

# Some hematological parameters of Wistar rats treated with *Chromolaena odorata* leave extracts

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## Abstract

This study was designed to investigate the effects of the different extracts of *Chromolaena odorata* leave on the hematopoietic system of Wistar rats. Solvent extraction was used for the ethanol and aqueous extractions while decoction method was used for the crude extraction. Fifty Wistar rats of both sexes weighing 140-180 g were used for this study. They were divided into ten groups each containing five rats. The animals were fed the extracts by oral gavage once daily for 21 days. Blood sample was collected

via cardiac artery. Hematological parameters were analyzed using automation method. The ethanol extract gave the highest extract yield. The aqueous, ethanol and crude extraction had median lethal toxicity (LD<sub>50</sub>) of 2738.6 mg/kg, 1581.1 mg/kg and 224.7 mg/kg, respectively. Significant difference (P<0.05) in the total white blood cell count was observed in the 75 mg/kg ethanol and 300 mg/kg crude extracts when compared with control group. Significant difference (P<0.05) in the hemoglobin concentration was observed in the 150 mg/kg ethanol extracts when compared with the control group. Significant difference (P<0.05) in the packed cell volume was seen in the 75 mg/kg aqueous, 150 mg/kg aqueous and 75 mg/kg ethanol extracts in respect to the control group. The mean cell volume, the mean platelet volume and platelet large cell ratio of the 75 mg/kg aqueous extract were significantly different (P<0.05) when compared with the control group. The present study showed possible treatment-induced hematopoietic function of *C. odorata* leave extracts.

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Contributions: OHU conceived the study; OHU, UEK and OIM are responsible for the experimental design; OHU, AJI and OIM participated in the leaf extraction process; OHU, OIM and UEK participated in the laboratory analysis of the haematological parameters; OHU performed the data analysis, and prepared the manuscript; UEK, OIM and AJI edited the manuscript. All authors read and approved the final manuscript.

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## Introduction

Therapeutic use of plants is as old as mankind. The connection between man and the search for drugs in nature dates from far past.<sup>1</sup> Ever since ancient times people looked for drugs in nature. The beginning of medicinal plant use were instinctive as is the case with animals.<sup>2</sup> Awareness to medicinal plant use is due to many years of struggles against illness in which man learned to pursue drugs in barks, seeds, fruits and other plant parts.<sup>1</sup> Plant components used in therapeutics are termed phytochemicals and they represent naturally occurring chemical compounds present in plants that produce health benefits other than those attributed to micronutrients and macronutrients.<sup>3</sup> *Chromolaena odorata* is a rapidly growing perennial herb with multi stemmed shrub and grows up to 2.5 m in open areas.<sup>4</sup> Its common name is Siam weed. It has many local names depending on the tribe; Akwokko akpa (Ibibio), Bienqua (Ijaw), Obu inenawa (Igbo), Ewe Akintola (Ijebu). In Nigeria *Chromolaena odorata* is commonly called independent plant or Awolowo plant or Elizabeth plant.<sup>5-7</sup> *Chromolaena odorata* leave decoction have been used in folk medicine for wound healing, stoppage of bleeding, and even in some communities where it was reported to be used in pile ailment.<sup>8</sup> Moreso, it has acquired the reputation of medicinal herb for variety of ailments including malaria, dysentery, toothache, and fever.<sup>9</sup> However, its homeostatic property have been reported *in vivo* and *in vitro* study.<sup>10</sup> Despite immense literature on the homeostatic and antimicrobial activities of *C. odorata*, not much information is available on the effect of the leaf extracts on hematological parameters.

## Materials and Methods

### Preparation of plant parts

Fresh leaves of *C. odorota* were harvested from farms along University of Calabar Staff Quarters road and were identified by Dr. J.K. Ebigwai of Botany Department, University of Calabar. The leaves were rinsed with distilled water and dried under shade. The dried leaves were ground into powder after which 200 g of the coarse powder was suspended in 1200 ml of ethanol (98.67% BDH) and 1200 mL of distilled water for the ethanol and aqueous extractions, respectively and agitated thoroughly for about 10 minutes with an electric blender. The suspensions were allowed overnight in a Westcool fridge (4°C). Twenty four hours later, the mixtures were filtered with chess cloth and the filtrates concentrated *in vacuo* (rotary evaporator) to 10% of the original volume at 40°C. These concentrates were kept in water bath at 37°C for complete dryness. These extracts were reconstituted in normal saline to an appropriate concentration before administration.<sup>11</sup> Fresh leaves for the crude extraction were ground using manual grater followed by decoction and then gravimetric analysis.<sup>12</sup>

### Animal treatment

A total of 50 apparently healthy Wistar rats of both sexes weighing 140-180 g obtained from the animal house, College of Medical Sciences University of Calabar were used for hematological evaluation while 39 mice weighing 60-80 g were used for the acute toxicity study. All the experimental animals were fed with standard animal feed and water *ad libitum*. All experiments were performed in accordance with the guidelines for care and use of laboratory animals of Faculty Animal Research Ethics Committee (FAREC-FBMS) Faculty of Basic Medical Sciences University of Calabar.<sup>13</sup> Animal use protocols were approved by the Faculty Animal Research Ethics Committee (FAREC-FBMS) Faculty of Basic Medical Sciences University of Calabar with reference number 001B4216.

### Acute toxicity

The acute toxicity study was performed using the method described by Lorke (1998).<sup>14</sup>

### Phytochemical analysis

#### Test for alkaloids

Presence of alkaloid in the extracts was estimated by Mayer's, Wagner' and Dragendorff's tests. Each of the extracts was stirred with 1% aqueous hydrochloric acid on water bath and filtered. Two milliliters of the filtrates (in each case) were added to Mayer's reagent, Wagner's reagent and Dragendorff's reagent in separate tubes. Alkaloids react with each of the reagents to form precipitate. The turbidity of the precipitation with either of the reagents is proportional to the quantity of alkaloid present.<sup>15</sup>

#### Test for tannins

Ferric Chloride and Lead Acetate tests were used to detect the presence of tannins. The extracts were stirred with distilled water and filtered. Two milliliters of the filtrates were then added to 10% ferric chloride and 10% lead acetate in separate tubes. Tannin in the extract reacts with the ferric chloride and lead acetate to form a blue-black, green or blue-green precipitate (in each case).<sup>15</sup>

#### Test for saponins

Emulsifying and Frothing tests were used to detect the presence of Saponins. The extracts were warmed with distilled water and filtered. Three drops of olive oil was added to 2 ml of the filtrate for the emulsifying test, while 4 ml of distilled water was added to 1ml of the filtrate for the frothing test. Both were shaken. Presence of brown emulsion (for the emulsifying test) and frothing (for frothing test) confirmed presence of saponins.<sup>15</sup>

#### Test for anthraquinone

Presence of anthraquinone was detected by modified Borntrager's test. Ten milliliters of concentrated chloroform was used to dissolve the extracts. The resulting solution was then filtered. Two and half milliliters of the filtrate was then added to 2.5 ml ammonia solution and shaken vigorously. Presence of pink to red precipitates confirmed the presence of anthraquinones.<sup>15</sup>

#### Test for glycosides

Half a gram of the extracts was boiled with 5 ml of dilute hydrochloric acid and the resulting solution was neutralized with sodium hydroxide solution and filtered. Two milliliters of the resultant filtrate was then added to Fehlings solution 1 and 2, mixed thoroughly and boiled in water bath for 2 minutes. The appearance of reddish-brown precipitates indicated a positive result.<sup>15</sup>

#### Test for flavonoids

The presence of flavonoid in the extracts was determined by sodium hydroxide method. The extracts were dissolved in distilled water and filtered. Two milliliters of the resultant filtrate was then added to two different tubes. Three drops of sodium hydroxide were added to the first tube and allowed to stand for 2 minutes. Three drops of sodium hydroxide and 3 drops of 0.5 N hydrochloric acid were added to 2 ml of the filtrate in the second tube. Both mixtures were observed for precipitation and colour change. Production of yellow coloration in the first tube and transition from yellow to colourless in the second tube confirmed presence of flavonoids.<sup>15</sup>

### Study protocol

The experimental animals were divided into ten groups and each group consisted of five animals. Group I served as control and received 0.2 ml of normal saline vehicle. Group II animals received 75 mg/kg of aqueous extract. Group III received 150 mg/kg of aqueous extract. Group IV received 300 mg/kg of aqueous extract. Group V received 75 mg/kg of the ethanol extract. Group VI received 150 mg/kg of the ethanol extract. Group VII received 300 mg/kg of the ethanol extract. Group VIII received the 75 mg/kg of the crude extract. Group IX received 150 mg/kg of the crude extract. Group X received the 300 mg/kg of the crude extract. Extracts for the test groups were dissolved in normal saline vehicle with respect to their concentration (extract dry weight per kg of the animal weight) and animal body weight. All dosages above were administered once per day via oral gavage for 21 days.

### Sample collection analysis

At the end of the study, the animals were sacrificed and blood collected via cardiac artery into EDTA container and analyzed immediately using automated SYSMEX KX-2IN by Sysmex America Inc. (IL, USA) using Stromalyser reagent also produced by Sysmex America Inc.

## Statistical analysis

All values were expressed as Mean±SEM. Statistically difference between groups were determined using one way Analysis of variance (ANOVA) followed by Least significant difference test in significant cases using SPSS version 20 software. Differences were considered to be statistically significant when  $P < 0.05$ .

## Results

The percentage yield of the ethanol and the aqueous extracts were 3.9% and 4.7% respectively while the crude extract gave yield of 0.046 mg/ml (Table 1). The crude extract contains Alkaloids, Tannins, Flavonoids, Saponins, Anthraquinones, Glycosides and Polyphenols. Alkaloids and Glycosides were absent in the aqueous extract while Anthraquinone was absent in the ethanol extract (Table 2). The acute toxicity ( $LD_{50}$ ) of the aqueous, ethanol and crude extracts were 2738.6 mg/kg, 1581.1 mg/kg and 1224.7 mg/kg, respectively (Table 3).

The red cell count and the hemoglobin concentration of the 150 mg/kg of the aqueous extract were significantly ( $P < 0.05$ ) higher than the control. The packed cell volume of the 75 mg/kg and 150 mg/kg aqueous extracts were significantly ( $P < 0.05$ ) higher than that of the control group. The mean cell volume, the mean platelet volume and the platelet large cell ratio of the 75 mg/kg aqueous extract were significantly ( $P < 0.05$ ) greater than that of the control group (Table 4). The total white cell count, the red cell count, the hemoglobin concentration and packed cell volume of the 75 mg/kg ethanol extract were significantly ( $P < 0.05$ ) higher than the control group (Table 5). The total white cell count of the 300 mg/kg crude extract was significantly ( $P < 0.05$ ) higher than the control group (Table 6).

## Discussion

Hematological indices provide physiological information on the blood picture and the reticuloendothelial system.<sup>16</sup> The present study observed higher yield in the aqueous extraction than the ethanol extraction. This observation is at variance with earlier report by Mbajuka *et al.* (2014),<sup>17</sup> who had higher value in the

ethanol extraction. This study showed presence of bioactive phytochemicals such as Alkaloids, Tannins, Flavonoids, Saponins, Anthraquinones, Glycosides and Polyphenols in the crude extract with Alkaloids and Glycosides absent in the aqueous extract and Anthraquinones absent in the ethanol extract. This observation is

**Table 1. Plant extract yield based on solvent used.**

Plant	Solvent	Yield
<i>C. odorata</i>	Ethanol	3.90%
<i>C. odorata</i>	Aqueous	4.76%
<i>C. odorata</i>	Crude	0.046 mg/mL

*C. odorata, Chromolaena odorata.*

**Table 2. Phytochemical result of crude, ethanol and aqueous extracts of *Chromolaena odorata* leaves.**

Phytochemical	Aqueous extract	Ethanol extract	Crude extract
Alkaloids	-	+	+
Tannins	+	+	++
Flavonoids	+	++	+++
Saponins	++	++	++
Anthraquinone	++	-	++
Glycosides	-	+	++
Polyphenols	++	++	++

+++ , present in appreciable quantity; ++, present; +, present in low quantity; -, not present.

**Table 3. Results of acute toxicity study of the various leaf extracts of *Chromolaena odorata*.**

Type of extract	$LD_{50}$ (mg/kg)
Aqueous	2,738.6
Ethanol	1,581.1
Crude	1,224.7

**Table 4. Some hematological parameters of aqueous leaf extract of *Chromolaena odorata* treated rats.**

Parameter	Control	75 mg/kg	150 mg/kg	300 mg/kg
WBC ( $\times 10^3/\mu\text{L}$ )	9.5±0.6	8.4±0.8	7.0±0.5	7.8±0.7
RBC ( $\times 10^6/\mu\text{L}$ )	7.1±0.2	6.8±0.1	8.5±0.3*	7.1±0.2
Hb (g/dL)	12.5±0.4	13.7±0.1	15.6±0.6*	11.6±1.0
PCV (%)	38.7±1.3	44.7±0.2*	48.4±1.8*	40.2±1.2
MCV (fL)	54.4±0.9	66.0±0.4*	55.7±1.9	57.0±1.6
MCH (pg)	17.7±0.9	20.2±0.2	18.3±0.8	16.4±1.4
MCHC (g/dL)	32.5±1.2	30.7±0.2	32.2±0.6	28.7±1.9
Platelet ( $\times 10^3/\mu\text{L}$ )	682.2±89.6	654.0±35.1	510.5±82.2	616.3±47.1
MPV (fL)	7.5±0.2	8.6±0.1*	7.8±0.4	7.7±0.1
P_LCR (%)	10.4±1.9	16.8±0.8*	13.6±2.3	12.2±1.5

Data expressed as mean±SEM; \*significant difference from control ( $P < 0.05$ ).

in the consonance with earlier report by Afolabi *et al.* (2007),<sup>7</sup> and Inyang and Adegoke (2008),<sup>18</sup> and in variance with report of Anyasor *et al.* (2008).<sup>19</sup> This variation is possibly due to differences in methodology. The result of this study showed that *C. odorata* did not negatively alter white cell indices rather, the 75 mg/kg ethanol, 300 mg/kg crude extract significantly increased the total white cell count in *C. odorata* treated rats. This rise in total white cell count may be due to hyper stimulation of hematopoietic regulatory elements which regulates proliferation by the extracts.<sup>20</sup> Higher quantity of flavonoids in the ethanol and crude extract might be the reason for this observed leucocytosis as flavonoids have been reported to increase intracellular vitamin C synthesis and leucocytosis.<sup>21</sup> This study showed significant increase in erythrocyte count in the 150 mg/kg aqueous and the 75 mg/kg ethanol extracts. This is confirmed by the increase in the haematocrit [packed cell volume (PCV)] and hemoglobin (Hb) since PCV and Hb are functions of total red blood cell as against expected anemia due to the presence of Saponin. The result of this study strongly suggests the possible role *C. odorata* leaf extract may have in treatment – induced erythropoietic activity. *Chromolaena odorata* leaf extract could be incorporated into pharmaceutical products intended to boost erythropoietic activity. This suggestion is affirmed by earlier report of high iron content in the leave of *C. odorata*.<sup>22</sup> The Saponin paradox is explained by the fact that

hemolytic saponins are toxic when given via intravenous route.<sup>23,24</sup> This study observed increase in mean platelet volume in the 75 mg/kg aqueous extracts showing improved platelet quality, hence its possible role in hemostatic as earlier reported.<sup>10</sup> Increased mean platelet volume is an indication of larger and more reactive platelets resulting in increased platelet turnover. Larger platelets are more adhesive and tend to aggregate more than small platelets and contain more secretory granules.<sup>25</sup>

## Conclusions

Both the aqueous and the ethanol leaf extracts of *Chromolaena odorata* significantly increased red cell count, hemoglobin concentration and packed cell volume in the experimental animal, suggesting its possible treatment induced hematopoietic activity

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**Table 5. Some hematological parameters of ethanolic leaf extract of *Chromolaena odorata* treated rats.**

Parameter	Control	75 mg/kg	150 mg/kg	300 mg/kg
WBC ( $\times 10^3/\mu\text{L}$ )	9.5 $\pm$ 0.6	14.5 $\pm$ 1.2*	10.9 $\pm$ 0.7	13.9 $\pm$ 2.3
RBC ( $\times 10^6/\mu\text{L}$ )	7.1 $\pm$ 0.2	8.3 $\pm$ 0.2*	6.5 $\pm$ 0.1	7.5 $\pm$ 0.1
Hb (g/dL)	12.5 $\pm$ 0.4	13.4 $\pm$ 0.9*	12.7 $\pm$ 0.2	13.0 $\pm$ 0.4
PCV (%)	38.7 $\pm$ 1.3	46.9 $\pm$ 1.7*	38.3 $\pm$ 1.2	40.8 $\pm$ 0.3
MCV (fL)	54.4 $\pm$ 0.9	56.6 $\pm$ 1.3	57.6 $\pm$ 1.6	54.4 $\pm$ 1.1
MCH (pg)	17.70.9	16.3 $\pm$ 1.4	19.1 $\pm$ 0.2	17.3 $\pm$ 0.8
MCHC (g/dL)	32.5 $\pm$ 1.2	28.9 $\pm$ 2.7	33.2 $\pm$ 0.7	31.8 $\pm$ 0.9
Platelet ( $\times 10^3/\mu\text{L}$ )	682.2 $\pm$ 89.6	879.4 $\pm$ 117.1	800.8 $\pm$ 77.9	656.0 $\pm$ 30.8
MPV (fL)	7.5 $\pm$ 0.2	7.5 $\pm$ 0.5	7.1 $\pm$ 0.2	7.1 $\pm$ 0.3
P_LCR (%)	10.4 $\pm$ 1.9	11.4 $\pm$ 2.8	8.3 $\pm$ 1.0	8.3 $\pm$ 1.1

Data expressed as mean $\pm$ SEM; \*significant difference from control (P<0.05).

**Table 6. Some hematological parameters of crude leaf extract of *Chromolaena odorata* treated rats.**

Parameter	Control	75 mg/kg	150 kg/mg	300 mg/kg
WBC ( $\times 10^3/\mu\text{L}$ )	9.5 $\pm$ 0.6	11.6 $\pm$ 0.8	10.8 $\pm$ 1.7	16.0 $\pm$ 5.4*
RBC ( $\times 10^6/\mu\text{L}$ )	7.1 $\pm$ 0.2	7.2 $\pm$ 0.2	7.7 $\pm$ 0.2	6.9 $\pm$ 0.9
Hb (g/dL)	12.5 $\pm$ 0.4	12.9 $\pm$ 0.8	13.7 $\pm$ 0.7	12.7 $\pm$ 2.4
PCV (%)	38.7 $\pm$ 1.3	40.2 $\pm$ 1.3	42.1 $\pm$ 1.0	37.1 $\pm$ 6.9
MCV (fL)	54.4 $\pm$ 0.9	55.7 $\pm$ 0.2	54.0 $\pm$ 0.8	53.5 $\pm$ 2.7
MCH (pg)	17.7 $\pm$ 0.9	17.9 $\pm$ 0.6	17.6 $\pm$ 1.0	18.2 $\pm$ 0.9
MCHC (g/dL)	32.5 $\pm$ 1.2	32.1 $\pm$ 1.1	32.6 $\pm$ 1.5	34.1 $\pm$ 0.1
Platelet ( $\times 10^3/\mu\text{L}$ )	682.2 $\pm$ 89.6	711.3 $\pm$ 76.5	781.0 $\pm$ 84.1	814.0 $\pm$ 63.0
MPV (fL)	7.5 $\pm$ 0.2	7.1 $\pm$ 1.2	6.7 $\pm$ 0.2	7.3 $\pm$ 0.5
P_LCR (%)	10.4 $\pm$ 1.9	8.1 $\pm$ 0.5	6.6 $\pm$ 0.7	8.8 $\pm$ 1.5

Data expressed as mean $\pm$ SEM; \*significant difference from control (P<0.05).

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