



Potential of Ethanol Extracted Secondary Metabolites of Plants from Thal Desert (Pakistan) for *In vitro* Changes in Haematological Indices

**Ghulam Yasin^{1*}, Mubasharah Sabir¹, Iqra Anwar¹, Adeela Altaf²,
Syeda Amna Batool³, Ikram Ul Haq⁴, Ali Noman⁵ and Khalid Hussain⁶**

¹Department of Botany, Bahauddin Zakariya University, Multan, Pakistan.

²Department of Environmental Sciences, Bahauddin Zakariya University, Multan, Pakistan.

³Department of Botany, University of Narowal, Narowal, Pakistan.

⁴Institute of Biotechnology and Genetic Engineering (IBGE) University of Sindh, Jamshoro, Pakistan.

⁵Department of Botany, Government College University, Faisalabad, Pakistan.

⁶Department of Botany, University of Gujrat, Pakistan.

Authors' contributions

This work was carried out in collaboration among all authors. Authors GY, MS and IA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AA and SAB managed the analyses of the study. Authors IUH, AN and KH managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Plants are adapted to stressful environments by synthesizing secondary metabolites as antioxidant having either toxicological or medicinal properties. Before utilizations of metabolites one should explore reality about their nature. By testing their *in vitro* effects on blood, their curing or toxic potential for influence on physiology and health status of an individual can be evaluated. In this experiment, secondary metabolites of some shrubs of Thal desert of Pakistan were extracted in ethanol and used to explore their *in vitro* effects on some hematological attributes as enlisted in tables. Results were statistically analyzed using Analysis Of Variance. Means of data were compared to control by DMR (Duncan's multiple range) procedure. Ethanolic extract increased the leucocytes count granulocytes percentage, monocytes lymphocytes MCV, MCH and platelets except extract of Haloxylon stem. HGB were decreased by extract of stems of Leptadenia,

*Corresponding author: E-mail: yasingmn_bzu@yahoo.com;

Calligonum and Salsola. Leptadenia stem extract also decreased the platelets. Lymphocytes, monocytes, eosinophils and RBC were increased by most of the plant extracts. The practical application of the plant secondary metabolites needs careful further extensive study.

Keywords: Secondary metabolites; potential; ethanolic extracts; Thal desert; haematological indices.

1. INTRODUCTION

Plants have developed some special mechanism for adjustment in changing environments [1-3] by synthesis of secondary metabolites playing a number of roles in changing environmental conditions [4]. These changes may include light, temperature, humidity, local geo-climatic, seasonal changes and developmental processes [4-5]. These compounds play different roles in plant itself such as protecting plants from herbivores [6-7], pathogen [8-9], and ultraviolet radiations [10-11]. Human diet containing plant secondary metabolites can affect the metabolism and health status. These compounds have been reported to be used as food additives to improve the quality of nutrition [12].

Some of the secondary metabolites have negative effects on consumer because of having harmful toxins [13] while others have been shown to have positive effects [14-15]. Some secondary molecules are produced from primary ones and are present in plant. Their production can be induced under *in vitro* conditions when applied biotic and abiotic stresses [16]. Human has used such metabolites for making drugs to cure many diseases [17-18]. Secondary metabolites have antioxidant [19-20], anti-inflammatory [21-22], antifungal [23-24], hepatoprotective [25] and neurological [26] effects. The uses of plants in cure is too old to date back 1550 BC [27-28]. The increasing demand of secondary metabolites needs the technology of transgenic plant production [29]. A number of medicinal plants containing secondary metabolites as flavonoids and alkaloids are used in natural medicine. Flavonoids like secondary metabolites are phenolic compounds that are the most bioactive secondary metabolites [30]. Lipid oxidation is slowed down by phenolics [31]. Flavonoids have the pharmacological effects of antimicrobial, antiviral, anti-inflammatory and antihepatotoxic properties [32]. Similarly, alkaloids are well known for their pharmacological effects [33]. Plants of the deserts face stressful conditions and synthesize secondary metabolites in response to excess reactive oxygen species. The practical uses of secondary metabolites for medicinal purposes need their prior knowledge of toxicological or

medicinal potential. The medicinal use of plant products without proper scientific studies can raise health issues [34].

Blood determines the physiological and pathological status of an organism. The health condition of a person is assessed mainly by evaluating blood attributes. Hence, the use of herbal products on various hematological parameters can be useful for toxicological or medicinal nature of plant metabolites extracts [35]. Commonly animals are used for deducing nature of herbal products [7]. However, *in vitro* technique application can be employed for directly assessment on human blood. This provoked the idea of designing a project to investigate the potential of Ethanolic extract secondary metabolites of desert plants for human haematological indices

2. MATERIALS AND METHODS

2.1 The Aims of Investigations

The choice of our investigation is aimed to explore the potential of Ethanol soluble secondary metabolites [36] resourced from desert plants for *in vitro* changes in human blood characteristics. This assessment will discriminate the ethno-pharmacological and toxicological validation of plant secondary metabolites [37]. The selection of desert plants is based on two criteria: first, due to arid environmental conditions, these synthesize and store secondary metabolites for adaptation to stressful environment [38]. The second criterion is that desert plants have been traditionally used by local peoples in the treatment of some diseases and free radicals related disorders but with no proper documentation of their side effects [39]. Blood of an organism reveals its health and can be a useful index for determining practical application of plant extract for human health [35]. Although toxic effects of plants are judged on animals but *in vitro* human blood can be used directly for deducing results [7].

2.2 Experimental Plan

Shrubs of the Thal desert were selected for secondary metabolites extraction. Ethanol was

used as solvent for extraction procedure [40]. The Ethanol used was of high purity. Human blood from a healthy volunteer was utilized for *in vitro* assessment of haematological indices [41-42]. Means of three replicates for each sample were used to reduce the error for comparison with normal blood characteristics.

2.3 Field Survey and Plant Sample Collections

A preliminary survey of Thal desert was conducted. During this survey, meetings with local peoples were arranged to know local plant names of the plants. Identification of the plant was performed by specialists and by matching them with the labelled herbarium of the department (Dr. Mumtaz Bukhari herbarium) of Botany Department Bahauddine Zakarya University, Multan Pakistan and/or the literature [43]. Data and specimens were collected keeping in view the uniformity among size and age of plants. Further processing of collected specimens was carried out in laboratory of the department.

2.4 Crude Herbal Extract Preparation

After washing specimens with running water and 2% ethanol, were dried at room temperature. Afolayan et al. [44] method was used for extract preparation. Plant material of 50.0 g was extracted in 200ml of Ethanol using orbital shaker. The extract was stored at -4°C.

2.5 Blood Sampling and *In Vitro* Analysis

After approval from BOS human O+ blood was obtained from a healthy volunteer aged 25 years. Informations about using no antioxidant supplementation and not taking any medications or addictive substances were ensured from donor before taking blood. Ethanol (5ml) was added to each extract for dilution. After reviewing literature, the ratio of mixing blood to extract was determined by trial of mixing various ratios and finding the appropriate dose when no coagulation occurred. Finally, 4.0 ml blood was mixed with 1ml extract and was shaken smoothly. The complete blood count tests (CBC) of samples was taken by using Automated Hematology Analyzer machine.

2.6 Statistical Analysis

One way ANOVA was used for data analysis at 5% level of significance using Costat statistical

software. Data were presented along with standard deviations (Mean \pm SD). Means of data were compared to control by Duncan's Multiple Range Test [45].

3. RESULTS

3.1 *Crotollaria Burhia* Root

Index of variability in blood parameters revealed a significant difference in leukocyte count (270.5%), granulocyte count (17130%), monocyte count (1452.55%), lymphocyte (250.74%), monocyte (5000%), HGB (14.76%), HCT (17.44%), MCV (34.69%), MCH (30.80%), RDW (64.23%) and platelets (14.48%). Extract did not increase MPV, Eosinophils MCHC, lymphocytes count and granulocyte parameters. Blood parameters MPV (35.06%), MCHC (3.38%), lymphocyte count (5.29%), eosinophils count (85.71%) and granulocyte (40%) revealed a significant decrease.

3.2 *Crotollaria Burhia* Stem

The extract increased leukocyte count (258.7%), granulocyte count (51515.38%), lymphocyte (250.74%), HGB (6%), HCT (17.18%), MCV (27.89%), MCH (22.98%), RDW (42.33%) and platelets (17.02%). The observations are excluded from the ongoing trends for MPV, MCHC, lymphocyte count and Eosinophils parameters. The significant decrease was MPV (20.20%), MCHC (3.38%), lymphocyte count (5.21%), eosinophils count (100%). Although not statistically justified, considerable extent of decrease monocyte count (100%), granulocyte (100%), monocyte (100%) and Eosinophils (100%) respectively was observed when extract was applied.

3.3 *Leptadaenia Pyrotechnica* Stem

The greater effect of extract was for leukocyte count (76.90%), granulocyte count (29515%), monocyte count (236.73%), lymphocyte count (107.46%), monocyte (361.53%), MCH (46.77%) and RDW (69.34%). The increasing augmentation could not have pace for MPV, MCHC, MCV, lymphocyte count and Eosinophils count parameters. Extract showed a decreased in MPV (100%), platelets (100%), MCHC (6.36%), MCV (55.98%) lymphocyte count (23.30%) and Eosinophils count (89.28%). Although not statistically justified, but to a substantial level decrease

Table 1a. *In vitro* effect of Ethanolic extract of shrubs of Thal on human heamatology [values represent mean±standard daviation; n=3]

Name of species	leukocyte count (10×3/u L)	Granulocyte (%)	Lymphocyte (%)	Monocyte (%)	Eosinophils (%)	Granulocyte (10×3/L)
Normal blood	5.63 ± 0.05k	0.13± 0.05o	47.93 ± 0.05i	1.96 ± 0.05m	2.76 ± 0.05a	0 ± 0 d
<i>Crotollaria burhia</i> (r)	20.86± 4.70a(+270.5)	22.4 ± 1.212g(+17130)	45.366 ±1.10defg(-5.290)	30.43 ± 0.85b (+1452.55)	0.4 ± 0.1b(-85.71)	0.4 ± 0.1 d(-40)
<i>Crotollaria burhia</i> (s)	20.2± 0.97a (+258.7)	54.1 ± 1.25b (+41515.38)	45.43 ± 0.81 defg (-5.21)	0 ± 0m(-100)	0 ± 0 e(-100)	0 ± 0 d(-100)
<i>Leptadaenia pyrotechnica</i> (s)	9.96± 0.35fg(+76.90)	38.5 ± 1.25 d(+29515)	36.76± 26.90gh(-23.30)	6.6 ± 1.08k(+236.73)	0.3 ± 0.1bc(-89.28)	3.76 ± 1.10c(-376)
<i>Haloxylon stockii</i> (s)	0± 0l(-100)	0 ± 0o(-100)	0 ± 0i(-100)	0 ± 0m(-100)	0 ± 0e(-100)	0 ± 0 d(-100)
<i>Salsola imbricata</i> (s)	8.4 ± 0.5hij(+49.20)	30.6 ± 1df(+23438.4)	41.43 ± 1.20fgh(-13.507)	25.53 ± 1.02d(+1202.5)	0.3 ± 0.1bc(-89.28)	2.56 ± 1.10 c(-256)
<i>Calligonum polygonoides</i> (s)	8.3 ± 0.55hij(+47.42)	8.36 ± 0.55l(+6330.76)	41.4 ± 1.20fgh(-13.569)	25.53 ± 1.20 d(+1202.5)	0.3 ± 0.01 bc(-89.28)	2.46 ±1.15 c(-246)
<i>Calligonum polygonoides</i> (r)	18.16 ± 0.65b(+222.5)	44.13 ± 0.80c (+33846.15)	40.4 ± 0.72 fgh(-15.657)	16.43 ± 1.00h(+738.26)	0 ± 0 e(-100)	7.66 ± 1.16a(-766)

Values sharing the different letters represent significance difference in respective row; values in parenthesis represents percentage difference over control group; LSD= least standard deviation; r=root; s=stem; f=flower

Table 1b. *In vitro* effect of Ethanolic extract of shrubs of Thal on human heamatology [values represent mean±standard daviation; n=3]

Name of species	Lymphocyte count (10×3/L)	Monocyte count (10×3/L)	Eosinophils count (10×3/L)	RBC (10×6/ uL)	HGB (g/dL)	HCT (%)
Normal blood	2.68 ± 0.02j	0.13± 0.05g	50.33 ± 0.57h	5.14 ± 0.005c	8.33 ±0.05 d	22.73 ± 0.05h
<i>Crotollaria burhia</i> (r)	9.4 ± 0.76 a (+250.74)	6.63 ± 1.069 a (+5000)	1.8 ± 0.1defg (-96.42)	4.51 ± 1.11 bc (-12.42)	9.56 ± 1.02 abc (+14.76)	26.66 ± 1.09 cd (+17.44)
<i>Crotollaria burhia</i> (s)	9.4± 0.62 a (+250.74)	0 ± 0 g (-100)	0 ± 0 h (-100)	4.62 ± 0.72 bc (-10.11)	8.83 ± 0.90 bc (+6.00)	26.6 ± 1.17 cd (+17.18)
<i>Leptadaenia pyrotechnica</i> (s)	5.56 ± 1.19 cdef (+107.46)	0.6 ± 0.1 fg (+361.53)	0 ± 0h (-100)	2.83 ± 1.001 c (-44.95)	6.76 ± 1.05 d (-18.55)	19.83 ± 0.90 h (-12.64)
<i>Haloxylon stockii</i> (S)	0± 0 j (-100)	0± 0 g (-100)	0± 0 cd (-17.14)	3.61± 1.22 bc (-29.76)	8.5± 0.88 bcd (+2.04)	20.7± 1.01 h (-8.93)
<i>Salsola imbricata</i> (s)	3.7 ± 0.91 fghi (+38.05)	2.6 ± 1.13 bcd (+1900)	3 ± 1 cd (-94.03)	3.77± 1.16 bc (-26.65)	8 ± 1 cd (-3.614)	21.6 ± 1.05 gh (-4.97)
<i>Calligonum polygonoides</i> (s)	3.6 ± 1.05 ghi (+34.32)	2.43 ± 1.23 bcd (+1769.23)	0 ± 0 h (-100)	3.77 ± 0.11 bc (-26.65)	8 ± 1 cd (-3.614)	21.56 ± 1.10 gh (-5.02)
<i>Calligonum polygonoides</i> (r)	5.36+ 2.92 cdefg (-100)	3 ± 1 bc (+2207.69)	3 ± 1 cd (-94.03)	3.60 ± 0.95 bc (-29.96)	8.63 ±0.94 bcd (+3.60)	25 ± 1f de (+9.98)

Values sharing the different letters represent significance difference in respective row; values in parenthesis represents percentage difference over control group; LSD= least standard deviation; r=root; s=stem; f=flower

Table 1c. *In vitro* effect of Ethanolic extract of shrubs of Thal on human hematology[values represent mean±standard deviation; n=3]

Name of species	MCV (FL)	MCH (PG)	MCHC (%)	RDW (%)	Platelets (10×3/uL)	MPV (10×3/L)	RDW
Normal blood	44.13 ± 0.05 k	16.13 ± 0.05 h	36.63 ± 0.05 j	13.71 ± 0.02 l	642.33 ± 0.57i	6.93 ± 0.05 a	17.41 ± 0.02 d
<i>Crotollaria burhia</i> (r)	59.4 ± 1.1 def (+34.69)	21.06 ± 0.80 defg (+30.80)	35.36±1.15defgh (-3.38)	22.5 ± 0.96 fghi (+64.23)	735 ± 4.509 g (+14.48)	4.5 ± 0.4 fg (-35.06)	21.73 ± 1.25 fg (-24.81)
<i>Crotollaria burhia</i> (s)	56.4 ± 2.00 fgh (+27.89)	19.8 ± 0.65 g (+22.98)	33.16 ± 0.55 hi (-9.39)	19.5 ± 1.01 j (+42.33)	751.66 ± 8.08 g (+17.02)	5.53±0.30 bcdef (-20.20)	20.56± 1.00fg (-18.09)
<i>Leptadaeni apyrotechnica</i> (s)	19.5 ± 1.05 k (-55.78)	23.63 ± 0.96 abc (+46.77)	34.3 ± 1.55 ghi (-6.36)	23.2 ± 0.96 efgh (+69.34)	0 ± 0 m (-100)	0 ± 0 h (-100)	22.5 ± 0.96 fg (-29.23)
<i>Haloxylon stockii</i> (S)	59.5 ± 1.05 def (+34.92)	23.6 ± 1.15 ab (+46.58)	39.3 ± 0.95c (+7.28)	20.46 ± 1.19 ij (+49.34)	1304 ± 18.7a (+103.23)	5.5 ± 0.6bcdef (-20.63)	23.76 ± 1.15 fg (-36.47)
<i>Salsola imbricata</i> (s)	55.4 ± 0.98 ghi (+25.62)	20.63 ± 1.20 efg (+27.89)	37.63 ± 1.78 cd (+2.73)	19.6 ± 1.85f (+43.06)	680.66±11.015h (+5.96)	5.26 ± 0.60 cdef (-24.09)	20.43 ± 20.56 fg (-17.34)
<i>Calligonum polygonoides</i> (s)	55.43 ± 1.02 ghi (+25.69)	20.66 ± 0.95 efg (+28.32)	36.3 ± 3.05 defg (-0.819)	19.66 ± 2.03 j (+43.50)	678.33 ± 7 h (+5.60)	5.5 ± 0.67 bcdef (-20.63)	20.56 ± 1.07 fg (-18.09)
<i>Calligonum polygonoides</i> (r)	65.4 ± 1.1 b (+48.19)	22.66 ± 0.95 abcd (+40.74)	33.6 ± 0.96 hi (-8.196)	28.36 ± 1.19 a (+106.85)	1175 ± 21.19 c (+82.02)	6.03±0.80abcde (-12.98)	22.63 ± 1.25 fg (-29.98)

Values sharing the different letters represent significance difference in respective row; values in parenthesis represents percentage difference over control group; LSD= least standard deviation; r=root; s=stem; f=flower

in HCT (12.64%), HGB (18.55%), RBC (44.95%) and Eosinophils (100%) respectively was found when extract was applied.

3.4 *Haloxylon Stockii* Stem

The significant increase was in HGB (2.04%), MCV (34.92%), MCH (46.58%), MCHC (7.28%), RDW (49.34%) and platelets (5.96%). Extract role was not in favour of increase in MPV, leukocyte count, Eosinophils count, Eosinophils, RBC, RDW and MPV parameters. The application of extract seemed to decrease significantly MPV (20.63%), leukocyte count (100%), eosinophils count (100%), eosinophils (17.14%), RBC (29.76%), MPV (24.09%) and RDW (36.43%). Although not statistically justified, considerable extent of decrease in granulocyte count (100%), lymphocyte count (100%), monocyte count (100%), granulocyte (100%), lymphocyte (100%), monocyte (100%) and HCT (8.93%) respectively was observed when extract was applied.

3.5 *Salsola Imbricata* Stem

Ethanol extract proved its significant influence of enhancing leukocyte count (49.20%), granulocyte count (23438.4%), monocyte count (1202.5%), lymphocyte (38.05%), monocyte (1900%), MCV (25.62%), MCH (27.89%), MCHC (2.73%), RDW (43.50%) and platelets (5.96%). An exception in this correlation was found MPV, HGB, RBC, HCT, Eosinophils, lymphocytes and Eosinophils count parameters. Blood parameters Eosinophils count (89.28%), lymphocyte count (13.56%), granulocyte (256%), Eosinophils (94.03%), RBC (26.65%), HGB (3.614%), HCT (4.97%) and MPV (24.09%) revealed a significant decrease.

3.6 *Calligonum Polygonoides* Stem

Extract has strongly influenced leukocyte count (47.42%), granulocyte count (6330.76%), monocyte count (1202.5%), lymphocyte count (34.32%), monocyte (1769.23%), MCV (25.69%), MCH (28.32%), RDW (43.50%) and platelets (5.60%) respectively. Ethanol extract affected MPV, MCHC, RBC, HGB, HCT, lymphocyte count, eosinophils count, granulocyte and eosinophils with different degrees thereby lowering values from control. Blood parameters MPV (20.63%), MCHC (0.8195), lymphocyte count (13.56%), eosinophils count (89.28%), granulocyte (246%), Eosinophils (100%), RBC (26.65%), HGB (3.614%), and HCT (5.02%)

revealed a significant decreased when treated with extract.

3.7 *Calligonum Polygonoides* Root

Mean values revealed enhancing role of Ethanol extract for leukocyte count (222.5%), granulocyte count (33846.15%), monocyte count (738.26%), monocyte (2207.69%), HGB (3.60%), HCT (9.98%), MCV (48.19%), MCH (40.74%) and RDW (106.85%). An exception in this correlation was found MPV, MCHC, RBC, Eosinophils count, lymphocyte and Eosinophils count parameters. Blood parameters MPV (12.98%), MCHC (8.196%), Eosinophils count (100%), granulocyte (766%), lymphocyte count (15.65%), lymphocyte (100%), Eosinophils (94.03%), and RBC (29.96%) revealed a significant decreased when treated with extract.

4. DISCUSSION

Blood status determine the physiology of an organism [46]. The effect of any foreign compound in body is analyzed by evaluating blood characters [47]. The results of present study revealed the effects of ethanol extracts of plant specimens on various hematological attributes. The results are in harmony and contradict with the earlier findings (Lohar *et al.* [48]. As already investigated some plants compounds are non-specific in effects [49]. The plant extracts contains high concentration of antioxidants like phenolics compounds. Phenolics are antioxidants playing role in diseases cure [50].

Erythrocytes (RBC) which are the most abundant cells blood play role in morphology and physiology of body. Any foreign agent in the form of medicine has more affects on Erythrocytes [51]. Results showed that Ethanol extracts of specimens reduced the number of RBCs. Major target of ROS are RBC due to high concentrations of unsaturated fatty acids in their membrane [52]. ROS also have oxidative damaging effects on erythrocyte due to hemolytic activity. This releases hemoglobin from these cells. These factors, cause deterioration of RBC membrane, which may, perhaps, be the reason of their breakdown [53].

In present findings MCHC were decreased while MCH and MCV increased. The MCHC is for haemoglobin concentration in blood and MCH is for haemoglobin concentration in each cell [54]. An increased in MCV and a decrease in MCHC

reflects reduced osmotic fragility of membrane. Mean cell volume (MCV) is volume or size of a red blood cell. Iron deficiency causes low MCV [55]. When MCV is high it means RBC is of larger size.

Anemia also causes an increase in MCH [56]. Mean corpuscular hemoglobin concentration (MCHC) is also average concentration of hemoglobin inside a single red blood cell. MCV is size of red blood cells while MCH and MCHC are for concentration of hemoglobin. A low MCHC reflects iron deficiency abnormal hemoglobin synthesis. White blood cells (WBC) play a vital role in immune function. A high number of eosinophils are due to a variety of disorders [57]. The size of RBC depends on MCV. High MCV mean larger RBC (macrocyte) and low MCV is for smaller RBC (microcytes). Normal RBCs normocytic. Anemia is categories on these classes of RBC size. A significant reduction in MCV might be due to its utilization in iron uptake by hemoglobin. Hemolysis increases free hemoglobin in blood which is called hemoglobinemia [58]. Low levels of RBC and hemoglobin is owed to iron deficiency or blood cell destruction which causes anemia [59]. Hematocrit (PCV) (called Packed Cell Volume (PCV) is used to diagnose anemia [57]. Decline in platelet results in lymphoma and myeloma [60-61].

There was reduction in platelets by Ethanol extracts of some specimens. Blood platelet might be changed by antiplatelet activity of antioxidants [62]. Therefore, antioxidative activity of compound might inhibit platelet function. Platelet reduction might be due to aggregation of platelets induced by ROS [63]. Platelets reduction may be beneficial because platelets reduce the blood viscosity which adds positively to blood pressure. Increase in haemoglobin concentration (MCH, MCHC) among all the haematological parameters may be due to the presence of active principles that stimulate haemopoiesis, or support in availability of iron for haemopoiesis, or agents for chelating iron are may be weakly present or completely absent in the plant extract which decreased the hemolysis of RBC [48]. The increase in haemoglobin (MCH and MCHC) causes oxygen transport to the tissues. An increase or decrease in blood attributes might be owed to free radical scavenging activity of extract, anticoagulation by extract [41]; anti-glycosylation [64]; thrombolytic potential [42] or by genotoxicity [65]. The practical application of the plant secondary

metabolites should be based upon their careful and extensive study regarding medicinal and toxicological nature.

5. CONCLUSION

Ethanol extracts of desert plants containing secondary metabolites revealed diversified results about their effects on haematological attributes. Hence a much careful and extensive study based criteria should be employed before their practical utilization.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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