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Anti-anaemic activity of *Jatropha tanjorensis* Ellis & Saroja in Rabbits.

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Objective: *Jatropha tanjorensis* Ellis & Saroja (Euphorbiaceae) is a common weed of field crops. The leaf is a commonly consumed vegetable in many parts of Southern Nigeria. The anti- anaemic, acute toxicity and proximate analysis of *J. tanjorensis* was investigated in rabbits.
Materials and methods: Routine methods were used in this study.
Results: The results revealed that proximate analysis of the sample showed that all the macronutrients were present with protein being the most abundant (41.65%). There was also significant decrease in Packed Cell Volume in groups B, C and D by day 3 of the experiment and subsequent increase by day 14 of the experiment after treatment with aqueous suspension of *J. tanjorensis* leaves. The results suggests that crude extract of *J. tanjorensis* improved the anaemic condition of the treated animals (Groups B, C and D) when compared with the phenyl hydrazine induced but untreated animals (Group E).
Conclusion: *J. tanjorensis* contains important elements which if used in recommended doses could be of immense benefits to man.

Keyword: Anti-anaemic, Acute toxicity, proximate analysis, *Jatropha tanjorensis*, Rabbits.

1. Introduction

Anaemia is a medical condition characterized by lowered haemoglobin level. There are over 400 types of anaemia, with haemolytic anaemia being the most frequent [1]. More than half of the world's population experience some forms of anaemia in their life time [2]. The incidence of anaemia is higher in the third world than in developed countries [3].

A study in a rural population of Nigeria reported that 19.7% of the children were anaemic [4]. Such prevalence has been attributed to various aggravating factors such as poor nutrition, high prevalence of blood parasites for example, plasmodium, trypanosome and helminthes infection [3]. Prolonged use of non-steroidal anti-inflammatory drugs as well as exposure to toxic chemicals such as phenyl hydrazine have also been implicated to cause the condition [5, 6, 7]. Due to the high prevalence and possibility of even further increase [2], there is the need to prevent it or seek for more cost effective and better treatment strategies. Some plants have been studied for their anti-anaemic properties [1, 3, 8].

Jatropha tanjorensis Ellis & Saroja (Euphorbiaceae) is a common weed of field crops, bush re-growth, road sides and disturbed places in the higher rainfall forest zones of West Africa.

It is commonly called 'hospital too far', catholic vegetable, 'Iyana-Ipaja' or 'lapalapa' [9]. The leaf is a commonly consumed vegetable in many parts of Southern Nigeria. It is also popular as a natural remedy against diabetes in this region [10]. Phytochemical screening of *J. tanjorensis* leaf revealed that it contains bioactive principles such as alkaloids, flavonoids, tannins, cardiac glycosides, anthraquinones, and saponins [11].

Although plant based natural medicines are popularly acclaimed to be safe, the need for adequate toxicity testing have been emphasized [12-14]. This present study aimed to evaluate the anti-anaemic, acute toxicity and proximate analysis of *J. tanjorensis*.

2. Materials and methods

2.1 Plant samples

The leaves of *J. tanjorensis* were freshly collected from BDPA Estate, Ugbowo, Benin City, Nigeria. The sample was identified and authenticated in the herbarium unit of the Department of Plant Biology and Biotechnology, University of Benin. They were air dried for 3 days, after which they were kept in the oven to dry at 40 °C for few hours. The crispy leaves were reduced to powder using an electrically powered engine. The powdered sample was stored in a moisture free, air-tight container until further use.

2.2 Proximate analysis of plant samples

The standard procedure as outlined in Horwitz (2000) was employed for the determination of the percentage proximate composition of carbohydrate, fats and oil, protein and other nutrients of the dry ground leaves of *J. tanjorensis*. The moisture content was determined by heating 2 g of ground air-dried sample in a vacuum oven at 70 ^{o}C under pressure of 95 mmHg to a constant weight.

The crude fat content was determined as follows: the oven-dried samples obtained from the moisture content determination was then extracted with petroleum ether (60-80 ^{o}C) for 16 hours in Soxhlet-type extractor. The ether was evaporated and the residue dried to a constant weight at 95-100 ^{o}C and then cooled in a desiccator. The weight loss expressed as percentage gave the crude fat content.

The percentage nitrogen was determined by the improved Kjeldahl method and the nitrogen content was converted to crude protein by multiplying with 6.25 [15].

The ceramic fibre filter method was used to analyze for the crude fibre content. Briefly, 2 g ground sample was defatted with petroleum either then digested with 1.25% (v/v) H_2SO_4 and 1.25% (v/v) NaOH. The residues were ignited at 130 ^{o}C for 2 hours, cooled in a desiccator and weighed. The ash content was determined using AOAC recommended method.

The carbohydrate content of the sample was determined by subtracting the sum of the percentages of moisture, crude fat, fibre, protein and ash from 100.

2.3 Major Trace Elements and Mineral Evaluation of plant samples

Sodium (Na) and Potassium (K) were determined by the Flame photometry (Jenway Ltd., Dunmond, Essex, UK) while phosphorus (P) was determined by Vanadomolybdate method using Corning colorimeter 253. Other minerals were determined after wet digestion with a mixture of sulphuric, nitric and per chloric acids using Atomic Absorption Spectrophotometer (Buck scientific, 200A East Norwalk, CT 06855, USA).

2.4 Experimental animals

Fifteen healthy rabbits of both sexes, weighing between 1.2 kg and 1.9 kg, purchased from 'Aduwawa' market in Benin City. They were kept in standard rabbit cages and were allowed unrestricted access to normal rabbit chow (Bendel Feeds and Flour Mill Ltd., Ewu, Edo State, Nigeria) and water, and allowed to acclimatize for 3 weeks.

The rabbits were divided into five groups, each consisting of three rabbits and baseline values determined. Marker pen was used to distinctly label each animal for easy identification. Group A rabbits served as the control and were neither induced with anaemia nor treated with the plant sample. Group B, C and D rabbits were induced with anaemia and were treated with the plant material at different dosage. Group E rabbits were induced with anaemia but were not treated with the plant material, and served as test control.

Anaemia was induced in the rabbits according to the method described by Harris and Kugler (1971), via subcutaneous administration of 2.5% neutralized phenyl hydrazine hydrochloride (Fisher scientific company, New Jersey, USA) at a dose of 30 mg kg-1 body weight with a maintenance dose of 15 mg kg-1body weight of the same drug 2 days after administration of the first dose.

2.5 Blood Collection and Haematological Analysis

About 3 ml of blood was collected from each rabbit into a 5 ml EDTA tubes by puncturing the prominent ear vein with syringe needles. The haematological analyses were carried out within 24 hours of blood collection using an automatic haemato-analyser (Sysmex, KX-21, Japan).

The first set of blood samples of all the animals in the five groups were collected before the induction of anaemia (day 1). The second set of blood samples were collected 3 days after the induction of anaemia (day 3). The third set of blood samples was collected 11 days later (day 14). The parameters included PCV (packed cell volume), WBC (white blood cell count), RBC (red blood cell count), Hb (haemoglobin concentration), MCV (mean corpuscular volume) and MCHC (mean corpuscular haemoglobin concentration).

2.6 Treatment of Phenyl Hydrazine-Induced Anaemia with Plant Material

Group B, C and D rabbits were treated by oral administration of 10 ml of aqueous suspension of *J. tanjorensis* leave powder 10 g/100 ml for group B rabbits, 7.5 g/100 ml for group C rabbits and 5 g/100 ml for group D rabbits twice daily from day 3 of the experiment.

2.7 Acute toxicity

The mortalities were counted from the day administration of plant sample started (day 3) to the end of the experiment (day 14) and the Lethal Dose (LD50) was determined.

2.8 Statistical analysis

The group means SEM was calculated for each analyte and the level of significance for the differences between means were calculated using students test SPSS 16. The level of significance was at 0.05

3. Results

3.1 Proximate Analysis

The proximate analysis of the sample showed that all the macronutrients were present with protein being the most abundant (41.65%).

Table 1: Percentage proximate composition of *Jatropha tanjorensis* leaves.

Macronutrient	Composition (%)
Protein	41.65
Fats and oil	36.73
Ash	4.71
Fibre	3.46
Total soluble carbohydrate	5.43
Starch	5.35
Moisture	9.98

3.2 Major Trace Elements and Mineral Evaluation of the Plant Sample

Table 2 indicates the presence of major trace elements and minerals in the plant sample, in relatively high concentrations. It had the highest concentration in potassium (K) (5.19 g kg-1) and the lowest concentration was in zinc (Zn) (0.99 g kg-1).

Table 2: Major trace elements and mineral content of dried *Jatropha tanjorensis* leaves

Parameters	Concentration (g kg-1)
Calcium (Ca)	4.56
Magnesium (Mg)	4.83
Sodium (Na)	1.41
Potassium (K)	5.19
Iron (Fe)	3.11
Zinc (Zn)	0.99
Manganese (Mn)	1.07

3.3 Haematology Analysis

Table 3 shows significant decrease in Packed Cell Volume in groups B, C and D by day 3 of the experiment and subsequent increase by day 14 of the experiment after treatment with aqueous suspension of *J. tanjorensis* leaves.

Table 3: Effect of administration of aqueous suspension of *Jatropha tanjorensis* leaves on Packed Cell Volume (PCV) % in phenyl hydrazine-induced anaemic rabbits.

	Duration of experiment (days)		
Groups	**1**	**3**	**14**
A	35.70±0.23	36.29±0.14	37.36±0.06
B	34.54±0.06	16.15±0.05	39.26±0.04
C	26.09±0.11	13.81±0.29	42.41±0.39
D	32.09±0.21	15.04±0.24	36.68±0.21
E	40.62±0.28	18.24±0.06	25.16±0.04

*Values are mean ± SEM; n=3

Table 4 shows significant decrease in Red blood cell count in groups B, C and D by day 3 of the experiment and subsequent increase by day 14 of the experiment after treatment with aqueous suspension of *J. tanjorensis* leaves.

Table 4: Effect of administration of aqueous suspension of *Jatropha tanjorensis* leaves on red blood cell count (RBC) x1012L-1 in phenyl hydrazine-induced anaemic rabbits.

	Duration of experiment (days)		
Groups	**1**	**3**	**14**
A	5.69±0.13	5.46±0.06	5.69±0.03
B	5.83±0.07	3.01±0.19	4.71±0.08
C	3.58±0.02	2.98±0.02	4.34±0.16
D	5.75±0.07	3.28±0.08	6.04±0.06
E	6.68±0.06	2.27±0.13	3.26±0.06

*Values are mean ± SEM; n=3

Table 5 shows significant increase in White blood cell count in groups B, C and D by day 3 of the experiment and subsequent decrease by day 14 of the experiment after treatment with aqueous suspension of *J. tanjorensis* leaves.

Table 5: Effect of administration of aqueous suspension of *Jatropha tanjorensis* leaves on White Blood Cell Count (WBC) x109L-1 in phenyl hydrazine-induced anaemic rabbits

	Duration of experiment (days)		
Groups	**1**	**3**	**14**
A	3.44±0.02	3.62±0.03	3.71±0.01
B	4.71±0.05	6.90±0.01	5.02±0.06
C	4.07±0.02	6.33±0.02	5.02±0.06
D	3.50±0.07	5.51±0.09	0.93±0.07
E	5.09±0.11	7.28±0.12	6.02±0.08

*Values are mean ± SEM; n=3

Table 6 shows significant decrease in Haemoglobin Concentration in groups B, C and D by day 3 of the experiment and subsequent increase by day 14 of the experiment after treatment with aqueous suspension of *J. tanjorensis* leaves.

Table 6: Effect of administration of aqueous suspension of *Jatropha tanjorensis* leaves on mean Haemoglobin Concentration (Hg) g/dl in phenyl hydrazine-induced anaemic rabbits

	Duration of experiment (days)		
Groups	**1**	**3**	**14**
A	11.96±0.06	12.07±0.03	12.34±0.03
B	11.85±0.08	5.63±0.06	10.94±0.06
C	7.18±0.02	4.01±0.19	11.68±0.32
D	10.71±0.08	4.92±0.12	9.34±0.20
E	13.56±0.08	7.03±0.16	6.82±0.02

*Values are mean±SEM; n=3

Table 7 shows significant decrease in Mean Corpuscular Volume in groups B, C and D by day 3 of the experiment and subsequent increase by day 14 of the experiment after treatment with aqueous suspension of *J. tanjorensis* leaves.

Table 7: Effect of administration of aqueous suspension of *Jatropha tanjorensis* leaves on Mean Corpuscular Volume (MCV) in phenyl hydrazine-induced anaemic rabbits.

	Duration of experiment (days)		
Groups	**1**	**3**	**14**
A	62.72±0.35	63.81±0.09	65.56±0.02
B	59.18±0.02	30.06±0.03	83.28±0.02
C	64.29±0.01	25.49±0.01	97.60±0.09
D	55.62±0.09	28.04±0.10	90.51±0.09
E	60.37±0.03	29.96±0.03	39.01±0.01

*Values are mean ± SEM; n=3

Table 8 shows significant decrease in Mean Corpuscular Haemoglobin concentration in groups B, C and D by day 3 of the experiment and subsequent increase by day 14 of the experiment after treatment with aqueous suspension of *J. tanjorensis* leaves.

Table 8: Effect of administration of aqueous suspension of *Jatropha tanjorensis* leaves on Mean Corpuscular Haemoglobin Concentration (MCHC) g/dl in phenyl hydrazine- induced anaemic rabbits

	Duration of experiment (days)		
Groups	**1**	**3**	**14**
A	33.39±0.14	33.12±0.16	32.97±0.04
B	34.25±0.10	18.10±0.12	27.80±0.14
C	30.82±0.20	15.03±0.20	25.92±0.20
D	33.43±0.10	14.92±017	25.46±0.10
E	33.25±0.10	18.01±0.11	30.82±0.21

*Values are mean ± SEM; n=3

3.4 Toxicity Study

Table 9 shows the acute toxicity data of treated rabbits Groups B, C and D from day 3 to day 14 of the experiment. It was observed that the aqueous suspension of *J. tanjorensis* leaves showed no visible signs of toxicity within the dose range used.

Table 9: Acute toxicity study in rabbits after 24 hours – 12 days of administration of 10 ml aqueous suspension of *J. tanjorensis* dried ground leaves twice daily.

Groups	Dose	Initial mean weight (kg ± SEM on day 1	Final mean weight (kg) ±SEM on day 12	Mortality
B	10 g/100 ml	1.08±0.08	1.67 ± 0.85	Nil
C	7.5 g/100 ml	1.05± 0.16	1.82± 0.68	Nil
D	5 g/100 ml	1.30 ±0.62	1.45±0.84	Nil

*Values are mean ± SEM; n=3

4. Discussion and Conclusion

The data on the proximate constituent of the plant sample, represented in Table 1, indicates clearly its potential as food. The crude protein content of the sample was 41.65% and this value compared favorably with and in some cases surpassed those reported for legumes grown in West Africa [16, 17].

The data on the proximate analysis also showed that the crude fibre content was comparable to the reported fibre content of *T. occidentalis* (4.60%) [18] which have been reported to possess anti-anaemic potentials [3]. Nutritionally, this is of beneficial effect since it had been reported that food fibre aids absorption of trace elements in the gut and reduce absorption of cholesterol [19]. This also makes the plant suitable for combating anaemia in pregnant women.

The ash content is a measure of a plants mineral content. The ash content (4.71%) of *J. tanjorensis* leaves indicates that the leaves contain appreciable amount of mineral element. The value was higher than 3.25-4.16% reported for seeds of some species of *Mucuna* [20].

From Table 2, it can be seen that *Jatropha tanjorensis* contains 4.56 g kg-1 of calcium. Calcium is necessary for the coagulation of blood, the proper functioning of the heart and nervous system and normal contraction of muscles. Its most important function is to aid in the formation of bones and teeth. The calcium content is very high compared to the reported values for other higher plant sources that are already in conventional use as medicines and in human/animal diet [21-22].

The value of sodium (1.41 g kg-1) is however low compared to the reported values of *Telfera occidentalis,* having sodium content of 8.2 g kg-1 [24] but was still comparable to the reported values for other plant sources that are already in conventional use as medicines and in human/animal diets [21-22]. The result reflects abundant level of potassium (5.19 g kg-1) compared to the reported value of *T. occidentalis* having potassium content as 3.7 g kg-1, [24] and has been reported to possess anti-anaemic potentials [3]. Sodium and Potassium are closely related in the body fluids, they regulate the acid-base balance. Sodium remains one of the major electrolytes in the blood. Without sodium the body cannot be hydrated, it would dry up [23].

The value of iron (3.11 g kg-1) is high compared to reported value of *T. occidentalis* which has iron content of 0.90 g/kg [24] which has been reported to possess anti anaemic potentials [3]. Iron is important for the building up of red blood cells essential for formation of haemoglobin the oxygen carrying pigment in red blood cells. Iron is used against anaemia, tuberculosis and disorder of growth [25]. Iron is an energizer but excess can cause fatigue but we hardly have excess if taken from natural source [23]. The availability of iron in a diet may modulate the regenerative response [26].

The value of zinc (0.99 g kg-1) is comparable to the reported value of *T. occidentalis* which has zinc content of 0.7 g kg-1 [24] which has been reported to possess anti-anaemic potentials [3]. Zinc is very important for nerve function and male fertility. It is important for normal sexual development especially for the development especially for the development of testes and ovaries, it is also essential for reproduction. Zinc stimulates the activity of vitamins, formation of red and white corpuscles [25] healthy functioning of the heart and normal growth [27].

The activity of manganese is noticed in the metabolism of food which is incorporated into the bone. According to [25], manganese is necessary for the functioning of the pituitary gland, the pineal gland the brain, it promotes hepatorenal function, combats anaemia and also essential for growth.

Haemolytic anaemia was diagnosed by a more than 50% reduction in PCV values the rabbits in groups B, C, D and E from the baseline values by three days after phenyl hydrazine administration. Phenyl hydrazine has been reported to induce haemolytic anaemia in rabbits at a dose of 30 mg kg-1 body weight two days after administration [3].

The rabbits in group A which served as control and were not induced with anaemia or treated, had PCV, RBC, WBC, Hb, MCV and MCHC concentrations within the normal range throughout the duration of the experiment.

The rabbits in group B, C and D which were induced with anaemia had significant ($P<0.05$) decrease in the values for PCV, Hb, RBC, MCV and MCHC concentration by day three of the experiment indicating anaemia, and also a significant increase ($P<0.05$) was also observed for WBC concentration. This group of rabbits was treated by oral administration of 10ml of the aqueous suspension of *J. tanjorensis* leaves powder (10 g/100 ml for group B, 7.5 g/100 ml for group C and 5 g/100 ml for group D) twice daily. Treatment with the plant sample significantly ($P<0.05$) improved the PCV, Hb, RBC, MCV and MCHC levels (Table 3) of the animals in these groups and also significantly ($P<0.05$) reduced the WBC level by day 14 of the experiment bringing it back to acceptable normal value.

Rabbits in Group E (test control) were induced with anaemia but were not administered the aqueous suspension of *J. tanjorensis* leaves. They also showed a significant decrease ($P<0.05$) in the values for PCV, Hb, RBC, MCV and MCHC counts and increased WBC count value by day 3 of the experiment. However, there seems to be slight recovery from anaemia by day 14, probably due to decreasing effect of the drug.

It has been reported that phenyl hydrazine causes oxidative damage to red cells by increasing the formation of reactive oxygen species [28, 29]. However, alkaloids and flavonoids protect cells as powerful antioxidants which prevent or repair damage done to red cells by free radicals or highly reactive oxygen species [3]. Previous phytochemical screening of *J. tanjorensis* leaf revealed the presence of alkaloids, flavonoids, tannins, cardiac glycosides, anthraquinones and saponins [11]. Thus, it appears that the presence of these antioxidants in the plant sample reverse the damaging effect of phenyl hydrazine.

The acute toxicity study in rabbits (Groups B, C and D) within the administered dose (Group B- 10 ml of 10 g/100 ml, Group C- 10 ml of 7.5 g/100 ml and Group D 10 ml of 5 g/100 ml aqueous suspension of the plant sample twice daily) did not show any sign of acute toxic effect. No mortality was recorded in the treated groups as shown in Table 4. This suggests the high tolerance and safety of the plant sample within the administered doses. It can therefore be deduced that the Lethal Dose (LD50) for the plant sample is greater than 10 g/100 ml since up to this dose no death was recorded.

In this study, it has been shown that the crude extract of *J. tanjorensis* improved the anaemic condition of the treated animals (Groups B, C and D) when compared with the phenyl hydrazine induced but untreated animals (Group E). It shows that *J. tanjorensis* contains important elements which if used in recommended doses could be of use to man. And further investigation to determine the lethal dose of *J. tanjorensis* is also recommended.

5. Conflict of interest: The authors declare that there are no conflicts of interests as regards the publication of this article.

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