

RESEARCH ARTICLE

JATROPHA TANJORENSIS LEAF EXTRACT EFFECT ON THE EXPRESSION PATTERN OF RUNX1 MRNA AND ETV6 IN AMELIORATING ANAEMIA

EO Osime¹, MO Erhunmwunsee², AI Aruomaren³

¹Professor of Hematology, Department of Medical laboratory science, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City. – Nigeria

²Medical Laboratory Intern, Department of medical laboratory science, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City. – Nigeria

³Senior Lecturer, Department of Medical laboratory science, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City. – Nigeria

Received: 21 July, 2026 /Revision: 03 August, 2026 /Accepted: 25 August, 2026

ABSTRACT: *Jatropha tanjorensis* is a plant that is known to possess various bioactive compounds with potential medicinal properties. Investigating its role in haemopoiesis and the associated molecular pathways could offer useful insights into its therapeutic potential for conditions related to blood cell synthesis and their related disorders. The objective of this study was to determine the effects of aqueous *Jatropha tanjorensis* leaf extract on RUNX1 mRNA and ETV6 mRNA in mediating haemopoiesis using male Wistar rats. A total of thirty (30) male adult wistar rats were selected into five (5) groups. The groups were control group, phenyl hydrazine group, 1000mg/Kg of *Jatropha tanjorensis* group, 2000mg/Kg of *Jatropha tanjorensis* group and 4000mg/Kg of *Jatropha tanjorensis* group. RNA extraction and semi-quantitative polymerase chain reaction (PCR) was used to isolate RNA from rat bone marrow; complimentary DNA was synthesized and was subsequently amplified using polymerase chain reaction. Gel electrophoresis was used to determine the bands of the genes. The findings revealed that the mRNA expression pattern of RUNX1 was significantly higher ($p < 0.001$) in the untreated, 1000mg/kg and 2000mg/kg when compared to the control group. The expression pattern of RUNX1 was significantly higher in 4000mg/kg group when compared to control group ($p < 0.05$). The expression pattern of RUNX1 was significantly higher in groups treated with 1000mg/kg, 2000mg/kg and 4000mg/kg when compared to untreated group ($p < 0.05$). The mRNA expression pattern of ETV6 was significantly lower ($p < 0.001$) in untreated, 1000mg/kg and 4000mg/kg groups when compared to the control group. Among the treated groups, only 2000mg/kg showed a significantly higher ($p < 0.05$) expression pattern of ETV6 when compared to both untreated and 1000mg/kg groups. In conclusion, *Jatropha tanjorensis* significantly increased RUNX1 and ETV6. These results suggest that *Jatropha tanjorensis* could influence haemopoiesis by modulating the expression of the key regulating factor.

KEY WORDS: *Jatropha Tanjorensis*, Runx1 Mrna, Etv6, Anaemia

Corresponding Author:

Dr. Osime Evarista Odaburhine,

Professor of Hematology

Department of Medical laboratory science, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City. – Nigeria

Email: evarista.osime@uniben.edu



INTRODUCTION:

Background of Study

Jatropha tanjorensis (belonging to the family of Ehuphorbiaceae) is a common weed of field crops, bush re-growth, roadsides, 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'.^[1] The leaf is a commonly consumed vegetable in many parts of Southern Nigeria. It is also popular as a natural remedy against anaemia and diabetes in this region.^[2] Phytochemical screening of *Jatropha tanjorensis* leaf revealed that it contains bioactive principles such as alkaloids, flavonoids, tannins, cardiac glycosides, anthraquinones, and saponins.^[3] *Jatropha tanjorensis* leaf has anti anaemic effect (blood replenishing potentials). The leaf was found to contain some important biogenic compounds that are important for rapid haemopoiesis in the bone marrow.^[3] Hence, it can be used in the management of anaemia.

Haemopoiesis, the process of forming new blood cellular elements in vertebrates, starts at the stem cells becoming committed progenitor cells that eventually develop into fully mature differentiated cells in blood. Thrombopoiesis, in particular, is haemopoiesis that leads to the formation of thrombocytes.^[4]

Thrombopoiesis involves a sequence of complex cellular events in mature bone marrow megakaryocytes, culminating in the generation of proplatelet extensions that release platelets into circulation.^[5]

Runt-related transcription factor 1 (RUNX1), is a protein that in humans is encoded by the RUNX1 gene.^[6] RUNX1 is a transcription factor that regulates the differentiation of haematopoietic stem cells into mature blood cells.^[7] Like other transcription factors, the RUNX1 protein attaches (binds) to specific regions of DNA and helps control the activity of particular genes. This protein interacts with

another protein called core binding factor beta or CBF β (produced from the CBFB gene), which helps RUNX1 bind to DNA and prevents it from being broken down. Together, these proteins form one version of a core binding factor (CBF) complex.^[8] The RUNX1 protein activates genes that help control the development of blood cells (haematopoiesis). In particular, it plays an important role in the development of haematopoietic stem cells. These early blood cells have the potential to develop into all types of mature blood cells such as white blood cells, red blood cells, and platelets.^[9] In addition, it plays a major role in developing the neurons that transmit pain.¹⁰ It belongs to the Runt-related transcription factor (RUNX1) family of genes which are also called core binding factor- α (CBF α).^[10] RUNX1 regulates the development of platelet granules through interaction with genes involved in the biogenesis of platelet granules such as the nuclear factor erythroid 2 (NF-E2). In addition, RUNX1 can also regulate genes related to platelet functions.^[11]

ETS variant transcription factor 6 (ETV6) is a member of the ETS family of transcription factors, indispensable for bone marrow haematopoiesis and required for normal megakaryopoiesis. ETV6 encodes the E26 Transformation-Specific (Ets) family transcription repressor and tumour suppressor variant 6. ETV6 (i.e. translocation-Ets-leukaemia virus) protein is a transcription factor that in humans is encoded by the ETV6 (previously known as TEL) gene. The ETV6 protein regulates the development and growth of diverse cell types, particularly those of haematological tissues. ETV6 has classically been described as a key transcriptional regulator of haematopoiesis.^[12, 13]

Jatropha tanjorensis is known for its traditional medicinal uses. Investigating its effects on gene expression can provide valuable insights into its therapeutic potential and mechanisms of action, contributing to the development of novel natural remedies or

pharmaceutical agents. In gene expression regulation, RUNX1 and ETV6 are critical transcription factors involved in various cellular processes, including haemopoiesis, immune response, and cell differentiation. Exploring how *Jatropha tanjorensis* influences the expression of these genes can elucidate its impact on cellular and molecular pathways, shedding light on potential health benefits.

MATERIALS AND METHOD:

Experimental Design

Thirty (30) male adult Wistar rats were selected into group 1 (control), group 2 (untreated group), and three experimental groups 3, 4, and 5. Each group consisted of six animals each. Afterward, they were fed for 1 month following the established feed formulation. Anaemia was induced in groups 2 to 5 using 40mg of phenyl hydrazine administered intraperitoneally. Group 1 was used as the control group while Group 2 was the positive control group (untreated). Group 3, Group 4, and Group 5 were administered with 1000mg/Kg, 2000mg/Kg, and 4000mg/Kg respectively of *Jatropha tanjorensis* crude extract.

Animals in group 1 were fed with grower mash and water only.

Animals in group 2 were given grower mash and water and were administered 40mg phenylhydrazine (PHZ) to induce anaemia.

Animals in group 3 were given grower mash and water, administered 40mg phenylhydrazine (PHZ), and treated with 1000mg/kg body weight of *Jatropha tanjorensis* crude extract after 7 days of administering phenylhydrazine.

Animals in group 4 were given grower mash and water, administered 40mg phenylhydrazine and treated with 2000mg/kg extract of *Jatropha tanjorensis* after 7 days of administering phenylhydrazine.

Animals in group 5 were given grower mash and water, administered with 40mg phenylhydrazine, and treated with 4000mg/kg crude extract of *Jatropha tanjorensis* after 7 days of administering phenylhydrazine.

The experimental period lasted for five (5) weeks and the body weight of animals was taken weekly.

RNA Extraction and Semi-Quantitative Polymerase Chain Reaction (PCR)

RNA was isolated from the rat blood and bone marrow with TRIzol Reagent (ThermoFisher Scientific) and converted to cDNA using ProtoScript First Strand cDNA Synthesis Kit (NEB). RUNX1 mRNA and ETV6 mRNA PCR amplification was done using OneTaq® 2X Master Mix (NEB).

Procedure:

After sacrificing the animals, the bone marrow harvested was embedded in 0.3mL of TRIzol (Invitrogen Life Technologies, Inc., Carlsbad, CA) reagent inside an Eppendorf tube for proper tissue preservation. The tissues were homogenized using a plastic pestle. RNA lyase buffer was added to the homogenate to further break down the tissue cell membranes, after which it was spun at 10000 rpm for 10 minutes. The supernatant which contains the RNA was carefully removed and placed in a separate Eppendorf tube. The RNA precipitating buffer was centrifuged at 10000 rpm for 30 minutes. The supernatant was carefully removed remaining the RNA precipitate at the bottom of the tube. RNA wash buffer was added and centrifuged again at 10000 rpm for 5 minutes. This step was repeated 3 times to remove excess solutions and buffers previously added. Nuclease-free water was added to break down the RNA in a pellet form and the phosphodiester bond of the RNA. The solution also had a nuclease inhibitor, which removes other DNA or RNA contaminants from the medium. The RNA was

quantified using a UV spectrophotometer at 260nm.

Complimentary DNA (cDNA) synthesis:

All components of the cDNA kit were added to the RNA following the manufacturer's instruction, these components included random primer, oligonucleotide, deoxynucleotides, and reverse transcriptase buffer. After adding all components to the RNA, the mixture was then incubated at 42^{0C} in a thermocycler for 1 hour. Then the temperature was increased to 75^{0C} to denature the reverse transcriptase. Thereafter all the RNA was converted to cDNA. For RQ-PCR on rats sample the expression levels of all six genes were normalized to the levels of the GAPDH housekeeping gene.

Gene amplification:

An equal volume of both forward and reverse primer was added, with PCR mix (master mix), taq polymerase, and magnesium added. The mixture was placed in a thermocycler for amplification. The thermo-cycler was programmed for 30 cycles.

Gel electrophoresis:

After the PCR process, the DNA gel loading dye was added to the mixture. The agarose gel was prepared by dissolving 1% of agarose gel in TBE buffer. The gel was then allowed to solidify and then the sample was loaded and the gel connected for electrophoresis. Thereafter, a snapshot was taken. The image was then transferred to ImageJ. The intensities of the bands from agarose gel electrophoresis were then quantified densitometrically using ImageJ software.

Statistical Analysis

Data obtained from this research was presented and analysed using a graph pad prism (version 8.02, California, USA). Analysis of variance (ANOVA) was used to compare means and results were expressed in Mean±Standard error of mean. $p < 0.05$ was considered significant.

International Journal of Medical Laboratory Research

RESULTS:

Fig 1: Relative Expression of RUNX1 mRNA using Male Wistar Rats Administered Aqueous Extract of *Jatropha tanjorensis* Leaves.

RUNX1(Runt-related transcription factor 1) is a key transcription factor that plays a critical role in haemopoiesis. RUNX1 mRNA serves as a template for the synthesis of the RUNX1 protein. RUNX1 is a transcription factor, which regulates the expression of other genes. The presence of sufficient RUNX1 protein is essential for the proper regulation of genes involved in haemopoiesis.

Figure 1 shows the expression pattern of RUNX1 gene as represented by gel electrophoresis and internal control (Glycealdehyde-3-Phosphate Dehydrogenase {GAPDH}) of control, untreated, 1000mg/kg, 2000mg/kg, and 4000mg/kg groups (Group 1,2,3,4 and 5 respectively). The expression pattern of RUNX1 was significantly higher ($p < 0.001$) in group 2 (untreated), group 3(1000mg/kg), and group 4(2000mg/kg) when compared to the control group. The expression pattern of RUNX1 was significantly higher in group 5 (4000mg/kg) when compared to the control group($p < 0.05$). The expression pattern of RUNX1 was significantly higher in group 3(1000mg/kg), group 4(2000mg/kg), and group 5(4000mg/kg) when compared to the untreated group ($p < 0.05$). The expression pattern of RUNX1 was significantly lower in group 5(4000mg/kg) when compared to group 3(1000mg/kg) and group 4(2000mg/kg). The expression pattern of RUNX1 was significantly higher in group 4(2000mg/kg) when compared to group 3(1000mg/kg).

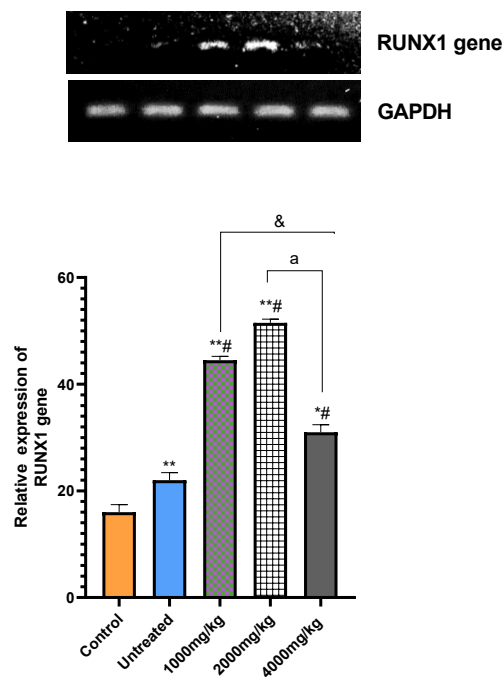
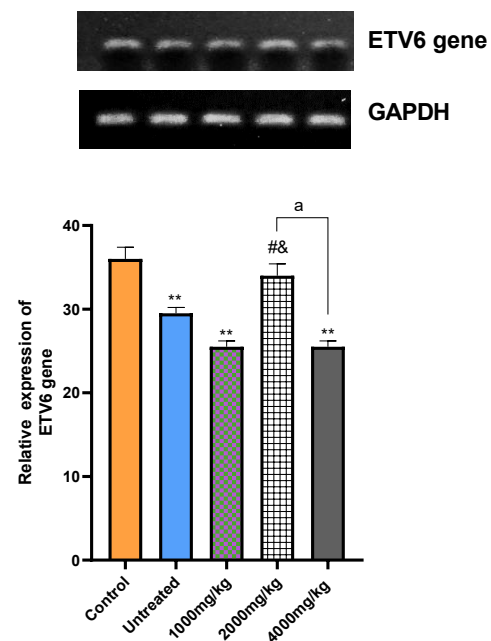


Figure 1: Relative expression of RUNX1 mRNA in male Wistar rats administered aqueous extract of *Jatropha tanjorensis* leaves. * Represents significance with control ($p < 0.05$). ** represents significance with control ($p < 0.001$). # represents significance with the untreated group ($p < 0.05$). & represents significance with 1000mg/kg of *Jatropha tanjorensis* extract. a represents significance with 2000mg/kg of *Jatropha tanjorensis* extract.

Fig 2: Relative Expression of ETV6 mRNA using Male Wistar Rats administered Aqueous Extract of *Jatropha tanjorensis* Leaves.

Figure 2 shows the expression pattern of ETV6 gene as represented by gel electrophoresis and internal control (Glyceraldehyde-3-Phosphate Dehydrogenase {GAPDH}) of control, untreated, 1000mg/kg, 2000mg/kg, and 4000mg/kg groups (Group 1,2,3,4 and 5 respectively). The expression pattern of ETV6 was significantly lower ($p < 0.001$) in untreated, 1000mg/kg, and 4000mg/kg groups when compared to the control group. Among the treated groups, only 2000mg/kg shows a significantly higher ($p < 0.05$) expression pattern of ETV6 when compared to both untreated and 1000mg/kg groups. The

expression pattern of ETV6 was significantly



lower in the 4000mg/kg group when compared to the 2000mg/kg group.

Figure 2: Relative expression of ETV6 mRNA in male Wistar rats administered aqueous extract of *Jatropha tanjorensis* leaves. ** represents significance with control ($p < 0.001$). # represents significance with the untreated group ($p < 0.05$). & represents significance with 1000mg/kg of *Jatropha tanjorensis* extract. a represents significance with 2000mg/kg of *Jatropha tanjorensis* extract.

DISCUSSION:

Jatropha tanjorensis leaf extract is usually use in rural settings in the treatment of anaemia. Studies have shown that the leaf extract has some haemopoietic activities, but there is paucity of data on the exact mechanism in which it's haemopoietic activity is exhibited.¹⁴ These studies revealed that aqueous *Jatropha tanjorensis* leaf extract has haemopoietic effects by influencing the haematological parameters, including those associated with thrombocytes.^[15] Phytochemical screening of *Jatropha tanjorensis* leaf revealed that it contains some essential biogenic components that are important for rapid haemopoiesis in the bone marrow, such as alkaloids, flavonoids, tannins, cardiac glycosides, anthraquinones,

and saponins.^[4] Research has shown that the process of haemopoiesis is highly regulated by several molecular pathways and signaling mechanisms. These pathways involve genes such as RUNX1 and ETV6 that acts as master regulators in guiding haemopoietic stem cells (HSCs) toward distinct lineages.^[7] During megakaryopoiesis, a process of generating megakaryocytes, which are the precursors of platelets, RUNX1 collaborates with other transcription factors critical for platelet formation.^[16] ETV6 ensures that the appropriate number and type of blood cells are produced to meet the body's demands by regulating the expression of genes that control their development into specific lineages.^[17] By influencing gene expression, RUNX1, and ETV6 contribute to the proper functioning of haemopoiesis and cellular differentiation process as it ensures that the right genes are turned on or off at the right time.^[18] This research, therefore, was propelled by the need to understand the haemopoietic benefits of aqueous *Jatropha tanjorensis* leaf extract, particularly its effects on the expression pattern of genes relating to haemopoiesis such as RUNX1 mRNA and ETV6 mRNA.

The results obtained from the gene analysis of this study showed that the expression of RUNX1 was markedly increased after the administration of 40mg phenyl hydrazine (a chemical compound often used in scientific research to induce anaemia in rat models). This may be attributed to the fact that the body may be compensating for the anaemia induced by Phenylhydrazine. The expression of RUNX1 was markedly increased in all the groups treated with aqueous *Jatropha tanjorensis* leaf extract, with the group treated with 2000mg/kg aqueous *Jatropha tanjorensis* leaf extract showing the highest expression. This may indicate that mild dose concentration of this treatment encourages receptor saturation which may invariably lead to increase expression of RUNX1 gene. Various research conducted on RUNX1 indicates that the expression of this gene is up regulated in times of increased

demand.^[13] This shows the expression of the gene was increased when anaemia was induced. This may be due to the direct effect of leave extract on erythropoietic cells, stimulating its activation and subsequent production of red Blood cells to compensate for anaemia. The result also indicates that aqueous *Jatropha tanjorensis* leaf extract has positive effect on the expression pattern of RUNX1 mRNA. The expression of ETV6 was significantly reduced after the administration of 40mg phenyl hydrazine as well as in groups treated with 1000mg/kg and 4000mg/kg aqueous *Jatropha tanjorensis* leaf extract. This may indicate that the production of red Blood cells is not due to the activation of ETV6. This may also indicate that *jatropha tanjorensis* leaf extract inhibits the expression of ETV6 genes thereby not affecting the production of red blood cells. It was also observed that the expression was markedly increased in the group treated with 2000mg/kg aqueous *Jatropha tanjorensis* leaf extract when compared to the untreated group. This marked increase might be indicative of a threshold dosage as the expression of RUNX1 also showed the most significant increase at this dosage. The increase in the expression pattern of RUNX1 with administration of *Jatropha tanjorensis* leaf extract aligns with the hypothesis that aqueous *Jatropha tanjorensis* leaf extract has an effect on the expression pattern of RUNX1mRNA and inhibits ETV6 expression may also be due to the bioactive compounds contained in the plant.^[14] From studies carried out.^[7] It was shown that the production of an adequate number of proper functioning blood cells is maintained by the upregulation of these genes in regulating the proliferation and differentiation of haemopoietic stem cells.

Following a therapeutic approach, the study suggests that further work is necessary to explore the benefits of *Jatropha tanjorensis* leaf extract in enhancing haemopoiesis and to elucidate the precise signaling pathway and mechanism by which the plant affects the expression of the RUNX1 and ETV6 genes.

Additionally, modulating the expression of RUNX1 and ETV6 may be helpful in the treatment of anaemia, thrombocytopenia, and other haematological conditions linked to impaired blood cell synthesis, such as anaemia and thrombocytopenia. Additionally, it is critical to carefully consider any adverse reactions and unforeseen consequences that might result from the modulation of these genes.

CONCLUSION:

The study's findings show that exposure to phenylhydrazine is linked to higher levels of RUNX1 and lower levels of ETV6 expression. Additionally, all treatment groups substantially increased RUNX1 expression following treatment of aqueous *Jatropha tanjorensis* leaf extract; however, the group receiving 2000 mg/kg of the plant's extract had the greatest expression. Only in the group given 2000 mg/kg of aqueous *Jatropha tanjorensis* leaf extract did the expression of the ETV6 gene rise; in contrast, other treatment groups showed a reduction in the gene's activity. These findings explain the ameliorative effects of *jatropha tanjorensis* leave extract in managing anaemia.

REFERENCES:

- [1]. Iwalewa EO, Adeumi CO, Omisore NOA, Adebajji OA, Azike CK, Adesina AO. Pro-antioxidant Effects and Cytoprotective Potentials of Nine Edible Vegetables in South-West Nigeria. *Journal of Medicinal Food*. 2005;8(4):539-544.
- [2]. Olayiwola G, Iwalewa EO, Omobuwajo OR, Adeniyi AA, Verspohi EJ. The Antidiabetic Potential of *Jatropha Tanjorensis* Leaves. *Nigerian Journal of Natural Products and Medicine*. 2004; 8:55-58.
- [3]. Ehimwenma SO, Osagie AU. Phytochemical Screening and Anti-Anaemic Effects of *Jatropha Tanjorensis* Leaf in Protein Malnourished Rats. *Plant Archives*. 2007;7(2):509-516.
- [4]. Schulze H, Shivdasani RA. Mechanisms of Thrombopoiesis. *Journal of Thrombosis and Haemostasis: JTH*. 2005;3(8):1717-1724.
- [5]. Machlus KR, Italiano JE. The Incredible Journey: From Megakaryocyte Development to Platelet Formation. *The Journal of Cell Biology*. 2013;201(6):785-796.
- [6]. Chang CC, Tsou HK, Chang HH, Chan LY, Zhuo GY, Maeda T, Lin CY. Runx1 Messenger RNA Delivered by Polyplex Nanomicelles Alleviate Spinal Disc Hydration Loss in a Rat Disc Degeneration Model. *International Journal of Molecular Sciences*. 2022;23(1):565.
- [7]. Dubey S, Dubey PK, Umeshappa CS, Ghebre YT, Krishnamurthy P. Inhibition of RUNX1 Blocks the Differentiation of Lung Fibroblasts to Myofibroblasts. *Journal of Cellular Physiology*. 2022;237(4):2169-2182.
- [8]. Goyama S, Mulloy JC. Molecular Pathogenesis of Core Binding Factor Leukemia: Current Knowledge and Future Prospects. *International Journal of Hematology*. 2011;94(2):126-133.
- [9]. Lam K, Zhang DE. RUNX1 and RUNX1-ETO: roles in hematopoiesis and leukemogenesis. *Frontiers in Bioscience*. 2012;17(4):1120-1139.
- [10]. Chen CL, Broom DC, Liu Y, de Nooij JC, Li Z, Cen C, Samad OA, Jessell TM, Woolf CJ, Ma Q. Runx1 determines nociceptive sensory neuron phenotype and is required for thermal and neuropathic pain. *Neuron*. 2006;49(3):365-377.
- [11]. Yuan H, Liu Y, Zhang J, Dong J, Zhao Z. Transcription factors in megakaryocytes and platelets. *Frontiers in Immunology*. 2023; 14:1140501.
- [12]. Hock H, Meade E, Medeiros S, Schindler JW, Valk PJM, Fujiwara Y, Orkin SH. Tel/Etv6 is an essential and selective regulator of adult hematopoietic stem cell survival. *Genes & Development*. 2004;18(19):2336-2341.
- [13]. Wang Q, Stacy T, Binder M, Marin-Padilla M, Sharpe AH, Speck NA. Disruption of the Cbfa2 Gene causes Necrosis and

- Hemorrhaging in the Central Nervous System and Blocks Definitive Hematopoiesis. Proceedings of the National Academy of Sciences of the United States of America. 1996;93(8):3444–3449.
- [14]. Idu M, Igbafe G, Erhabor J. Anti-anaemic Activity of *Jatropha tanjorensis* Ellis and Saroja in Rabbits. Journal of Medicinal Plants. 2014;2(1).
- [15]. Osayande EA, Ihemeje VI, Osime EO. *Jatropha Tranjorensis* Leaf Extract Modulation on Glycoprotein IX, 1B Alpha and 1B Beta in Albino Wistar Rats. Sokoto Journal of Medical Medical Laboratory Science. 2025;10(1):20-28.
- [16]. Lu C, Yang Z, Yu D, Lin J, Cai W. RUNX1 Regulates TGF- β Induced Migration and EMT in Colorectal Cancer. Pathology-Research and Practice. 2020;216(11):153142.
- [17]. Palanichamy JK, Sharma G, Nidhi T, Chopra A, Bakhshi S. Role of IGF2BP1 and Target Genes in ETV6-RUNX1 Positive B-Acute Lymphoblastic Leukemia. Blood. 2019; 134:3818.
- [18]. Mayumi A, Yoshida H, Mitsuno K, Nishida N, Osone S, Yasuda T, Imamura T. Leukemic Cells Expressing ETV6-Frk Identified in a Refractory B-ALL Patient Are Sensitive to Dasatinib in Vitro. Blood. 2021; 138:4335.

Cite of article: Osime EO Erhunmwunsee MO Aruomaren AI. *Jatropha Tanjorensis* leaf extract effect on the expression pattern of RUNX1 MRNA and ETV6 in Ameliorating Anaemia. Int. J. Med. Lab. Res. 2026; 11(3): 7-14.
<http://doi.org/10.35503/IJMLR.2026.11302>

CONFLICT OF INTEREST: Authors declared no conflict of interest

SOURCE OF FINANCIAL SUPPORT: Nil

International Journal of Medical Laboratory Research (IJMLR) - Open Access Policy

Authors/Contributors are responsible for originality of contents, true references, and ethical issues.

IJMLR publishes all articles under Creative Commons Attribution- Non-Commercial 4.0 International License (CC BY-NC). <https://creativecommons.org/licenses/by-nc/4.0/>