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HAEMOSTATIC EFFECT OF CRUDE AQUEOUS EXTRACT AND FRACTION OF SOLANU NIGRUM BERRIES IN A LEUKAEMIC ALBINO RAT MODEL

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ABSTRACT: *Leukaemia is a malignant disorder affecting bone marrow progenitor cells, leading to uncontrolled production of abnormal white cells (blasts) that crowd the bone marrow and peripheral blood. This disease imposes a significant economic burden on patients and families due to treatment costs. Solanum nigrum berries (ssp. villosum) are known for their nutritional and medicinal properties. This study investigated the haemostatic effects of Solanum nigrum berries' crude aqueous extract and fractions in a leukaemic albino rat model. Fresh green berries were sourced from markets in Enugu Metropolis, shade-dried, and ground into fine powder. One thousand grams of the powder were extracted using cold maceration. Forty grams of the crude aqueous extract underwent saponification and fractionation using N-hexane and ethyl acetate solvents at the National Agency for Food and Drug Administration and Control (NAFDAC) laboratories. Phytochemical analyses were conducted at the Projects Development Institute (PRODA). Sixty male albino rats (250–300 g) were used, with 15 for acute toxicity testing (Lorke method) and 45 divided into nine groups for the main study. Group A was the normal control (food and water only), while Groups B and C received 250 mg and 500 mg aqueous extracts, respectively. Groups D and E received 250 mg and 500 mg N-hexane fractions, and Groups F and G received 250 mg and 500 mg ethyl acetate fractions. Group H served as the positive control (150 mg cyclophosphamide), and Group I served as the negative control (75 mg ethylnitrosourea). Leukaemia was induced using 75 mg/kg body weight of ethylnitrosourea for three weeks, followed by 28 days of extract and fraction administration. Blood samples were collected on days 8 and 28 for analysis of prothrombin time, activated partial thromboplastin, and fibrinogen levels, while bone marrow and peripheral blood smears were examined under ×100 oil immersion. Qualitative phytochemical analysis revealed the presence of tannins, alkaloids, phenols, glycosides, and flavonoids in varying concentrations. Quantitative analysis showed tannins ranged from 0.362–0.724 mg/100 g, saponins from 0.0692–0.680 mg/100 g, alkaloids from 20–40 mg/100 g, phenols*

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from 1.283–1.312 g/kg, and glycosides from 0.060–3.630 mg/100 g across different solvents. Acute toxicity testing (LD50) was 4,850 mg/kg. Significant changes ($p < 0.05$) in prothrombin time, activated partial thromboplastin, and fibrinogen levels were observed across groups. The findings suggest that Solanum nigrum berries possess haemostatic properties and could help combat bleeding in leukaemia.
Keywords: *Leukaemia, Haemostatic, Solanum nigrum and Phytochemicals*

Introduction

Haemostasis is the process whereby blood coagulation is initiated and terminated in a tightly regulated manner, together with the removal of clots as part of vascular remodeling. As such, haemostasis describes the global process by which vessel integrity and patency are maintained over the whole organism for its lifetime (Versteeg et al., 2011). While coagulation is one of the processes in haemostasis, it is the process through which a blood clot forms in response to tissue injury, preventing further blood loss from the injury (Versteeg et al., 2011).

Leukaemia is a malignant haematological disorder characterised by the excessive proliferation of immature or abnormal blast cells in the bone marrow. This overproduction hinders the formation of normal blood cells, leading to an accumulation of abnormal white blood cells in the peripheral circulation. The condition results from malignant transformations that commonly arise in pluripotent stem cells, while it may also impact committed stem cells with limited self-renewal potential. These modifications induce abnormal cell proliferation, clonal expansion, impaired differentiation, and reduced apoptosis, ultimately converting healthy blood cells into malignant blasts (Ashkan, 2022)

It is a worldwide health issue affecting individuals in both developed and developing nations, characterised by elevated morbidity and mortality rates. It has been acknowledged for centuries and transmitted through generations.

Leukaemia, akin to other cancer forms, results from genetic anomalies in the DNA of white blood cells, red blood cell and platlets.

- There are four primary types of leukaemia:
- Acute Myeloid Leukaemia (AML), alternatively referred to as Acute Myelogenous Leukaemia.
- Acute Lymphoblastic Leukaemia (ALL) is alternatively referred to as acute lymphoid leukaemia.
- Chronic Myeloid Leukaemia (CML), also referred to as Chronic Myelogenous Leukaemia.
- Chronic lymphocytic leukaemia (CLL).

The Function of Herbal Medicine

Herbal medicine is increasingly favoured for its possible advantages, especially in mitigating adverse effects linked to orthordox treatments. Solanum nigrum, commonly referred to as Black Nightshade, Garden Nightshade, or "Ahuha" in Igbo, is a notable plant. This ephemeral perennial plant flourishes in agricultural plains, forested areas, and disrupted habitats globally. It is a prevalent wild plant with

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significant medicinal properties (Shep et al., 2009; Home Grown, 2012). The berries yield high amounts of iron, calcium, vitamin B, approachable amount of vitamin B12, vitamin C and carotene (Shep et al. 2009). This plant has a long history of medicinal uses and has been used as a traditional folk medicinal for treating various ailments such as inflammation, fever, pain and liver disorders (Ong et al.,2003).

Solanum nigrum, a species from the Solanaceae family of the genus Solanum, is among the largest and most diverse groupings within the genus.

Classification of Solanum nigrum Berries:

Scientific Classification: Kingdom: Plantae; (Unranked): Angiosperms; Order: Solanales; Family: Solanaceae.

Genus: Solanum

Species: S. Nigrum

Subspecies S. Villosum

2.19 Taxonomy of Solanum nigrum (Villosum):

Solanum nigrum, commonly referred to as garden nightshade, black nightshade, or red makoy, is a short-lived perennial species that can attain a height of up to 50 cm, and in some instances, may reach 60 cm. The berries measure between 6 to 8 millimetres in diameter and exhibit a transformation to a crimson or orange hue upon reaching maturity. The seeds measure approximately 1 millimetre in length, and the embryos undergo epigeal germination. The plant possesses alternate, ovate leaves measuring between 1.6 and 3.0 inches in length and 1 to 2 inches in width. It is adorned with clusters of cream, white, and violet flowers, featuring striking yellow anthers. The plant is a diminutive, upright herb, typically ranging from 12 to 47 inches in height, characterised by its fragile disposition, as evidenced by its smooth and tender stems and branches. The plants are widely cultivated across Africa and West Africa for both nutritional and commercial purposes, particularly in tropical and subtropical agro-climatic regions. Although it may be regarded as a weed in various European and Western nations, it thrives in a diverse range of arid, shallow, or deep soils throughout India (Rathore & Gypta 2013).

Toxicological Profile of Solanum nigrum.

The toxicity of Solanum nigrum exhibits significant variability among its different variants; certain forms are hazardous, whereas others are benign. The toxins are especially abundant in immature green berries (Schep et al. 2009). Some comprises of glycoalkaloids, with a particular emphasis on solanine. The toxicity of Nigrum is contingent upon its subspecies. Symptoms indicative of severe poison are diarrhea, fever and dizziness.

Anticancerous properties

Tang et al. (2008) emphasise the anti-cancer properties of Solanum nigrum. This plant is recognized as a herbal remedy with potential anti-cancer properties, with diosgenin being identified as the active

compound. Methanolic extracts of Solanum nigrum fruits demonstrated significant cytotoxic effects on the HeLa cell line (Sanjay 2009). Moreover, research on its aqueous extract has demonstrated its capacity to impede the progression of hepatoma, enhance survival rates, and mitigate liver injury as well as early hepatocarcinogenesis induced by 2-acetylaminofluorene. These effects are most likely attributable to elevated levels of glutathione S-transferase, antioxidant enzymes, and steroidal glycosides (Hsu, 2009).

Properties of Anti-Ulcer and Antioxidant Activity

Research indicates that extracts derived from Solanum nigrum berries demonstrate gastroprotective properties through their free radical scavenging activity, implying potential therapeutic significance for the management of gastrointestinal disorders (Mallika Janu, 2006). For instance, the administration of berry extract in the treatment of gastric mucosal injury induced by aspirin demonstrated antiulcer effects through the inhibition of acid secretion, presumably by the suppression of H+/K+-ATPase activity and a reduction in gastrin release. The ulcer-healing properties may also be attributed to its anti-secretory effects. Moreover, the berries are rich in vitamin B12, which may enhance their protective attributes.

Prothrombin Time (PT)

Prothrombin time is a diagnostic assessment that measures the duration required for plasma to coagulate, serving as an evaluation of the functionality of specific components within the coagulation system. It serves to complement the partial thromboplastin time (PTT), which assesses an alternative pathway within the coagulation cascade.

Activated Partial Thromboplastin Time (APTT).

The Activated Partial Thromboplastin Time (APTT) test evaluates the duration required for blood to coagulate following the introduction of specific reagents, focusing on the functionality of coagulation factors. This assessment is essential for evaluating an individual's coagulation ability and identifying potential hemorrhagic or thrombotic disorders.

Upon the occurrence of tissue or vascular injury, the process of hemostasis is initiated. Platelets adhere to the site of injury and aggregate, subsequently initiating the coagulation cascade in a sequential manner. This process establishes a fibrin network that stabilises the injury, minimises haemorrhage, and facilitates the healing process.

Fibrinogen

Fibrinogen, often referred to as factor I, is a glycoprotein synthesised in the liver and present in the circulatory system of all vertebrates (de Moerloose, Casini, & Neerman-Arbez, 2013). In the event of tissue or vascular injury, thrombin catalytically transforms fibrinogen into fibrin, thereby establishing the structural basis of a thrombus. These aggregates are typically employed to occlude blood vessels and inhibit haemorrhaging. Furthermore, fibrin engages with thrombin, thereby diminishing its activity through a mechanism referred to as antithrombin I, which facilitates the process of thrombus

formation (de Moerloose, Casini, & Neerman-Arbez, 2013). Fibrin facilitates the distribution of blood platelets and endothelial cells, enhances fibroblast proliferation, promotes the formation of capillary tubes, and plays a crucial role in angiogenesis. These functions are essential for the processes of tissue repair and wound recovery.

Certain medical conditions may lead to diminished or defective fibrinogen levels, resulting in infrequent inherited or acquired disorders. These complications may result in significant haemorrhaging or thrombosis, which can be addressed through the administration of fibrinogen or therapies aimed at limiting coagulation (Casini, 2016; Undas, 2011). Furthermore, abnormalities in fibrinogen levels have been linked to specific hepatic and renal disorders. Fibrinogen, classified as an acute-phase protein, exhibits an elevation in concentration as a physiological response to systemic inflammation, tissue injury, and certain pathological conditions, including malignancies such as cancer. Increased fibrinogen levels resulting from inflammation or malignancy may contribute to thrombosis and vascular damage under such conditions (Davalos, 2012; Repetto, 2017).

ETHYLNITROSOUREA:

Ethylnitrosourea (ENU), also known as N-ethyl-N-nitrosourea (chemical formula $C3H_7N_3O_2$), is a highly potent mutagen. For a given gene in mice, with a molar mass of 117.108 g.mol. ENU can induce one new mutation in every 700 loci. It is also toxic at high doses. Preferred IUPAC name (N-Nitroso-N-ethylurea, 2021), N-Ethyl-N-nitrosourea, other names N-Ethylnitrosourea, Ethyl-1-nitrosourea, Nitrosoethylurea, N-Nitroso-N-Ethylurea. The chemical is an alkylating agent, and acts by transferring the ethyl group of ENU to nucleobases (usually thymine) in nucleic acids. Its main targets are the spermatogonial stem cells, from which mature sperm are derived.

CYCLOPHOSPHAMIDE (CP)

Cyclophosphamide (CP), also known as cytophosphane among other names,
(Cyclophosphamide, 2016) is a medication used as chemotherapy and to suppress the immune system. As chemotherapy it is used to treat lymphoma, multiple myeloma, leukaemia, ovarian cancer, breast cancer, small cell lung cancer, neuroblastoma, and sarcoma (Cyclophosphamide, 2016). As an immune suppressor it is used in nephrotic syndrome, granulomatosis with polyangiitis, and following organ transplant, among other conditions. (Pagnoux, 2016**)**. it is taken by mouth or injection into a vein (Pagnoux, 2016)

Most people develop side effects. (American retrieval 2016, Pagnoux, 2016) Common side effects include low w, 2016, its blood cell counts, loss of appetite, vomiting, hair loss, and bleeding from the bladder. ^ Other severe side effects include an increased future risk of cancer, infertility, allergic reactions, and pulmonary fibrosis. (Pagnoux, 2016). Cyclophosphamide is in the alkylating agent and nitrogen mustard family of medications. (Pagnoux, 2016). It is believed to work by interfering with the duplication of DNA and the creation of RNA. (Pagnoux, 2016).

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Cyclophosphamide was approved for medical use in the United States in 1959. (Pagnoux, 2016). It is on the World Health Organization's List of Essential Medicines, the most effective and safe medicines needed in a health system. (WHO, pdf, 2015). The wholesale cost in the developing world is about US$3.65-14.30 per 1 g vial. In the United Kingdom this dose costs the NHS about 17.06 pounds. In the United States this dose by mouth is about $19.56. (Brayfield, 2017).

Statement of the problem

Leukaemia being one of the haematological malignancies is on the increase and its motality rate is high (Omoti *et al,* 2012). There is a progressive increase of leukaemia in Nigeria from 95 cases to 179 cases between 1996 – 2012 giving an average increase of 84%. (Omoti *et al,* 2012) This increase in leukaemia cases might be due to high level of environmental pollution, radiation pollution, other epigenetic factors (Hoffbrand *et al,* 2005).

Treatment of Leukaemia is a problem which is mainly through chemotherapy, targeted therapy, interferon therapy, radiation therapy, and stem cell transplantation (Camp – sorrel 2012). The cost of treating leukaemia is very high and it is not at the reach of the common man. Moreover, the side effects of chemotherapy are debilitating and hard to manage. Based on the cost of treatment with the above methods and the unbearable side effects, there is need to search for alternative herbal treatment to the existing ones. Such alternatives may include the use of *Solonum Nigrum* berries.

Justification of the work.

Bleeding is one of the m ajor symtoms of leukaemia being cancer of the blood and Solanum nigrum berries has been well investigated for its therapeutic properties in other cancers and anaemia nevertheless, no research in industrialised nations has specifically examined its haemostatic effects in leukaemia. Factors including the association between anaemia and leukaemia, the disease's elevated mortality rate, the exorbitant costs and significant adverse effects of treatments such as chemotherapy and radiotherapy have prompted numerous patients to pursue safer and more standardised herbal medicinal alternatives (Eden, 2010; Kabeel et al., 2018).

The berries of Solanum nigrum have demonstrated potential in the treatment of anaemia and haemorrhage, conditions commonly linked to leukaemia. Neverthel. This study seeks to evaluate the haemostatic effect by measuring the prothrombin time test, activated partial thromboplastic test and fibrinogen level of crude aqueous extracts and fractions of Solanum nigrum berries in a LEUKAEMIC albino rat model

Aims and objectives: The general aim of this research was to evaluate the haemostatic effects of crude aqueous extract and fractions of Solanum nigrum berries in a leukaemia albino rat model.

The specific objectives are;

1. To determine the prothrombin time level
2. To measure the Activated partial thromboplastin time &
3. To measure the Fibrinogen level.

MATERIALS AND METHODOLOGY

3.1 Materials

3.1.1 Acquisition of Plant Materials

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The berries of Solanum nigrum were procured from both prominent and minor markets in Enugu, specifically from Ugwuogonike, Nkwo-Agu, New Relief, and Ogbete. Professor C.C. Onyeke, a taxonomist, identified and authenticated the fruit. In the Department of Plant Biotechnology at the University of Nigeria, Nsukka (UNH No). A total of 665 voucher specimens are preserved in the University of Nigeria Herbarium for future reference.

3.1.2 Experimental Subjects and Housing Conditions.

The investigation utilised adult male albino rats approximately three months of age. The animals were acquired from the Animal Husbandry Department at the University of Nigeria, Nsukka. The rodents exhibited a weight range of 250 to 300 grammes. They were accommodated in the Animal House of the College of Medicine at the University of Nigeria, Enugu Campus. They were permitted a two-week acclimatisation period under standard husbandry conditions. They were provided with commercially available rat meal as a primary source of nutrition and granted unrestricted access to water.

3.2 Methodology.

3.2.1 Preparation of Plant Extracts and Fractions

The berries of Solanum nigrum were subjected to washing, subsequently shade dried, and then finely pulverised into a powder. In a 10-litre vessel, 1,500 grammes of pulverised berries were subjected to maceration in 2.5 litres of distilled water for a duration of 24 hours. The extraction process endured for a duration of six hours, characterised by periodic mingling and agitation. Subsequent to a 24-hour period, the mixture was subjected to sieving using muslin cloth and subsequently filtered through Whatman No. 1 filter paper. The filtrate was concentrated by positioning it on a thermostatically controlled heating plate set to 20°C for a duration of seven hours. Subsequently, the water evaporated, yielding a semi-solid aqueous extract of the berry, which was then collected, weighed, and stored at 4°C until required for use. This was designated as a crude extract. A portion of the crude extract was suspended in distilled water at a concentration of 1 g per 10 mL, specifically 40 g in 400 mL, and subjected to sonication for a duration of 15 minutes at a temperature of 37°C. The aqueous suspension was partitioned into equal volumes of n-hexane and ethyl acetate.

The residue and solvent fractions were subjected to evaporation until dried utilising a rotary evaporator, specifically the Buchi Rotavapor R-200, equipped with the 'V' assembly developed by Lyman C. Craig. The extractive values and percentage yields of the various fractions were ascertained and util

3.2.3 Phytochemical Examination.

The photochemical constituents of the aqueous extract and fractions of Solanum nigrum berries were analysed at the Project Development Institute (PRODA) in Enugu, as well as at the National Agency for Food and Drug Administration and Control (NAFDAC) in Agulu.

3.2.4 Induction of Leukaemia.

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Zeller (1979) elucidated the methodology employed in the experimental induction of leukaemia. Some of the rats were administered intraperitoneally with 75 mg/kg body weight of Ethylnitrosourea on a weekly basis for a duration of three weeks. The induction of acute leukaemia was substantiated through observations of weight loss, mortality, total and differential white blood cell (WBC) counts, the presence of over 20% blast cells in the peripheral blood film and bone marrow of the rats, as well as the identification of atypical lymphocytes (Kabeel et al., 2018).

3.2.5 Design of Experiments

This was accomplished utilising the Vadivel Method, as delineated in 2009. The investigation comprised forty-five (45) male albino rats, each weighing between 250 and 300 grammes. The subjects were divided into nine (9) groups, designated A through I, and accommodated in plastic cages. Each cage accommodates seven (7) Albino Rats per cohort.

Group A received DMSO, along with sustenance and water, for a duration of 30 days and functioned as the standard control group.

Group B received a crude aqueous extract (CAE) at a dosage of 250 mg/kg body weight, administered once daily for a duration of thirty days.

Group C received a crude aqueous extract (CAE) at a dosage of 500 mg/kg body weight, administered once daily for a duration of 30 days.

Group D received a daily administration of n-hexane fraction (HF) at a dosage of 250 mg/kg body weight for a duration of 30 days.

Group E received the n-hexane fraction at a dosage of 500 mg/kg body weight once daily for a duration of 30 days.

Group F was administered an ethyl acetate fraction (EAF) at a dosage of 250 mg/kg body weight once daily for a duration of 30 days.

Group G received an administration of ethyl acetate fraction (EAF) at a dosage of 500 mg/kg body weight, once daily, over a duration of 30 days.

Group H was administered the standard pharmacological treatments of cyclophosphamide and prednisone for a duration of 30 days.

Group I was administered 75 mg/kg body weight of ethylnitrosourea on a weekly basis for a duration of 21 days, followed by a recovery period.

3.2.6 Ethical Considerations:

This investigation was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki, established by the World Medical Association, pertaining to medical research involving animal subjects. All animals utilised in this study were treated in accordance with ethical standards, as sanctioned by our institution, the University of Nigeria, Nsukka (UNN).

3.2.7 Collection of Samples:At about 10 :00 am in the morning three(3) mls of blood was collected from each rat through the retro-orbital sinus of the median canthus of the eye with capillary tubes and

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transferd into 0.45mls of trisodium citrate anticoagulant bottle for Prothrombin time , Activated partial thromboplastin ,and Fibrinogen levels.

3.4 Determination of clotting tests

1. Prothrombin time test: Prothrombin Time is an indicator of the extrinsic blood coagulation mechanism. Deficiencies of prothrombin co factors V, VII, and X give rise to a prolonged clotting time

3.4.1 Principle of the Test: -

Tissue thromboplastin in the presence of calcium (ca+) activates extrinsic pathway of human blood coagulation cascade. Activation time is proportional to the concentration of individual clotting factors taking part in the coagulation cascade. When thromboplastin regent TI added to citrated plasma, clotting cascade is initiated forms gel clot. The time required for clot formation would prolong if the is a deficiency of factor(s) activity in the extensic pathway of the coagulation cycle.

3.4.2 Procedure for prothrombin time

Sample Collection: Collect 9 parts of blood in a plastic tube containing 1 part of 3.2tr-soidum citrate and mix gently

Centrifuge immediately for 15 minutes at 5000 rpm to obtain platelet poor plasma. Transfer supernatant plasma into a 2ml plastic tube immediately; do not disturb the buffy coat while collecting the supernatant plasma. This test was done within two hours of collection.

2. The PTreagents made up of tissue thromboplastine and calcium was prewarned at 37^{o}C for 10 minutes
3. 100ml of platelet poor plasma was dispensed into a plastic tube with a micro pipette in a water bath at 37^{o}C and incubated for 3minutes
4. 200vl of pre-warmed PT reagent was added into the, test tube, the PP plasma in the water bath.
5. The stop watch was started and the time taken for formation of a clot was recorded in seconds.

3.5 ACTIVATED PARTIAL THROMBOPLASTIN TEST

3.5.1 Principle: The test measures the clotting time of plasma after the activation of contact factor but without the addition of tissue thromboplastin and so expresses the overall efficiency of the intensic pathway. To standardise the activation of contact factors the plasma is first preincubated for a set period with contact activator such as kaolin, ellagic acid during this phase of the test factor X1as but coagulation does not proceed beyond this in the absence of calcium. After recalacication, factor Xla activated factor IX and coagulation follow. A standardized phospholipid is provided to allow the test to be performed on platelet poor damage, the test depends not only on contact factor and on factor VIII and IX but also on the reactions with factors X, V, prothrombin, and fibrinogen. It is also sensitive to the presence of circulating anticoagulants (inhibitors) and heporin. (Dacie & Lewis 2010).

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In the presence of calcium ions cephaloplastin activates, coagulation factors of intrinsic pathway in plasma leading to clot formation clotting time proportional to the concentration of factors VIII, IX, XI and XII as well as common pathway factors II V, and X.

Reagents/components

APTT Reagent 1 = Calcium chlorid solution (0.020ml)

APTT regents 2 = Rabbit rain cephalin, Ellagic acid activator, Buffer stabilizers and preservative,

Trisodium citrate = (1 x 10ml) trisoduin citrate (0.09m/l)

Storage & stability the sealed reagents are stabile up to the expiring date stated on the label, when stored at 2-8$^{oC.}$ Keep regent at 37^{oC} for 10 minutes prior to use.

3.5.2 Procedure:

The tests as done in duplicate measure: - ie APTT of each test is determined at each level twice. (Dacie & Lewis 2010)

i. Gently swirl reagents visa bring to room temperature before use.

ii. Mix equal volumes of the phospholipid reagent & Koalin suspension and leave in a glass tube in the water bath at 37^{oC}

iii. Add 0.1ml of plasma into new glass tube

iv. Add 0.1ml APTT reagent/pre warned at 37^{oC})

v. Add 0.1ml pre-warned calcium chloride, mix simultaneously and start the stop watch observe the formation of clot carefully and record the time for the appearance the first fibrin web.

Graph of mean double determination in seconds was plotted against each heparin concentrated on graph paper provided. Points were connected on straight lines. Normal reference value at between 21-38 seconds using a gappe diagnostics Switzerland in vitro diagnostic use. (Dacia & Lewis 2018).

3.6 FIBRINOGEN ASSAY

This was done using fibrinogen test kit from biobase.

Principle: The principle is based on immunotubidimetic method. When plasma samples mixed with reagents and, sample the fibrinogen specificity in antigen and antibody response form insoluble-antibody complex causing increased turbidity, under 340nm wavelength detection of the turbidity charge, change degree is proportional to the concentration of the sample of fibrinogen.

Applicable instrument is fully automatic biochemical analgzer

Reference range = 2.0-4.0g/l (Dacie & Lewis, 2018)

The reagent is intended for the invitro quantitate determination of fibrinogen in human plasma. Fibrinogen is a kind of multifactorial plasma globulin synthesis in the liver. The main physiological function is as a clotting factor I directly involved in the coagulation process in the body. With the development of the fibrinogen test technology, the research gradually through recent studies suggest that fibrinogen is associated with many diseases. The determination of fibrinogen has important

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clinical significance, increase in diabetes, acute mycordial intarction acute infectious diseases, connective tissue diseases, acute nephritis, shock, hand surgery, state of frequency, high character acute infection, emergency etc, produce common congenital low or no fibrinogen, Haematic disease, hereditary fibrinogen anamolies, DIC, primary fibrinolytic disease, severe hepatitis, and cirrhosis of the liver, etc.

Results

Table 4.1: Qualitative phytochemical analysis of *Solanum nigrum* berries extracts.

The results of the qualitative pytochemistry on table 4.1 showed trace (+) presence of tannin with water and n-hexane then moderate presence (++) with ethylacetate (++). Sapoinin showed trace (+) with n-hexane. Alkaloids showed large presence with water and ethylacetate and trace with n-hexane. Phenols showed moderate presence with water none with ethylacetate Glycosides showed moderate presence with water none with ethylacetate and n-hexane Steroids shows no presence in all. Flavonoids recorded trace with water and ethylacetate and heavy presence ($^{+++}$) with n-hexane

Table 4.1

Sample Identification	**Parameters**	**With Water**	**With Ethylacetate**	**With N-Hexnae**
***Solanum nigrum* berries**	Tannin	+	++	+
	Saponin	-	-	+
	Alkaloids	++	++	+
	Phenols	++	-	-
	Glycosides	++	-	-
	Steroids	-	-	-
	Flavonoids	+	+	+++

Table 4.2: Quantitative phytochemical determination of *Solanum nigrum* berries

The quantitative analysis on table 4.2 recorded that flavonoid had the highest quantity (14.70mg/100g) in the N-Hexane fraction followed by the ethylacetate fraction (4.90mg/100g) and water extract (4.90mg/100g).

Also, alkaloids had high quantities (40mg/100g) in the ethylacetate fraction and,water ,20mg/100g in n-hexane fraction.

Quantitative phytochemical determination of *Solanum nigrum* berries

Sample Identification	**Parameters**	**With Water**	**With Ethylacetate**	**With N-Hexnae**
***Solanum nigrum* berries**	Tannin mg/100g	0.362	0.724	0.372
	Saponin mg/100g	0.0692	0.680	0.138
	Alkaloids mg/100g	40	40	20
	Phenols g/kg	1.312	1.310	1.283

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	Glycosides mg/100g	3.630	0.060	0.060
	Steroids mg/100g	-	-	-
	Flavonoids mgQE/100g	4.90	4.90	14.70

Table 4.3: Some Coagulation parermeters of the test and control of leukaemic albino rat models on Day 8

Parameter	A control	B 250mg/kg bw Aq Ext	C 500mg/kg bw Aq Ext	D 250mg/kg bw N-hex	E 500mg/kg bw N-hex	F 250mg/kg bw eth acet	G 500 mg/kg bw eth acet	H Pos cont	I Negative Control
PT (Sec.)	16.40±0.87	36.18±3.09aa*	21.56±3.40 b	29.54±1.00 ** a	38.20±3.18 aaaa ***d +	16.25±3.04	10.16 ±1.71+	19.20±2.13	25.00 ±2.60
APTT Sec)	24.00±0.95aaa	35.28±2.54	35.64±5.05	28.00±0.84 a ++	36.20±0.73 *	29.50±2.18+	42.05 ±0.82 **	43.84±5.20	51.25 ±5.93
Fibrinogen (g/dl)	3.31±0.33aaa	2.77±0.20++	2.45±0.22+	2.15±0.10+ **	4.63±0.01 aaa**ddd +++	2.74±0.32	3.19±0.68	2.08±0.22	1.47±0.04

* Represents comparison with normal control * is p<0.05, ** is p<0.01, *** is p<0.0001
a represents comparison with positive control, a is p<0.05, aa is p<0.01, aaa p<0.0001
+ represents comparison with negative control, +p<0.05, ++ is p<0.01, +++ p<0.0001
b represents comparison with group B. b is p<0.05
d represents comparison with group D. d is p<0.05, dd is p<0.0001

The result showed that the prothrombin time test recorded significant prolongation (p<0.05) in 250mg/kg bwt Aqeous extract, (P<0.01) in 250mg/kg N-Hexane fraction, when compared to the nomal control. The prothrombin time was significantly increased (p<0.01) in 250mg/kg aqueous extract, (p<0.05) in 250mg/kg of N-Hexane fraction, and (p<0.001) when compared with positive control.

The activated partial thromboplastin time were significantly decreased (p<0.001) in normal control, (p<0.05) in 250mg/kg N-Hexane when compared with the positive control. There was also significant prolongation (p<0.01) in 500mg/kg of ethylacetate when compared to normal control.

Fibrinogen level was siginicantly increased (P<0.001) in the normal control, 500mg/kg of N-Hexane when compared positive control. Then when 250mg/kg of N-Hexane was compared with 500mg/kg

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of N-Hexane, there was a significant increase in 500mg/kg of N-Hexane fraction. Fibrinogen level was significantly increase ($p<0.01$) in 250mg/kg and 500mg/kg of N-Hexane fraction when compared in normal control.

The fibrinogen level was significantly increasing ($p<0.001$) in N-Hexane when compared with negative control.

Table 4.4: Some Coagulation parermeters of the test and control of leukaemic albino rat models on Day 28

Parameter	A Control	B 250mg/kg bw Aq Ext	C 500mg/kg bw Aq Ext	D 250mg/kg bw N-hex	E 500mg/kg bw N-hex	F 250mg/kg bw eth acet	G 500mg/kg bw eth acet	I Negative Control
PT (secs)	13.00±2.83	21.74±1.80	9.28±3.17 b ++	16.50±3.63	18.60±1.33	15.00±2.93+	14.00±1.45 +	25.00±2.60
APTT (secs)	24.40±2.42	29.40±2.46++	27.40±0.60++	32.72±0.96**++	28.80±0.20++	32.00±3.22+	30.00±1.14++	51.25±5.93
Fibrinogen (g/dl)	8.10±5.64	2.51±0.00++	2.95±0.22+++	3.57±0.25++	7.62±4.10++	3.32±0.52	2.90±0.34++	1.47±0.04

*** Represents comparison with normal control; ** is p<0.01**

+ represents comparison with negative control, +p<0.05, ++ is p<0.01, +++ p<0.0001

b represents comparison with group B. b is p<0.05

Table 4.7 showed the coagulation parermeters (PT, APTT, Fib) of test and control of leukaemic albino rat model on day 28.

The results of table 4.7 showed that prothrombin time test was significantly, decreased ($p<0.01$) in 500mg/kg of aquoues extract, ($p<0.01$) in 250mg/kg of ethylacetate and 500mg/kg of ethylacetate when compared to the negetive control.

Activated partial thromboplastin was significantly prolonged ($p<0.01$) 250mg/kg of N-Hexane when compared to the normal control, and significantly decreased ($p<.0.01$) in 500mg/kg of ethylacetate when compared to the negetive control.

Fibrinogen level was significanlty increased in ($p<.0.01$) in all the treatment groups when compared with the negetive control.

Table 4.13: Comparison of coagulation indices between day 8 and 28 of treatment in the different concentrations of the aqueous extract, fractions and the normal control. The result of the comparison of valuation

Parameter	Aqueous Extract		N-Hexane		Ethyl-Acetate		Control
	250mg/kg bw	500mg/kg bw	250mg/kg bw	500mg/kg bw	250mg/kg bw	500mg/kg bw	
PT (secs)	**<0.01**	**<0.05**	**<0.01**	**<0.0001**	**<0.05**	>0.05	>0.05

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APTT (secs)	>0.05	>0.05	**<0.01**	**<0.0001**	**<0.01**	**<0.0001**	>0.05
Fibrinogen (g/dl)	>0.05	>0.05	**<0.01**	**<0.0001**	>0.05	>0.05	>0.05

Showed that significant changes in Prothrombin Time of different concentrations 250mg and 500mg of in aqueous extracts ($p<0.01$) and ($p<0.05$) on day 8 versus day 28. Significant changes ($p< 0.01$ and $P<0.0001$) in prothrombin, activate partial thromboplastin and fibrinogen levels on day 8 verse day 28 of N-Hexane fraction ($p<0.01$ and $p< 0.0001$) respectively. 250mg/kgbdwt reveald significant changes in prothrombin time, ($p<0.05$), Activated partial thromboplastin ($p<0.05$), and significant change in 500mg/kgbwt in Activated partial thromboplastin ($p<0.0001$). 250mg/kg and 500mg/kg of ethylacetate showed significant variation ($p < 0.05$) in proibrombintin of 250mg, significant charge variation ($p < 0.01$) in activated partial thrombplastin a none in fibrngen level.

Table 4.17: Multiple comparison of coagulation parameters as stratified by fractions on day 8

Parameter	**250 mg/kg bw**			**500mg/kg bw**		
	Aqueous vs N-Hexane	Aqueous vs Ethyl acetate	N-Hexane vs Ethyl acetate	Aqueous vs N-Hexane	Aqueous vs Ethyl acetate	N-Hexane vs Ethyl acetate
PT (secs)	>0.05	**<0.01**	**<0.05**	**<0.01**	**<0.05**	**<0.0001**
APTT secs)	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
Fibrinogen g/dl)	>0.05	>0.05	>0.05	**<0.01**	>0.05	>0.05

The multiple comprism of coagulation parameters of 250mg of aqueous extract and 250mg of ethylacetate revealed significant changes ($p <0.01$) in prothrombin time test. 250mg of N-hexane fraction versus 250mg of ethylcacetate fraction revealed significant changes ($p<0.01$) in prothrombin time. 500mg of aqueous extract versus N-hexane fraction showed significant change ($p<0.01$) prothrombin time and in fibrinogen level.

Also, 500mg of aqueous extract versus, 500mg of ethylacetate fraction showed significant change ($p <0.05$) in prothrombin time test. Then 500mg of N-hexane fraction versus 500mg of ethylacetated showed significant change ($p<0.0001$) in prothrombin time test.

Table 4.19: Multiple comparison of coagulation parameters as stratified by fractions on day 28

Parameter	**250 mg/kg bw**			**500mg/kg bw**		
	Aqueous vs N-Hexane	Aqueous vs Ethyl acetate	N-Hexane vs Ethyl acetate	Aqueous vs N-Hexane	Aqueous vs Ethyl acetate	N-Hexane vs Ethyl acetate
PT (s)	>0.05	>0.05	>0.05	**<0.05**	>0.05	>0.05
APTT (s)	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

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Fibrinogen (g/dl)	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

On the comparism of 500mg/kg bwt of Aqueous Extract versus 500mg/kg of N-hexane fraction there was a significant change (p<0.05) in the protrombin time test p<0.05.

DISCUSSION AND CONCLUSIONS

This study shows that the aqueous extract & fractions of *Solanum Nigrum* (ssp villosoun) berries possess, heaemostatic activities on blood cells in leukaemia, also 75mg/kg body weight of ethylnitrsourea administration in albino Rats resulted in Acute Leukaemia.

Ethylnitrsourea administration caused mutation in the DNA of the white blood cells causing the bone marrow to produce abnormal white cells (blast cells) which populate the Bone Marrow and peripheral blood cells leading to abnormal increase in white blood cells and then leukaemia.

Treatment of leukaemia with the aqueous extract & N-Hexane and ethyl acetate fractions confers a very strong protection against, bleeding in leukaemia.. It equally brings about remissiosn on blastic cells. It has a better outcome in clotting parameters, more than some chemotherapy drugs such as cyclophosphamide that are used in the treatment of some cancers such as lymphoma, leukaemia, ovarian cancers & others (Radivoyevich et al, 2015).

It also contains protection on the coagulation properties of the blood as bleeding is also one of the symptoms of leukaemia.

From this study, the qualitative phytochemical analysis revealrd that the Berries are rich in Vit. B_{12}^{++} which are useful in haemopioesis helping in the synthesis of blood cells thereby combating the presence of anaemia which are indicated in leukaemia. It is also rich in Alkaloid $^{+}$, phenol^{++}, flavonoid^{++}.

The quantitative phytochemical and bioactive substances revealed that the *Solanum Nigrum* berries contains taninis (0.362 ± 0.2mg/100g), Alkaloids (40±2.0mg/100g), polyphenol 1.312±0.2g/kg), (flavoids 14.75mg/100g), & (Sapanin 0.0692mg/100g).

Some of the bioactive substances identified are (quarcetine 14.7mg/100g). Glycoside (3.60mg/100g). The oral acute toxicity testing (LD50) for the berries was 4.769g/kg body weight.

On day 8, the study revealed that prothrombin time was significantly Prolonged (P<0.05) in group B those that received 250mg of aqueous extract, (P<0.01) on group D those that received 250 mg of n-hexane fraction, and (P<0.01) on group E those that received 500mg of n-hexane fraction when compared with the normal control, Then significantly increased (p<0.01) on group B, (P<0.05) in group D, and(p< 0.0001) in group E as when compared to positive control. Prothrombin time was significantly decreased (P<0.05) in group E and G when compared with negative control.

There was a significant increase (P<0.01) in group B. those that were treated with 250mg/kg bdwt (36.18±3.09 seconds vs 16.40±0.87 secs) when compared with the normal control, there was a

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significant increase (P<0.01) in group D, those that were treated with 250mg/kg bdwt of N-hexane fraction, (29.54±1.0secs vs 16.40±0.87 sec), significantly increased (P<0.001) in group E those that were treated with 500mg/kg bdwt of N-Hexane fraction (38.20±3.18 seconds vs 16.40±0.87 secs when compared with the positive control. When 500mg/kg bdwt of N-hexane was compared with 250mg/kg bdwt of N-hexane. There was a significant increase (P<0.05) on day 8.

The activated partial thromboplastine Test was significantly increased (P<0.05) in 500mg/kg bdwt of N-Hexane group (36.20±0.72 vs 24.00±0.95). It was also significantly decreased (P<0.01) in group D those that received 250mg/kgbwt and group F those that were treated with 250mg/kgbwt of ethylacetate when compared with the negative control.

Significantly increased, (P<0.01) in group G, those that were treated with 500mg/kg bdwt of ethylacetate when compared with the positive control Group A. (42.05±0.82 vs 24.00 ± 0.95). On the comparism of 250mg/kg bdwt of N-hexane with 500mg/kg bdwt on day 8 (N-Hexane), there was a significant increase (P<0.05) on those that were treated with 500mg/kg bdw of N-hexane. This prolongation can be attributed to the higher doses of N-hexane fractions, which was not well tolerated by the animals.

Activated partial thromboplastin tests revealed significant decrease (P<0.01) in group D, those treated were treated with 250mg/kg bdwt, of N-Hexane fraction when compared with those treated with 150mg/kg bdwt of cyclophosphamide group H, on day 8. Significant decrease (P<0.01) in F those that were treated with 250mg/kg bdwt of Ethyl acetate fractions when compared with the Cyclophosphamide group. On comparisim with the negative control group I the activated partial thromboplastin test revealed significant decrease (P<0.01) in group D those that were treated with 250mg/kgbwt of n-hexane ftaction.It was significantly decreased (P<0.01) in group f those that were treated with 250 mg/kg bwt of ethlyacethete . This goes to prove that the N-hexane and Ethyacetate fraction are better treatment during bleeding, bringing the bleeding to a stop than with the positive control group H with longer prolongation, the APPT close to the normal value. These fractions can be used in patients that have bleeding tendencies to stop the prolonged APPT, revealing some coagulation deficiencies that normally happen in some diseases as a secondary disorder in Leukaemia.

The activated partial thromboplastin time is used to evaluate the coagulation factors XII, XI, IX, VIII, X, V., II (Prothrombin) and I (fibrinogen) as well as Priekallillikreim, (PK) and high molecular weight (2019) Kninogen (HK) While Prothrombin Time test evaluates the coagulation factors VII, X, V, II and I, (Fibrinogen).

The prothrombin Time and APPT can provide valuable information on Leukamia on further tests that may be needed.

On the comparison of the positive control group H and group A normal control there was a significant prolongation (P<0.0001) in the cyclophosphamide group (43.34±5.20 vs 24.00±0.95).

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The animals that were treated with150mg/kg bdwt of cyclophosphamide exhibited abnormal/prolonged APTT. These implies to the debilitating side effect of chemotherapy that is had to manage and can lead the early death of the patient.

On the analysis of the fibrinogen on day 8, the result showed a significant decrease ($P<0.01$) in group D, those that were treated with 250mg/kg bdwt of N-hexane fraction, significant increase in group E ($P<0.01$) those that were treated with 500mg/kgbwt of N-Hexane fraction when compared with the normal control. Significantly increased in group E ($P<0.001$) when compared with both the positive and negative control, significantly increased ($P<0.01$) in group B ($P<0.05$) when compared with the negative control. This points towards abnormal effect of Leukaemia on the fibrinogen level, there could be a decreased or a defective factor I, II, Vor X in Leukaemia.

Significant increase ($P<0.01$) in those that were given 500mg/kg bdwt of N-hexane fraction. This points to the fact that there is an increase or an effect on fibrinogen, prothrombin, (II), V or X in the plasma of the animals showing that this 500mg/kg bdwt of N-hexane can be given in cases of fibrinogen visa vis Leukaemia. Leukaemia affects fibrinogen levels, making them defective or decreased, therefore Solanum Nigrum berries can bring the levels to normal.

On the comparisim of the positive group A with the negative group H, (-ve group) those that were treated with 150mg/kg bdwt of cyclophosphamide, there was a significant decrease in ($P<0.001$) in H. impacting that cyclophosphamide drug has a debilitating effect on the fibrinogen level of leukaemic patient.

There was a significant increase ($P<0.001$) in those that were treated with 500mg/kg bdwt of N-hexane fraction when compared with group H in (cyclophosphamide group).

This still portrays the positive effect of the berry's extraction through N-hexane fraction having the ability to increase fibrinogen level in the blood and making them effective, thereby combarting bleeding episodes in Leukaemia (Vandiver & Vandracek 2019).

On day 28, studies on the coagulation parameter revealed a normal prothrombin time levels in all the groups when compared with the control. Activated partial thromboplastin revealed a significant increase ($p<0.01$) on group D 250mg/kgbwt of N-hexane fraction.

Coagulation studies on day 28:

On day 28, after the analysis the prothrombin time test, there was a significant decrease ($P<0.01$) in the protrombin time test of group C, significant decrease ($P<0.05$) in group F and G when compared with the negative control. When group B was compared with group C there was a significant decrease ($P<0.05$) in group C. This shows that the aqueous extract and fraction were able to correct the prolonged PT in leukaemia. The activated partial thromplastin also revealed corrected prolongation (normal seconds) in all the groups except in group D, those that received 250mg/kg bdwt of N-hexane there was a significant increase ($P<0.01$) when compared with the normal control, but when

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compared with negatve control there were significant increases (P<0.01) in group B, C, D, E and G and (P<0.05) in group F. Though still within the normal range.
The fibrinogen level also recorded significant increases (P<0.01) in group B, p<0.0001 in group C, (P<0.01) in group D, group E and group G in normal values in all the groups.
This goes to support the excellent effect of this berries on fibrinogen and some coagulation parameters of the blood.

Comparison of coagulation profile between day 8 and day 28 of treatment in the control, Aqueous Extract, N-hexane & Ethyl acetate fractions revealed that in 250mg/kgbdwt of Aqueous Extract, there was a significant change (P<0.01) prothrombin Time test, and no significant changes in activated partial thromboplastine and Fibrinogen level. The 500mg/kgbdut of Aqueous Extract also revealed a significant change. (P<0.05) in Prothrombin time test, no significant change (P>0.05) in Activated partial thromboplastin and fibrinogen levels respectively.

In the of N-hexane fraction group on comparism of day 8 and day 28 treatment, group D those that were treated with 250mg/kgbdwt of n-hexane revealed a significant change (P<0.01) on prothrombin Time test, activated partial thromboplastin and fibrinogen levels respectively. Also, the 500mg/kgbdwt of N-hexane fraction revealed a significant change P<0.0001 in prothrombin Time test, activated partial thromboplatin Time and fibrinogen levels respectively. This is a good discovery effect of the fraction on coagulation parameter. The Solanum nigrum berries have a good effect on Coagulation parameters can serve as a biomarker in coagulation studies.
The 250mg/kgbdwt of ethylacetate fraction revealed a statically significant change (P<0.05) in Prothrombin Time test, significant change (P<0.01) in activated partial thromboplastin (P<0.01) and no significant in fibrinogen level. The 500mg/kgbdwt of ethylacetate group G revealed a significant change in Activated partial thromboplastin p<0.0001 and no significant p>0.05 in prothrombin time and Fibrinogen level. The aqueous Extract and fractions of Solanum Nigrum berries is a good remedy for coagulation disorders.
The multiple comparison of coagulation parameter as stratified by fractions on day 28 revealed that of the dosage of the 500mg/kg bdwt doses on the comparison of aqueous versus N-hexane recorded a significant change on prothrombin time (p < 0.05) and none on APTT and fibrinogen. These effect on PT can serve as a bio marker in leukaemia, PT prolongation in leukaemia can be of immense diagnosis tool in cancer studies. Indicating that the bleeding in leukaemia could be linked to decrease prothrombin factor.

References

Ashkan Emodi MD, (2022), University of Maryland Jennie York, MD, University, of Maryland, title: acute lypmphoblastic leukaemia, school of medicine last review/ revision Jon 2022/modified

Brayfield, A, ed. (2017). "Cyclophosphamide: Martindale: The Complete Drug Reference". *Meditines Complete*. London, UK: Pharmaceutical Press. Retrieved 12 August 2017.

Casini, A., Undas, A., Palla, R., Thachil, J., & de Moerloose, P. (2018). Diagnosis and classification of congenital fibrinogen disorders: Communication from the SSC of the ISTH. *Journal of Thrombosis and Haemostasis*, 16(9), 1887–1890.

Dacie, J. V., & Lewis, S. M. (2016). *Dacie and Lewis Practical Haematology* (12th ed.). Churchill Livingstone

Davalos, D., & Akassoglou, K. (2012). Fibrinogen as a key regulator of inflammation in disease. *Seminars in Immunopathology*, 34(1), 43–62.

de Moerloose, P., Casini, A., & Neerman-Arbez, M. (2013). Congenital fibrinogen disorders: an update. *Seminars in Thrombosis and Hemostasis*, 39(6), 585–595

Eden I. (2010) Aetiology of Childhood leukaemia. Cancer treatment Reviews, 36, 286 -287 pub med.

Home grown (2012): Black Night shades (*Solanum Nigrum*) is Edible & Delicious: Root & Simple, http/www.root/single.com. https: / doi. Org/10.1016/0145-2126 (79) 90047 Leukaemia Research Volume 3, Issue 4, 1979, pages 239-243 245-248

Hsu, Y.-L., Kuo, P.-L., Lin, C.-C., & Lin, C.-C. (2009). Solanum nigrum L. extract inhibits 2-acetylaminofluorene-induced hepatocarcinogenesis through overexpression of glutathione S-transferase and antioxidant enzymes. *Journal of Agricultural and Food Chemistry*, 57(16), 6901–6908.

Kabeel M.M., Ahmed M G., Seham E. Mansy (2018) "Anti- Leukaemic activity of a four-plant mixture in a leukaemic rat model. *TheJournal of Basic and Applied Zoology:* 79, (7)2371https://doi.org/10.1186/s4/936-018-0019-5.

Ong H.C., Sguran Khassiat, Makanan Dan Ubatan (2003). Utisan publication Distributions 2nd ed, Fakuti Sains. University Malaga: 92-93.

Pagnoux C (2016). "Updates in ANCA-associated vasculitis". *European Journal of Rheumatology*. 3 (3): 122-133. doi:10.5152/eurjrheum.2015.0043. PMC 5058451. PMID 27733943.

Patel, S., Gheewala, N., Suthar, A., & Shah, A. (2009). In-vitro cytotoxicity activity of Solanum nigrum extract against HeLa cell line and Vero cell line. *International Journal of Pharmacy and Pharmaceutical Sciences*, 1(1), 38–46.

Rathore, V., & Gupta, D. (2013). In vitro clonal propagation of *Solanum nigrum*. *Research Journal of Chemical and Environmental Sciences*, 1(2), 32–33.

Repetto, O., & De Re, V. (2017). Coagulation and fibrinolysis in gastric cancer. *Annals of the New York Academy of Sciences*, 1404(1), 27–48.

Shep, I, J., Slaughter, I.J., Temple, W.A., (2009) contaminated Berries in Frozen vegetable. The New Zealand Medical Journal 122 (1292): 95-6PMID4487780http/www.nzma.org.nz/jssournal 122-1292/3547.

Tang, X., Gao, J., Wang, Y., Fan, L., & Pei, J. (2008). Review about mechanisms of anti-cancer of Solanum nigrum. *China Journal of Chinese Materia Medica*, 33(15), 1775–1777.

Undas, A., & Ariëns, R. A. S. (2011). Fibrin clot structure and function: A role in the pathophysiology of arterial and venous thromboembolic diseases. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 31(12), e88–e99.

Versteeg, H. H., Heemskerk, J. W. M., Levi, M., & Reitsma, P. H. (2013). New fundamentals in hemostasis. *Physiological Reviews*, 93(1), 327–358