Original Research Article

Phytochemical Profile and Haematological Indices in Toxicity Studies of *Maerua angolensis* and *Gliricidia sepium* Leaves Extracts

Keywords: *Maerua angolensis*, Phytochemical, Haematology, *Gliricidia sepium*, Toxicity

ABSTRACT

Background: What defines plants actions and activities in phytomedicine is their building chemicals. Exploiting plant phytochemistry is, therefore, a pointer to its likely medicinal activities. We studied the phytochemical profile of *G. sepium* and *M. angolensis* and their haematological interference in Wistar rats during acute and subacute toxicity testing.

Methodology: Standard methods were used for qualitative and quantitative phytochemical evaluation. 100g each of powdered leaves of *Gliricidia sepium* and *Maerua angolensis* were soaked in 100ml of Petroleum ether and 70% Ethanol respectively for 48 hours at room temperature. The filtered solutions were concentrated up to 50 ml and were used for the qualitative phytochemistry. Eighteen female rats were used for acute toxicity (limit test). They were divided into three groups (n=6). 2000mg and 5000mg of extract per kg body weight was administered orally to groups 2 and 3 respectively and monitored for 14 days. Group 1 was administered with 0.25ml of distilled water to serve as the control. Fifty Wistar rats (25 males and 25 females) were divided into five groups of equal sexes (n=10) for the subacute testing. Groups 2, 3, 4 and 5 were respectively administered with 125, 250, 500 and 1000mg/kg body weight of extract orally for 28 days. Group 1 was treated as done for the acute study. Animals were anaesthetized and EDTA blood samples were collected through cardiac puncture and processed using Sysmex Xn 1000 auto-analyzer.

Results: Both *G. sepium* and *M. angolensis* leaves extracts showed the presence of varieties of medicinal phytochemicals. Seasonal variations have little effect on their quantities and plants harvested during the wet season housed more phytochemicals than that of the dry season. The haematological profiles were not affected during the acute and subacute toxicity evaluation. *G. sepium* rather boosted the blood production by increasing the population of the circulating erythrocytes and red cell indices.

Conclusion: *G. sepium* and *M. angolensis* are candidate plants for medicinal phytochemicals, both extracts did not portray danger to the system and *G. sepium* extract could even be used for haematoprotective functions.

Comment [SN1]: Rephrasing is required

1. INTRODUCTION

Evidence abounds to substantiate the claim that the earliest people on earth were not without naturally endowed plants that they used in the treatment of diseases of their time [1,2,3]. However, advancements in science and technology gradually brought to the limelight, the screening to detect and isolate the bioactive compounds in these plants [4,5]. Till today, people

still use various herbs and concoctions believed to have therapeutic tendencies, without any recourse to the phytoconstituents of the plant(s) used. Even where literature evidence is available, majority of herbal users do it based on their inherent belief about the plant functions, not because of the results of the scientific screening of the plants.

Phytochemical study comprises the process of extraction, screening and identification of the active substances of medicinal importance in plants. Such bioactive substances include but are not limited to alkaloids, saponin, carotenoids, flavonoids, antioxidants, tannin, and phenols [6]. There are almost 35,000 species of plants that are currently being used in herbal therapies across the globe, out of which only 20% have been screened for their phytochemicals. With these large yet to be explored herbs, the future of phytomedicine seems to be very bright with the tremendous discovery of new and novel therapeutic products [7].

Evaluation of the toxicological effects of the investigational product on the blood and blood-forming organs is of utmost importance in toxicity studies. This is done by analyzing the haematological parameters to detect the variances from the normal. The candidate product does not need to be haematotoxic before the analysis is done, blood as the moving tissue travels continuously in the organ system and any effect on the blood can be easily carried to the other organs. Also, the haematopoiesis origin is pluripotent, it, therefore, requires due cognizance to ensure the safety of all peripheral blood cells from the blood-forming paths during toxicological studies [8]. Frequent interaction of inherited traits with some drugs is one of the most interesting reasons to study the haematology of a new product, glucose-6-phosphate dehydrogenase deficiency is a popular example.

Gliricidia sepium (Jacq.) Kunth ex Walp. belongs to the family Fabaceae with a traced origin to Central America. It is a leguminous tree whose different parts have been documented for medicinal purposes in many tropical and sub-tropical countries [9]. According to Rastrelli *et al*. [10], phytochemical screening of the plant yielded formosin, formononetin, medicarpin with antifungal property and other useful chemicals. Extracts of the leaf, flower and bark of *Gliricidia sepium* have been used in the treatment of patients with pathogenic bacterial infections [11], skin diseases [12,13] and nematodes [14]. Its antioxidants and insecticidal activity have also been reported [14,15]. Unfortunately, very little work has been done to ascertain the claimed antitumor activity of this plant especially with the plant species in Sub-Saharan Africa.

Maerua angolensis known as Leggal Mbaali by the Fulfude speakers is a small tree of usually 5-6 metres tall, a multipurpose tree harvested mainly as medicine [16]. Its parts are widely used traditionally to treat skin rashes, sores, womb cleansing, and sexually transmitted diseases [17]. The leaves of the plant are reportedly being used in the treatment of diabetes mellitus [18], convulsion [19], skin rashes and stomach ulcers [20], dysentery and epilepsy [21]. However, there were reports (verbal) of the use of the leaf of the plant for the cure of breast cancer in Yola, Adamawa State in the North-East part of Nigeria, by the herbalists. Though, no documented fact has been published on this report. A Survey of the users understanding of the function of this plant formed the first task of this project and it was generally confirmed that the plant is in use for treating cancer patients.

2. MATERIALS AND METHODS

2.1 Collection, Identification and preparation of the plants

Collection of leaves of *Gliricidia sepium* was done from the garden of Imam Ahmad Islamic Centre, Ilorin, Kwara State, Nigeria (Coordinate: 8°26'53.6"N 4°33'53.8"E) and *Maerua angolensis* leaves were collected from Duware town, Yola, Adamawa State, Nigeria (Coordinate: 9°11'16.1"N 12°31'33.7"E). The identification and authentication were done at the Herbarium of the Department of plant science, University of Ilorin, where Voucher numbers UILH/002/2019/752 and UILH/002/2019/1389 were given to *G. sepium* and *M. angolensis* respectively. The plant samples were deposited at the herbarium. The leaves were washed separately in tap water, rinsed in distilled water and air-dried at room temperature for 7 weeks. They were made into powder using BOXIYA multifunctional blender (BXY-3027) and stored at room temperature.

2.2 Phytochemical Analysis

Qualitative and quantitative chemical determination of the extracted constituents were done using extracts of two different solvents and from plants plucked at two different geographical seasons (wet and dry). Standard methods were used for both qualitative and quantitative analysis. From petroleum ether extracts, the presence of the following constituents was tested qualitatively: Basic alkaloids; Volatile oils; Sterols and Triterpenes; Anthracenoside aglycones (Emodols); Coumarins and Flavonic aglycones (flavonoid). Alcoholic extracts were used to test for the presence of Saponins, Tannins, Reducing compounds, Alkaloid salts, Anthracenosides, Coumarins, Flavonosides, Sterol glycosides and Anthocyanosides.

2.2.1 Qualitative determination of the phytoconstituents

The standard techniques used for these tests were as described by Ezeonu & Ejikeme [22] and Edeoga et al. [23] with little modifications. 100g each of ground leaves of *Gliricidia sepium* and *Maerua angolensis* was soaked in 100ml of Petroleum ether and 70% Ethanol respectively for 48 hours at room temperature. The filtered solutions were concentrated up to 50 ml and were used for the analysis. Alcohol extracts were used to test for the presence of the phytoconstituents as follows:

Identification of polyphenols (tannins)

3 drops of 0.1% ferric chloride were added to 5 ml of the filtrate in a test tube. The development of brownish-green or a blue-black colouration showed the presence of tannins [22].

Identification of reducing compounds

0.5ml of the filtrate was diluted with 1 ml of distilled water in a test tube. 0.5 ml of Fehling's solution A+B was added and heated for 5 minutes. Formation of a brick-red precipitate denoted the presence of reducing compounds [22].

Identification of alkaloid salts

1ml of the filtrate was dispensed into two test tubes A and B. 2 drops each of Mayer's and Dragendroff's reagents were added to tube A and B respectively. Formation of yellowish-white precipitate in tube A and a red precipitate in tube B showed the presence of Alkaloids [23].

Identification of polyphenolics glycosides (anthracenosides, coumarins and flavonosides)

The test to confirm the presence of these phytoconstituents is done with a part of hydrolyzed alcohol extract.15ml of 10% hydrochloric acid solution is added to 25ml of the alcohol extract by refluxing and heated up for half an hour. Due to the precipitation by different divisions of the glycosides, the solution became opalescent. The solution was allowed to cool and extracted 3 times in a separating funnel with 10 ml of ether. The 30ml ether extracts were put together and dehydrated using anhydrous sodium sulphate. An ether and aqueous solution were thereby formed.

The ether solution was used to test for the polyphenolics, steroid glycosides and triterpenes considering the chemical reaction characteristics of each group. The aqueous part was used to identify anthocyanosides.

Identification of anthracenosides

Using Borntrager's reaction, 4ml of ether filtrate is concentrated to 2ml, 1ml of 25% ammonia solution was added by shaking. Formation of a cherish-red colour of the alkaline solution confirmed the presence of anthracenoside.

Identification of coumarins

5ml of ether extract was evaporated to dryness. 2ml of distilled water was added to the residue and heated to dissolve. The solution was divided equally into two test tubes labelled A and B. 0.5ml of 10% ammonia solution was added to tube A and tube B was left as a reference. Appearance of a blue or green fluorescence under ultraviolet light, which is deeper in tube A showed the presence of coumarins.

Identification of flavonosides

Using Shibata's reaction, 5ml of ether extract was evaporated to dryness and the residue was dissolved in 2ml of 50% methanol by heating. Metal magnesium and 5 drops of concentrated HCl was added. Appearance of red colour confirmed the presence of flavonols while the orange colour confirmed the presence of flavanones.

Identification of sterol glycosides (cardiotonic)

Using Liebermann-Burchard's reaction, 10ml of ether extract was evaporated to dryness and the residue was successively dissolved in 0.5ml of acetic anhydride and 0.5ml of chloroform. The solution was transferred into a dry test tube and 1ml of concentrated HCl was pipetted to the bottom of the tube. A reddish-brown ring formed at the separating level of the two liquids indicated the presence of cardiotonic glycoside.

Identification of sterol glycosides (saponins)

10ml of the filtrate and 5ml of distilled water were mixed and was vigorously shaking for the development of a stable and persistent froth. The formation of emulsion on addition of three drops of olive oil confirmed the presence of saponins [22].

Identification of anthocyanosides

The acidic-aqueous part was used to identify the presence of anthocyanosides. The red solution turned to violet at a neutral $_{P}H$ and to blue in an alkaline medium confirmed the presence of anthocyanins.

The extract of the second solvent (petroleum ether) was also used to test for the presence of the phytoconstituents as follows:

Identification of basic alkaloids

10ml of the extract was evaporated to dryness and the residue dissolved in 1.5 ml of 2% HCl. The solution formed is divided into 3 equal volumes in test tubes labelled A, B, C. To tube A and B, 2 drops each of Mayer's and Dragendroff's reagents were added respectively, while tube C serve as the control. A yellowish-white precipitate in tube A and a red precipitate in tube B confirmed the presence of Alkaloids.

Identification of volatile oils

10 ml of the extract was evaporated to dryness in a conical flask. The residue with a characteristic pleasant odour was dissolved in 2 ml of alcohol by repeated elutions. One part of the solution was further evaporated to dryness. The residue with the same characteristic pleasant odour confirmed the presence of volatile oils.

Identification of triterpenes

10ml of the extract was evaporated to dryness and the residue was dissolved in 0.5ml of acetic anhydride and later in 0.5ml of chloroform. The solution was transferred into a dry test tube and 1ml of concentrated HCl was pipetted to the bottom of the tube. A reddish-brown ring was formed at the separating level of the two liquids. The turning of the supernatant layer to green or violet colour confirmed the presence of triterpenes.

Identification of emodols

3 ml of the extract was transferred into a test tube and 1 ml of 10% sodium hydroxide was added. The mixture was gently shaken and appearance of red colour showed the presence of emodols.

Identification of coumarins

3 ml of the extract was evaporated to dryness. The residue was dissolved in hot water and after cooling, the solution was divided into two test tubes labelled A and B. 0.5ml of 10% ammonia solution was added to tube A while tube B was left as a reference. Occurrence of an intense fluorescence under ultraviolet light indicated the presence of coumarins.

Identification of flavonoids

3 ml of the extract was evaporated to obtain residue which was later dissolved in 2ml of 50% methanol by heating. Metal magnesium and 4 drops of concentrated hydrochloric acid were added. Appearance of red or orange colour confirmed the presence of flavonoids.

Identification of carotenoids

10ml of the extract was evaporated to dryness and 2 drops of saturated solution of antimony trichloride in chloroform were added. Appearance of blue colouration which later turned red confirmed the presence of carotenoid pigments.

2.2.2 Quantitative determination of the phytoconstituents

Three of the phytochemicals that were qualitatively analyzed and found present in the extracts were also quantified.

Quantification of alkaloid:

In to 250 ml beaker, 5 g of each plant powder was put and 200 ml of 10% acetic acid in ethanol was added. The beaker was covered and allowed to stand for 12 hours. The solution was filtered and the filtrate was concentrated to 25% of the original volume on a regulated water bath. Precipitation was done by dropwise addition of concentrated ammonium hydroxide till it was completed. The solution was allowed to settle and the precipitate was carefully collected, washed with dilute ammonium hydroxide and filtered. The residue was dried and weighed as total Alkaloid [23].

Quantification of flavonoid:

100 ml of 80% aqueous methanol was used to extract 10 g of the plant powder repeatedly at room temperature. The pooled solution was filtered using Whatman filter paper No 42 (125 mm) and the filtrate was evaporated into dryness in a crucible on a water bath. The residue was made to attain a constant weight and weighed as the total flavonoids [23].

Quantification of total phenol:

0.5 ml of the extract was mixed with 0.5 ml of 1:1 Folin-Ciocalteu reagent and distilled water. The mixture was incubated at 22°C for 5 minutes after which 2 ml of 20% Na_2CO_3 was added and further incubated for 90 minutes at 22°C. Absorbance was measured spectrophotometrically at 650nm and with gallic acid as a standard, the total phenol was calculated in mg/ml [24].

2.3 Acute and Subacute Toxicity Studies

The procedures of limit test outlined in the guidelines of the Organization for Economic Co-operation and Development (OECD 423) was followed in carrying out the acute toxicity study as described by Aniagu et al. [25]. Eighteen female rats were divided into three groups (n=6). 2000mg and 5000mg of extract per kg body weight was administered orally to groups 2 and 3 respectively and monitored for 14 days. Group 1 was given 0.25ml of distilled water orally to serve as the control. Likewise, the doses of subacute toxicity study were administered as explained in Guideline 407 of OECD (Repeated Dose 28-Day Oral Toxicity Study in Rodents) [26]. Fifty Wistar rats (25 males and 25 females) were divided into five groups of equal sexes (n=10). Groups 2, 3, 4 and 5 were respectively administered with 125, 250, 500 and 1000mg/kg body weight of extract orally for 28 days. Group 1 was treated as done for the acute study.

2.4 Animal Sacrifice and Blood Collection for Haematological Evaluation

Animals were anaesthetized with chloroform and sacrificed by lumbar dislocation after blood collection through cardiac puncture. EDTA blood samples collected were used for the measurement of red blood cell count (RBC): Total RBC and RBC indices- Packed Cell Volume (PCV), Haemoglobin (HB), Mean Cell Haemoglobin (MCH), Mean Cell/corpuscular Volume

(MCV) and Mean Corpuscular Hemoglobin Concentration (MCHC); White blood cell count (WBC): Total WBC, Differential WBC- Lymphocytes (LYMP), Neutrophil (NEUT), Eosinophil (EOS) and Platelet count, using fully automated haematology analyzer (Sysmex Xn 1000).

2.5 Data analysis

The data obtained were analyzed using the statistical package for social science (SPSS) for Windows, version 21.0 (SPSS Inc., Chicago, IL, USA) and represented as Mean±SEM. Student T-test at 95% confidence interval was used to evaluate the significance of the difference between the mean values of the measured parameters in the respective test and control groups. A mean difference was considered significant at $p < 0.05$.

3. RESULTS

3.1 Phytochemical Analysis

The results of qualitative and quantitative analysis of both leaves harvested at two different seasons were presented in the tables below:

Table 1: Qualitative analysis of *Gliricidia sepium* and *Maerua angolensis* leaves ethanol extracts

Phytochemical	GS (Dry)	GS (Wet)	MA (Dry)	MA (Wet)
Saponins	-	-	+	+
Tannins	-	-	-	-
Reducing Compounds	+	+	+	+
Alkaloid salts	+	+	+	+
Starch	-	-	-	-
Anthracenosides	-	+	-	-
Anthocyanosides	+	++	-	+
Coumarins	+	++	+	++
Flavonosides	+	++	++	++
Steroid glycoside	+	+	+	+

GS (Dry) = extract of *Gliricidia sepium* leaves harvested during the dry season, GS (Wet) = extract of *Gliricidia sepium* leaves harvested during the wet season, MA (Dry) = extract of *Maerua angolensis* leaves harvested during the dry season, MA (Wet) = extract of *Maerua angolensis* leaves harvested during the wet season, + = Present, - = Absent.

Table 2: Qualitative analysis of *Gliricidia sepium* and *Maerua angolensis* leaves petroleum ether extracts

Test Parameter	GS (Dry)	GS (Wet)	MA (Dry)	MA (Wet)

Basic Alkaloids	+	+	+	+
Volatile Oils	+	+	+	+
Sterols & Triterpenes	+	+	+	+
Emodols	-	-	-	-
Coumarins	++	++	+	++
Flavonoid glycosides	++	++	+	++
Carotenoids	+	+	++	++

Table 3: Quantitative analysis of *Gliricidia sepium* and *Maerua angolensis* leaves extracts

Phytochemical	GS (Dry)	GS (Wet)	MA (Dry)	MA (Wet)
Alkaloids (%)	9.30± 0.1	9.81± 0.0	8.50± 0.0	9.10± 0.2
Flavonoids (mg/ml)	0.49± 0.0	0.53± 0.1	0.50± 0.0	0.49± 0.0
Total Phenol (mg/ml)	3.20± 0.5	3.89± 0.3	2.80± 0.2	2.77± 0.3

3.2 Haematological Analysis

Table 4: Acute oral intake of *G. sepium* leaf extract on the haematological indices

Parameters	Control	2000mg/kg	5000mg/kg	p-value
PCV %	39.0 ±1.7	40.3 ±2.1	40.0 ±1.0	.619
WBC ($x10^9$/L)	9.8 ±0.8	11.1 ±2.0	10.7 ±0.8	.492
LYMP %	87.7 ±2.5	74.3 ±6.0	88.7 ±1.5	.007*
NEUT %	11.7 ±2.9	23.0 ±6.2	10.3 ±1.5	.017*
EOS %	0.7 ±1.2	2.7 ±1.2	1.0 ±1.0	.137
PLT ($x10^9$/L)	326.7 ±30.0	404.3 ±91.9	307.0 ±17.5	.165
RBC ($x10^{12}$/L)	4.7 ±0.3	5.6 ±0.6	5.1±0.3	.091
HB (g/dl)	13.0 ±0.6	13.4 ±0.7	13.3 ±0.3	.619

MCV (fl)	8.4 ±0.2	7.2 ±0.5	7.9 ±0.7	.067
MCH (pg)	2.8 ±0.1	2.4 ±0.2	2.6±0.2	.067
MCHC (g/dL)	0.3 ±0.0	0.3 ±0.0	0.3 ±0.0	1.000

*=significant value

Comment [SN2]: Level of significance

Table 5: Acute oral intake of *M. angolensis* leaf extract on the haematological indices

Parameters	Control	2000 mg/kg	5000 mg/kg	p-value
PCV %	39.0 ±1.7	38.0 ±1.0	39.3 ±2.1	.579
WBC ($x10^9$/L)	9.8 ±0.8	9.8 ±0.3	9.7.7 ±0.5	.940
LYMP %	87.7 ±2.5	71.7 ±10.4	88.7 ±4.9	.061
NEUT %	11.7 ±2.9	24.3 ±9.5	10.0 ±3.6	.071
EOS %	0.7 ±1.2	4.0 ±1.0	1.3 ±1.5	.083
PLT ($x10^9$/L)	326.7 ±30.0	308.3 ±9.5	310.3 ±11.2	.485
RBC ($x10^{12}$/L)	4.7 ±0.3	5.2 ±0.3	4.8±0.3	.380
HB (g/dl)	13.0 ±0.6	12.7 ±0.3	13.1 ±0.7	.579
MCV (fl)	8.4 ±0.2	7.3 ±0.3	8.1 ±0.1	.064
MCH (pg)	2.8 ±0.1	2.4 ±0.1	2.7±0.0	.071
MCHC (g/dL)	0.3 ±0.0	0.3 ±0.0	0.3 ±0.0	1.000

Table 6: Subacute effect of *G. sepium* leaf extract on the haematological indices

Parameters	Control	125mg/kg	250 mg/kg	500 mg/kg	1000 mg/kg	p-value
PCV %	41.0±0.9	45.3±0.6	42.5±4.9	48.3±0.6	44.0±8.7	.264
WBC ($x10^9$/L)	10.1±0.3	8.5±0.4	8.4±1.8	7.2±0.6	9.0±0.3	.015*

LYMP %	86.9±1.7	81.0±3.6	84.0±8.5	83.3±5.8	88.7±2.3	.111
NEUT %	12.2±1.6	16.7±5.8	14.0±8.5	15.7±4.9	10.0±2.0	.270
EOS %	0.9±1.0	2.3±2.5	2.0±0.0	1.0±1.0	1.3±1.2	.544
PLT ($x10^9$/L)	316.5±30.0	308.0±9.2	305.0±8.5	308.0±9.8	320.3±8.6	.134
RBC ($x10^{12}$/L)	4.9±0.1	5.6±0.4	5.5±0.6	6.0±0.2	5.6±0.8	.188
HB (g/dl)	13.6±0.2	15.1±0.2	14.2±1.6	16.1±0.2	14.7±2.9	.264
MCV (fl)	8.9±0.3	8.1±0.4	7.8±0.1	8.1±0.2	7.8±0.4	.805
MCH (pg)	2.9±0.1	2.7±0.1	2.6±0.0	2.7±0.1	2.6±0.1	.805
MCHC (g/dL)	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	1.000

Table 7: Subacute effect of *M. angolensis* leaf extract on the haematological indices

Parameters	Control	125 mg/kg	250 mg/kg	500 mg/kg	1000 mg/kg	p-value
PCV %	41.0±0.9	41.7±3.2	44.3±4.0	42.0±3.0	45.0±0.0	.126
WBC ($x10^9$/L)	10.1±0.3	7.6±0.7	7.2±0.9	8.2±0.6	9.6±0.8	.002*
LYMP %	86.9±1.7	89.3±1.2	87.0±2.6	79.3±1.2	84.0±5.7	.017*
NEUT %	12.2±1.6	9.7±0.6	10.7±1.2	17.3±1.5	15.0±7.1	.068
EOS %	0.9±1.0	1.0±1.0	2.3±2.1	3.3±0.6	1.0±1.4	.104
PLT ($x10^9$/L)	316.5±30.0	313.0±24.3	304.7±16.3	310.7±19.3	322.0±45.3	.054
RBC ($x10^{12}$/L)	4.9±0.1	5.4±0.4	5.4±0.4	5.2±0.2	5.4±0.1	.370
HB (g/dl)	13.6±0.2	13.9±1.1	14.8±1.3	14.0±1.0	15.0±0.0	.126
MCV (fl)	8.9±0.3	7.8±0.4	8.2±0.1	8.0±0.3	8.4±0.1	.971

MCH (pg)	2.9±0.1	2.6±0.1	2.7±0.0	2.7±0.1	2.8±0.0	.971
MCHC (g/dL)	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	1.000

4. DISCUSSION

4.1 Phytochemical Analysis

The phytochemical screening of *Gliricidia sepium* and *Maerua angolensis* leaves extracts of dry and wet harvest revealed the presence of diverse, but rich secondary metabolites, which justified the use of the plants in tradomedical settings for the treatment of various diseases. The use of different parts of *G. sepium* has been reported in many countries for different purposes [9]. Oduola et al. [27] reported its Anti-sickling property, Reddy & Jose [11] reported its use for the treatment of pathogenic bacterial infections, skin diseases [13], anti-tumour [10] and so on. *M. angolensis* too is being used for treating sexually transmitted diseases [17], Diabetes mellitus [18], stomach ulcer [20], convulsion [19] and so on.

Apart from anthracenosides which was absent (undetectable quantity) in the dry season sample of *G. sepium* extract, other phytochemicals (Alkaloid salts, Reducing compounds, Anthocyanosides, Flavonosides, Coumarins and Steroid glycoside) were present in both wet and dry season samples. Although, anthocyanosides, coumarins and flavonosides were found in higher quantity in the wet season sample as compared to the dry season; this might be due to the full effect of photosynthesis when the plants are generally green and metabolites are produced in abundant without stress [28]. The presence of volatile oils and carotenoids were additionally confirmed from the PE extract sample. Carotenoids families are known for their natural pigments, provitamin and antioxidant activities. About 50 dozen carotenoids are recognized and studied from plants and animal sources [29]. One of their most significant functions is to mop up free radicals and inhibit oxidative stress [30]. According to Sinha [31] who studied the phytochemical and antioxidant activities of *Gliricidia sepium*; the plant possesses abundant beneficial metabolites like Alkaloids, phenols and flavonoids and has been described as a candidate plant for medicinal purposes. Alkaloids, which are present in both plants, are among the most important bioactive substances in phytomedicine with highly diverse group of compounds widely distributed in the plant kingdom [32]. Ephedrine and morphine are among the alkaloid compounds that have passed the clinical trials and have been developed into drugs for asthmatic relief and analgesic respectively. As a reservoir of drug discovery, many alkaloids compounds have been successfully developed into anticancer drugs, vinblastine is among the list. Rotenoids, evodiamine, berberine, matrine, sanguinarine, tetrandrine and piperine are also parts of the promising alkaloid compounds with potential antiproliferative properties in clinical trials [32].

Methanol leaf extract of *Maerua angolensis* has been reported to harbour secondary metabolites like Alkaloids, Saponins, Flavonoids and Tannins [33], the result of this work correspond with their study report, except for tannins, which was probably of undetectable quantity in this study. Seasonal variation and soil profile might be responsible for this difference. Seasonal variations of phytochemicals in plants have been attributed to the interaction between the species and their environmental conditions at a particular instance [34,35]. Because of the large influence that

these variations might have on the efficacy of medicinal plants, the geographical dispensation of the species ecosystem must be taken into consideration and this is the reason for seasonal harvesting considered in this study.
The conspicuous presence of some phytochemicals in *Maerua angolensis* harvested plants during the wet seasons and confirmation of additional metabolites which were absent during the dry season's harvest, have been attributed to the increased soil moisture levels due to rainfall [36]. Yola has an average annual rainfall of 850 mm - 1000 mm with over 41% of rain falling in August and September [37]. The plants in this area, therefore, experienced a long period of the dry season during which tree leaves are shed, the photosynthetic rate slowed down and translocation of food reserves from leaves to storage parts took place stressfully [38,39].

The quantitative analysis was done for Alkaloids, Phenols (total) and Flavonoids which are the targeted phytochemicals in this study. The results pattern showed higher, but non-significant values for quantity of phytochemicals in the wet season extracts from both plants, in relation to the dry season's. Alkaloids, which is one of the most important secondary metabolites in phytomedicine, is found in abundance in both plants (8.5-9.8% across seasons). Majority of the antiproliferative drugs in clinical trials are considering the characteristics of alkaloid derivatives in their studies [40]. Phenolics are, perhaps, the most extensively studied metabolites in chemopreventive studies. They can be categorized into simple phenols and phenolic acids, hydroxycinnamic acid derivatives and flavonoids [41]. This is the reason for their total quantification in phytochemical studies [41]. They play an indispensable role in plant reproduction and act as soldiers that defend plants against predators, parasites and pathogens [42]. Their antioxidant capacity and ability to prevent some diseases have been documented especially in cancer research [6]. The most prevalent simple phenols are monophenols, 3-ethylphenol, 3,4-dimethylphenol and diphenol. They are the major plant defence molecules in preventing the growth of infectious organisms [43]. Simple phenols are also the building blocks in the production of complex compounds like flavonoids and tannins [44].

Flavonoids are another major form of phenolic compounds known for their high antioxidant potential. Over 4000 types of flavonoids have been studied in plants and they have been linked to reducing main chronic diseases in humans. Flavanones, flavones, flavonols, flavanols, and isoflavonoids are derivatives of flavonoids with a difference in their structural units [6]. Most of the hydroxycinnamic acids and their derivatives originated from p-coumaric acid, ferulic acid and caffeic acid. They are usually esters and coumaric acid, caffeic and chlorogenic acid are their popular phytochemicals in plants [45]. Total phenol content is found to be 3.89 mg/ml in the *G. sepium* wet season extract, a value that is more than twice reported by Sinha [31]. Seasonal variation might be the determining factor for this difference. The antioxidants mop-up activities of phenols have been described as their major capacity in diseases prevention, including cancer [6]. Flavonoids values reported for *G. sepium* in this study was in tandem with the report by Sinha [31] and Akharaiyi et al. [14].

Sterols are phytochemicals found in the plant host that have structures similar to cholesterol. They possess additional hydrocarbon chain at the position of Carbon-24 that structurally differentiate them from cholesterol [46]. Plant sterols have protective effects on some chronic diseases like cardiovascular disorder, diabetes, and cancer. Sterols have shown antiproliferative effects on colon cancer, lung cancer and stomach cancer by inhibiting cell proliferation, inducing apoptosis and regulating endocrine tumor growth [47].

4.2 Haematological Analysis

Blood, as the wheel of life, travels continuously in the body system. Any haematotoxic materials are then easily carried from one organ to another. Also, due to the pluripotent nature of the blood-producing organs, safety of all circulating blood cells must be ensured before pronouncing a drug or compound to be safe for consumption [8].

In this study, *G. sepium* extract showed certain degree of haematoprotective characteristics (Table 4 &6). The extract increases the population of circulating red blood cells and white blood cells, as seen from the values of PCV, WBC, HB and RBC, though at a non-significant level when compared with the control at both acute and subacute toxicity study reports. Apart from the assurance of safety, this result also means non-interruption of oxygen supplied to the tissues when this extract was consumed. The statistically significant difference in the value of the circulating LYMP and NEUT in the acute and total WBC in the subacute studies was understandable. As the territorial soldiers of the body systems, mobilization to attack the foreign invaders is expected once a new compound has entered the body. These effects have been normalized at the highest dose, however. The circulating blood components always almost remain constant under normal circumstances, disruption by anemia, loss of blood and some poisons consumption that can cause brain drainage can be experienced. Marrow can, however, brings the number of circulating blood cells to normal by activating relevant factors to stimulate haemopoiesis [48]. If a toxic dose of a compound is administered into the body, changes in the blood parameters is a pointer towards a haematological disorder [49].

In most mammals, erythrocytes are biconcave-shaped, non-nucleated cells that are packed with haemoglobin, the oxygen-carrying pigment that gives the blood its colour. Red cells normally maintain a constant volume in circulation and a decrease in the volume is always associated with a disease condition like anaemia. Likewise, elevated RBC volume can be induced by a defect in marrow production (erythrocytosis) apart from factors like smoking, chronic kidney diseases, liver diseases, exposure to carbon monoxide and other toxins [49].

The RBC contains haemoglobin, the oxygen-binding protein that has a tetrapyrrole ring system, with ferrous iron at the centre. Each molecule of haemoglobin has four amino acid chains; a pair each of folded alpha and beta chains with each carrying a heam group which attached to a histidine residue at position 92 in the beta chains and position 87 in the alpha chains. This is the protein that transports oxygen from the pulmonary artery to the tissues and removes carbon dioxide from the tissues for exhalation by the lungs [49]. When there is haemorrhage, accelerated blood cell destruction or iron deficiency which could cause the release of immature reticulocytes, the haemoglobin level becomes low. Sickle cell disease also results from the alterations in haemoglobin molecules [50]. High levels of free haemoglobin in the blood might result during massive erythrocytes haemolysis which could be triggered by antibody attack on the red blood cells or inflammation [51].

PCV, also known as haematocrit, represents the percentage of erythrocytes volume of whole blood volume. It is a special indicator to detect or confirm anaemia and they are affected by any factor that can affect the red blood cells [49]. An abnormal increase in the PCV values signifies polycythemia under normal circumstances, which could be due to a high level of toxin, vitamin B12 / folate deficiency, hypoxia, alcoholism and so on. Abnormally low PCV signifies anaemia which might be due to a reduced number of circulating red blood cells, decrease amount of

haemoglobin in each red blood cell or both. This can occur in response to damage to the myeloid tissue by chemicals, radiations, toxins and so on [51]. MCH levels are the calculated average amount of haemoglobin that is inside each red blood cell, while MCHC level is a calculation of the average weight of the haemoglobin based on the volume of red blood cells. Both are indicators of health status of the blood haemoglobin. A lower value of MCH is common in thalassaemias, iron deficiency and other chronic diseases associated with anaemia, while higher values are common in macrocytic anaemias [49,51]. The Mean cell volume (MCV), also called mean corpuscular volume is a value that describes the average size of erythrocytes in a blood sample. Microcytosis and macrocytosis in red cells indices are information revealed by the MCV values. It is an important parameter in the early diagnosis of kidney disease [52]. Variations in erythrocytic parameters are, therefore, important indicators in determining the safety of drugs in toxicological studies.

Leucocytes are the human system protectors from foreign invaders, they provide immunity to the system against antigen invasion. WBCs originated from the myeloid stem cells except for lymphocyte which has a separate origin, the lymphoid stem cells. In humans, Leucocytes are divided into two: the granulocytes, which made up of eosinophils, neutrophils, and basophils described according to their cytoplasmic granules' staining characteristics. Agranulocytes are the lymphocytes and monocytes [48,53] The cytoplasmic granules of the granulocytes contain varying enzymes and other substances for effecting phagocytosis and inflammatory response. The primary function of polymorphs is phagocytosis; the function they performed majorly in the tissues, into which they pass between the capillary endothelial cells [48].

Neutrophils granules are known for their high alkaline phosphatase activity, it also contains acid phosphatase, myeloperoxidase and other acid hydrolases during their primary developmental stage [49]. Their secondary granules contain lysosomes, collagenase and lactoferrin. Neutrophils defend the body against microbial attack either by production of hydrogen peroxide and superoxide anion or by discharge of intracellular acid pH or enzymes lactoferrin and lysosome [49]. Increase value of neutrophils count (neutrophilia) is common in bacterial infections, tissue injury, steroid therapy and metabolic disorder. Low levels (neutropenia) are seen in both acquired and inherited disorders like leukaemia and Kostmann's syndrome. Vitamin deficiencies, Hepatitis A, B and C, HIV/AIDS, Malaria and direct toxicity are some of the other conditions which can lead to reduced neutrophils counts [54].
High eosinophils counts are frequently linked to disorders of allergic origin and parasitic infestations. Eosinophils regulate inflammation and destroy foreign invaders. Their granules have a variety of hydrolytic enzymes and high peroxidase activity. Health conditions like Hodgkin disease, autoimmune disease, Asthma and so on can be linked to eosinophilia. However, abnormally low eosinophils counts may be due to overproduction of certain steroids hormones such as cortisol and could also be due to alcoholic intoxication [55].

The dark cytoplasmic granules of the basophils which contain histamine and heparin clearly overlie the nucleus. The obscuring function of basophils is becoming clearer as the prime early producers of cytokines essential for allergy initiations and responses. They have IgE attachments site and serve as the main cellular source for early production of interleukins IL-4 and IL-13 [55]. Increased basophils value may connote chronic myeloid leukaemia or polycythemia [56].

Lymphocytes have been recognized as a cell with immunological potential to assist phagocytes in the defence of the body system. They are produced and develop primarily from the thymus and the bone marrow and hence named dividedly as T-lymphocytes and B-lymphocytes [56]. The specific immune responses are generated within the secondary lymphoid organs; lymph nodes, spleen, the lymphoid tissues of the GIT and Respiratory tracts. T-lymphocytes account for up to 75% of lymphocytes in the peripheral blood and carry out cell-mediated immunity while B-lymphocytes perform humoral immunity. Elevated lymphocytes count in the adult may be due to chronic infections like tuberculosis, lymphoma or leukaemia and acute infections like hepatitis, pertussis and infectious mononucleosis [57].

Maerua angolensis extract also showed no harmful effect as seen from the haematological parameters (Table 5&7). There was no significant difference between the control and experimental values in the acute study. Most importantly, the two extracts did not affect the clotting pathways as the platelet counts showed no significant difference from the control in the two investigated extracts. Platelets are produced by the fragmentation of megakaryocytes in the bone marrow. Each fragment enclosed by the cell membrane is referred to as a thrombocyte. They are the agents responsible for coagulation in the blood system. Elevated platelets count may suggest a stimulatory effect on thrombopoietin hormone by the investigational product which might suggest anti-haemophilia. Increased count in animals could also be due to inflammation or bleeding disorder [49].

In conclusion, both plants housed medicinal phytochemicals that are friendly with the body fluid tissue. They are candidates to harvest for pharmaceutical intervention.

Comment [SN6]: Conclusion should be under separate heading and needs more information / Clarity

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

NOTE:

The study highlights the efficacy of "Herbal" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

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Comment [SN7]: Incorporation of latest references is required

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