resende M, Chaves RF, Garcia RM, Garcia RM, Barbosa JA,Marques AS, et al. Benzoic acid and essential oils modify the cecum microbiota composition in weaned piglets and improve growth performance in finishing pigs. Livest Sci. 2020 Dec; 242:104311.

[45] Wang F, Yin Y, Yang M, Chen J, Fu C, Huang K. Effects of Combined Supplementation of Macleaya cordata Extract and Benzoic Acid on the Growth Performance, Immune Responses, Antioxidant Capacity, Intestinal Morphology, and Microbial Composition in Weaned Piglets. Front Vet Sci. 2021 Aug 18;8:708597.

[46] Zhang R, Wang X, Zhu J, Liu L, Liu Y, Zhu H. Dietary sanguinarine affected immune response, digestive enzyme activity and intestinal microbiota of Koi carp (cryprinus carpiod). Aquaculture. 2019; 502:72–9.

[47] Pu J, Chen D, Gang T, He J, Ping Z, Mao X, et al. Protective Effects of Benzoic Acid, Bacillus Coagulans and Oregano Oil on Intestinal Injury Caused by Enterotoxigenic Escherichia Coli in Weaned Piglets. Biomed Research International. 2018 Aug.

[48] Niu Y, Wan X, Zhang L, Wang C, He J, Bai K, et al. Effect of Different Doses of Fermented Ginkgo biloba Leaves on Serum Biochemistry, Antioxidant Capacity Hepatic Gene Expression in Broilers. Animal Feed Science and Technology. 2019 Feb; 248:132–140.

[49] Kumar A, Bansal D, Bajaj K, Sharma S, Srivastava VK. Synthesis of Some Newer Derivatives of 2-Amino Benzoic Acid as Potent Anti-inflammatory and Analgesic Agents. Bioorganic and Medicinal Chemistry, 2003 Nov; 11(23):5281–5291.

[50] Mizushina, Y.; Miyazaki, S.; Ohta, K.; Hirota, M.; Sakaguchi, K. Novel anti-inflammatory compounds from Myrsine seguinii, terpeno-benzoic acids, are inhibitors of mammalian DNA polymerases. Biochim. Biophys. Acta (BBA) Gen. Subj. 2000; 1475, 1–4.

[51] Manivannah R, Shopna R. Anti-microbial and Anti-inflammatory Activity of New 4-methoxy-3-(methoxymethyl) Phenol and (E)-N′-(5-bromo-2-methoxybenzylidene)-4-methoxy Benzohydrazide Isolated from Calotropis gigantean white. Natural product sciences. 2017 March; 23(1):69

[52] Wright DA, Payne JP. A Clinical Study of Intravenous Anaesthesia with a Eugenol Derivatives G.29.505. British Journal of Anaesthesia, 1962 June; 34(6):379-385.

[53] Gorzalczany S, Lopez P, Acevedo C, Ferraro G. Anti-Inflammatory Effect of *Lithraea molleoides* Extracts and Isolated Active Compounds. Journal of Ethnopharmacology, 2011 Nov; 133(3):994-998.

[54] Morucci, F., Lopez, P., Mino, J., Ferraro, G. and Gorzalczany, G. Antinociceptive Activity of Aqueous Extract and Isolated Compounds of Lithrea molleoides .Journal of Ethnopharmacology, 2012 May; 142(2):401-406.

[55] López P, Ferraro G, Anesini C. Comparative antioxidant activity of an extract of Lithraea molleoides and an isolated 5-alkyl resorcinol derivative. Effects on the proliferation of normal and tumoral lymphocytes. Phytother Res. 2011 Feb; 25(2):271-6.

[56] Catalano A, Lantaño B, Fabián L, and López P. New 5-Alkenyl Resorcinols from *Lithraea molleoides* (Vell). Eng. American Journal of Plant Sciences, 2020 June; 11(6):861-868.