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Research Article

Phytochemical Constituents and Effect on Haematological Parameters and Lipid Profile of Aqueous Extracts of *Eugenia jambolana* Leaves, Stem Bark and Root Bark in Normal Albino Rats

[1]O.A. Owolabi, [1]D.B. James, [1]E.B. Adejor, [1]N.Q. Nwaozuzu, [1]T. Oloba and [2]C.D. Luca
[1]Department of Biochemistry, Ahmadu Bello University, Samaru Zaria, Nigeria
[2]Department of Biochemistry, University of Jos, Nigeria

Abstract: This study was designed to investigate the effect of *Eugenia jambolana* on haematological parameters and lipid profile being a commonly used plant in Nigerian folklore medicine. Aqueous extracts of the leaves, stem bark and root bark of E*ugenia jambolana* were screened for phytochemicals and its effect on haematological parameters and lipid profile in normal albino rats were investigated. Twenty four Albino rats weighing between 150-200 kg body weights were divided into four groups of six rats each. Group 1 served as control while groups 2, 3 and 4 were administered (50 mg/kg body weight) of leaves, stem bark and root bark respectively for 21 days. Preliminary phytochemical screening revealed the presence of saponins, alkaloids, flavonoids, cardiac glycosides, tannins and carbohydrates and quantitative analysis by gravimetric method showed significantly ($p<0.5$) higher levels of saponins and alkaloids in the aqueous extracts of leaves and root bark respectively. The aqueous extracts (leaves, stem bark and root bark) had no significant ($p>0.05$) effect on haematological parameters. Aqueous extracts of the root bark significantly ($p<0.5$) increased TC, TG and LDL-c levels and the stem bark extract significantly ($p<0.05$) reduced HDL-c compared to the control. All three aqueous extracts significantly ($p<0.05$) lowered HDL-c/TC ratio, the root bark significantly ($p<0.05$) increased LDL-c/HDL-c ratio and both aqueous extracts of stem bark and root bark significantly ($p<0.05$) increased log (TG/HDL-c) of treated groups when compared to control.

Keywords: Albino rats, atherogenic factors, *Eugenia jambolana*, Haematological, lipid profile

INTRODUCTION

Eugenia jambolana belonging to the family Mytaceae is a large ever green and densely foliaceous tree with grayish brown thick bark and can grow up to 30m high. The leaves are leathery, oblong-ovate, smooth and shining with numerous nerves uniting within the margin. Annually the trees produce oblong or ellipsoid fruits (berries). They are green when raw and purplish black when fully ripe. The ripe fruits are luscious, fleshy and edible; it contains a single large seed.

It is widely distributed throughout India, Ceylon-Malaya, Australia and Nigeria. The plant is commonly known as black plum, purple plum or blackberry in English (Sagrawat *et al*., 2006). In Nigeria, its common name is *jambul*, *Malmo* (Hausa), *ube-une* (Igbo) and *dudu* (Yoruba).

All parts of this plant can be used medicinally and it has a long traditional use in alternative medicine. The fruit has been used in treating a wide variety of ailments, including, cough, (Sagrawat *et al*., 2006). It also enriches blood, strengthens teeth and gums and forms good lotion for removing ringworm infection on the head. The bark is digestible and is good for bronchitis and asthma. The seed can be used as an astringent and a diuretic. The plant is also known to possess, antibacterial, antidiabetic (Nadkarmi, 1954), anti-diarrheal, antifungal, anti inflammatory, CNS depressant activities as well as hepato and gastric ulcer protective activities.

Plants have provided mankind with herbal remedies for many diseases for many centuries till date. They continue to play a major role in primary healthcare as therapeutic remedies in developing countries. The role of plants in folklore medicine is attributed to the presence of phytochemicals; which are non nutritive plant chemicals that have disease preventing or curative properties.

The blood is a vital fluid, which contains the Red Blood Cell (RBC), White Blood Cell (WBC) and platelets suspended in the serum in homeostatic concentrations. The blood cells take up about 45% of the blood, while plasma constitutes about 55% (Guyton and Hall, 2000). The blood is important for pulmonary and tissue respiration, as a medium of endocrine and neurohumoral transmissions, biotransformation and metabolic excretion (Adebayo *et al*., 2005).

Lipid profile is the collective term given to the estimation of total cholesterol, high-density lipoprotein

Corresponding Author: E.B. Adejor, Department of Biochemistry, Ahmadu Bello University, Samaru Zaria, Nigeria

cholesterol, low-density lipoprotein cholesterol and triglycerides. An extended lipid profile may include very low-density lipoprotein. This is used to identify hyperlipidemia (various disturbances of cholesterol and triglyceride levels), which has been reported by Rerkasem *et al*. (2008) as one of the most important risk factors in the development and progression of atherosclerosis that lead to Cardiovascular Diseases (CVDs) and sometimes pancreatitis.

Due to the importance of Haematological parameters and lipid profile in the normal functioning of human system and or as a tool of diagnosis, *Eugenia jambolana*, a commonly used plant in Nigerian folklore medicine was chosen to investigate its effect on haematological parameters and lipid profile of normal albino rats.

MATERIALS AND METHODS

Collection of plant sample and identification: Leaves, stem bark and root bark of *Eugenia jambolana* were collected from the Department of Biological Sciences, Ahmadu Bello University Zaria Kaduna, Nigeria and identified at the Herbarium unit in Department of Biological Sciences, Ahmadu Bello University Zaria in the month of May, 2012 and given voucher number 900127 which was deposited for future reference.

Animals: Twenty four Rats weighing 150-200 g of both sexes were purchased from the Animal House, Department of Biochemistry, University of Jos. The animals were kept in stainless steel cages under standard laboratory condition of 12 h light/dark cycle in the Animal House, Department of Public Health, Veterinary Medicine, Ahmadu Bello University, Zaria. They were allowed to adjust to the laboratory environment for the period of 2 weeks before the commencement of the experiment. They were fed with pellet diet (grower's mash) and water *ad libitum*.

Preparation of plant: The collected plant parts were cut into pieces rinsed in clean water to remove dust particles and debris without squeezing, dried at room temperature for 16 days. Thereafter, the dried plant parts were ground into fine powder using a clean dry mortar and pestle, the powder obtained was then used to prepare the extracts.

Phytochemical screening: Phytochemical tests were conducted on the dry sample of leaves, stem bark and root bark of *Eugenia jambolana* to determine the presence of alkaloids, anthraquinone, tannins, terpenoids, saponins, flavonoids, cardiac glycosides and carbohydrates using standard protocols (Sofowora, 1993; Trease and Evans, 1993).

Extraction: 100 g of each plant part was dissolved in 500 mL of distilled water separately in a 2L round bottom flask and kept for 48 h before filtering. The plant solution was then filtered. The resulting filtrate was evaporated and concentrated to a paste dryness using water bath set at 45°C to obtain the extract. The concentrated extracts were stored in the refrigerator until required for analysis.

Lethal dose at 50 (LD_{50}): Lethal dose at 50 test involves the administration of a substance to a group of animals at increasing doses in order to determine the dose that kills 50% of the test subjects within a given time. The administration of *Eugenia jambolana* (leaves, stem bark and root bark) aqueous extracts were orally. The animals used for LD_{50} were grouped into 2 phases. All the phases had 3 groups with 3 animals in each group.

Animal Grouping: Twenty four rats were randomly grouped into 4 groups of 6 rats each.

Group 1: Animals given water and feed only. This served as the control group

Group 2: Animals given water, feed and 50 mg/kg body weight of aqueous extract of leaves of *Eugenia jambolana*.

Group 3: Animals given water, feed and 50 mg/kg body weight of aqueous extract of stem bark of *Eugenia jambolana*.

Group 4: Animals given water, feed and 50 mg/kg body weight of aqueous extract of root bark of *Eugenia jambolana*.

Sub chronic studies: The extracts' groups were given oral doses of 50mg/Kg body weight for 21 days, at the end of the 21 days; the animals were weighed, anaesthetized using chloroform and bled by cardiac puncture. The blood samples were collected in specimen bottles for determination of some biochemical and haematological parameters. Packed Cell Volume was determined by microhaematocrit method (Alexander and Griffins, 1993), White and Red Blood Cell Counts were determined by using Neubauer haemocytometer (counting chamber) under the light microscope. Serum Total Cholesterol (TC), High-Density Lipoprotein-Cholesterol (HDL-c) and Triglycerides (TG) were determined by enzymatic method as described by Stein (1987), Low-Density Lipoprotein cholesterol (LDL-c) was determined by the method of Friedewald (1972) and atherogenic risk factor was calculated using formula of Dobiasova and Frohlich (2001).

Statistical analysis: The data was analyzed by the analysis of variance (ANOVA). The difference between the various extracts and animal groups were compared using the Duncan Multiple Range Test. The results are expressed as mean±standard deviation. p-value less than 0.05 was taken as significant ($p<0.05$).

Table 1: Phytochemical constituents of aqueous extracts of *Eugenia jambolana*

Phytochemicals	Leaves	Stem bark	Root bark
Carbohydrates	+	+	+
Glycosides	-	-	-
Free anthraquinone	-	-	-
Cardiac glycosides	+	+	+
Saponins	+	+	+
Flavonoids	+	+	+
Tannins	+	+	+

Table 2: Quantitative analysis of phytochemical constituent of aqueous extracts of *Eugenia jambolana*

Phytochemicals	Leaves	Stem bark	Root bark
Flavonoids	0.05 ± 0.01^{a}	0.03 ± 0.01^{a}	0.10 ± 0.02^{b}
Saponin	0.82 ± 0.01^{c}	0.75 ± 0.03^{b}	0.71 ± 0.02^{a}
Cardiac glycosides	0.06 ± 0.01^{a}	$0.08\pm0.0\,1^{b}$	0.14 ± 0.01^{c}
Alkaloids	0.07 ± 0.01^{a}	0.19 ± 0.02^{b}	$0.\,27\pm0.01^{c}$
Tannins	0.04 ± 0.01^{a}	0.05 ± 0.01^{b}	0.06 ± 0.01^{b}

Values expressed as mean±SD; values with different superscript in the row differ significantly ($p<0.05$)

RESULTS AND DISCUSSION

The results in Table 1 show the presence of carbohydrate, saponin, alkaloids, flavonoids; tannins in the aqueous extracts (leaves, stem bark and root bark) of *Eugenia jambolana*.

Quantitative assessment of the various phytochemical constituents in Table 2, shows that saponins are significantly ($p<0.05$) higher in the aqueous extract of the leaves while alkaloid and cardiac glycosides are significantly ($p<0.05$) higher in the aqueous extract of the root bark.

Table 3 shows the haematological paramaters of control and *Eugenia jambolana* treated albino rats. There is no significant ($p>0.05$) difference in PCV, Red and White Blood Cell Counts as well as eosinophils, lymphocytes, neutrophils of treated groups compared to the control.

Lipid profile assessment is shown in Table 4; the aqueous extract of root bark significantly ($p<0.5$) increased TC, TG and LDL-c and stem bark extract significantly ($p<0.05$) lowered HDL-c compared to the control. The Atherogenic risk predictor indices of animals treated with the aqueous extracts of *Eugenia jambolana* is shown in Table 5. All three aqueous extracts significantly ($p<0.05$) lowered HDL-c/TC, ratio, root bark extract significantly ($p<0.05$) increased LDL-c/HDL-c ratio and both aqueous extracts of stem bark and root bark significantly ($p<0.05$) increased log (TG/HDL-c) when compared to control.

Discussion: Saponins are high molecular weight glycosylated plant secondary metabolites, consisting of a sugar moiety linked to a triterpene or steroid aglycone (Price *et al*., 1987). Detergent properties are the typical characteristics of saponins. They are also known to possess pesticidal activity (Irvine, 1961) and also used in the treatment of contaminated water (Hall and Walker, 1991). They are also known for their health benefits such as cholesterol lowering and anticancer properties (Gurfinkel and Rao, 2003; Kim *et al*., 2003).

Alkaloids are low molecular weight nitrogenous compounds. It is contained in 20% of plant species; mainly involved in plant defense against pathogens and herbivores. Alkaloids have been found to possess analgesic, antimalarial, antibacterial, antihypertensive (Dangi *et al*., 2002) properties.

Cardiac glycosides belong to triterpenoids class of compounds (Brian *et al*., 1985). They are cardioactive compounds with their inherent activity residing in the aglycone portions of their sugar moiety. They exert a number of effects on neural tissue and thus indirectly influence the mechanical and electrical activities of the heart, modifying vascular resistance and capacitance (Olaleye, 2007).

This study shows high amounts of saponin in aqueous extract of leaves and alkaloids and cardiac glycosides in aqueous extract of root bark which could be responsible for their medicinal purposes.

Table 3: Effect of aqueous extracts of *Eugenia jambolana* on haematological parameters of treated albino rats

Aq. Ext.	PCV (%)	Hb (g/dL)	WBCx10^{3}/uL	RBCx10^{6}/uL	Lym (%)	Neu (%)	Mon (%)	Eos (%)
Control	43.67 ± 3.51^{a}	14.47 ± 1.21^{a}	3.87 ± 1.86^{a}	7.17 ± 0.55^{a}	84.33 ± 4.04^{a}	13.33 ± 3.06^{a}	1.00 ± 0.01^{a}	1.33 ± 1.53^{a}
Leaves	46.33 ± 4.16^{a}	15.43 ± 1.40^{a}	3.60 ± 1.97^{a}	7.67 ± 0.72^{a}	86.00 ± 4.36^{a}	13.00 ± 4.36^{a}	1.00 ± 0.01^{a}	1.00 ± 0.01^{a}
Stem bark	48.67 ± 7.37^{a}	16.20 ± 2.48^{a}	3.50 ± 1.32^{a}	8.13 ± 1.22^{a}	84.00 ± 2.00^{a}	14.00 ± 2.00^{a}	1.33 ± 0.58^{a}	0.67 ± 0.58^{a}
Root bark	41.00 ± 2.65^{a}	13.67 ± 0.91^{a}	4.60 ± 1.97^{a}	6.77 ± 0.46^{a}	84.67 ± 4.16^{a}	13.33 ± 4.16^{a}	1.00 ± 0.01^{a}	1.00 ± 0.01^{a}

Values expressed as mean ± SD; values with different superscripts in the row differ significantly ($p<0.05$)

Table 4: Effect of aqueous extracts of *Eugenia jambolana* on serum lipid parameters of treated albino rats

Group (n = 6)	TC	TG	HDL-c	LDL-c
Control	45.50 ± 14.89^{ab}	57.17 ± 18.71^{a}	28.60 ± 2.25^{b}	12.28 ± 4.79^{a}
Leaves	45.50 ± 5.63^{ab}	44.91 ± 14.14^{a}	26.00 ± 2.25^{ab}	13.85 ± 0.43^{ab}
Stem bark	35.75 ± 5.63^{a}	57.17 ± 14.14^{a}	14.17 ± 2.14^{a}	12.93 ± 0.58^{ab}
Root bark	64.83 ± 5.92^{b}	98.00 ± 21.22^{b}	19.50 ± 6.75^{ab}	25.93 ± 4.88^{b}

Values expressed as mean ±SD; values with different superscripts in the row differ significantly ($p<0.05$)

Table 5: Effect of aqueous extracts of *Eugenia jambolana* on atherogenic risk predictor indices of treated albino rats

Group (n = 6)	HDL-c/TC	LDL-c/HDL-c	LOG[TG/HDL-c]
Control	0.72 ± 0.30^{b}	0.40 ± 0.13^{a}	0.26 ± 0.07^{a}
Leaves	0.30 ± 0.04^{a}	0.53 ± 0.03^{a}	0.23 ± 0.17^{a}
Root bark	0.37 ± 0.08^{a}	0.95 ± 0.11^{a}	0.62 ± 0.21^{b}
Stem bark	0.26 ± 0.07^{a}	1.93 ± 0.92^{b}	0.81 ± 0.08^{b}

Values expressed as mean ±SD; values with different superscripts in the row differ significantly ($p<0.05$); values of HDL-c/TC ratio <0.03 are antherogenic are undesirable, values of LDL-c/HDL-c >2.3 are antherogenic and undesirable (Ojiakor and Nwanjo, 2005)

The blood is a vital fluid found in humans and other animals that transports gases and nutrients to all body organs and tissues of the body and carries away waste materials as well as regulation of body temperature. Hence, transports any foreign body which might be harmful to all parts of the body leading to initiation and progression of diseases. The level of blood components is characteristic of one's health status.

This study reveals that aqueous extracts of leaves, stem bark and root bark of *Eugenia jambolana* have no significant ($p>0.05$) effect on PCV, RBC, WBC suggesting that the plant might not compromise the functional capacity of the blood neither exposing the body to opportunistic infection.

Hyperlipidemia is the elevation of lipids in the blood. It is characterised by the elevated levels of serum total cholesterol, triglycerides, low density and very low density lipoprotein cholesterol and decreased levels of high density lipoprotein cholesterol. It is a risk factor of Cardiovascular Diseases (CVD); which is a leading cause of morbidity and mortality all over the world (Yusuf *et al*., 2001). It has been established that reduction of total cholesterol or Low Density Lipoprotein cholesterol (LDL-c) is associated with decreased risk of atherosclerosis and coronary heart disease (Grundy *et al*., 2004). Further studies have also shown an inverse correlation between High Density Lipoprotein cholesterol (HDL-c) level and the risk of cardiovascular diseases (Wierzbicki, 2005).

TC, TG and LDL-c were increased by the aqueous extract of the root bark and HDL-c was lowered by the stem bark extract. Aqueous extracts of *Eugenia jambolana* (leaves stem bark and root bark) reduced HDL-c/TC, ratio, root bark extract increased LDL-c/HDL-c ratio and both stem bark and root bark extracts increased log (TG/HDL-c). This suggests that these parts of the plant increase lipid profile and consequently may increase the risk of cardiovascular diseases.

CONCLUSION

This study shows the presence of some phytochemicals such as carbohydrate, cardiac glycosides, tannins, saponins, alkaloids and flavonoids. Among all the phytochemicals, saponins and alkaloids have the highest values in the aqueous extracts of leaves and root bark respectively.

The aqueous extracts of leaves, stem bark and root bark of *Eugenia jambolana* were found not to have any adverse effect on the haematological parameters of albino rats but had negative effect on both lipid profile and atherogenic risk predictor indices.

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