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Phytochemical Screening and Haematological Evaluation of *Parkia biglobossa* and *Gongronema latifolium*

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ABSTRACT

The need to broaden raw material base of agro-allied industry coupled with the paradigm shift from the use of synthetic chemicals in food processing necessitates the search and evaluation of extinction-threatened spice plants such as *Parkia biglobosa* and *Gongronema latifolium*. Fermented seeds of *P. biglobosa* and leaves of *G. latifolium* were collected, sun-dried, ground into powder and extracted using 98% ethanol. They were phytochemically screened and administered orally to male rats at doses of 0, 200, 300 and 400mg/kg BW, respectively for 60 days. Phytochemistry of the plants showed that alkaloids, glycosides, reducing compounds and polyphenol were present in the different extracts though in varying proportions. Tannins and flavonoids were only present in the leaf extract of *G. latifolium* but completely absent in seed extract of *P. biglobosa*. Additionally, saponins, phlobatanins, anthraquinones and hydroxymethyl anthraquinones were not identified. Result on haematological parameters revealed that the administration of the extracts caused significant effects ($P < 0.05$) on all the blood parameters except the packed cell volume (PCV) that showed no significant difference ($P > 0.05$). Generally, our results indicate that the two spice plants can enhance the production of blood cells, especially at the doses of 200mg/kg and 300mg/kg BW, respectively though seed extract of *P. biglobosa* was seemingly more toxic than the leaf extract of *G. latifolium*. Implicitly, therefore, the conservation and domestication of these extinction-threatened spice plants becomes crucial given additional benefits inherent in them, their use as spices, notwithstanding.

KEY WORDS: Phytochemistry, haematology, *Parkia biglobosa*, *Gongronema latifolium*, conservation, domestication.

INTRODUCTION

It has been observed that plant foods, especially spices and vegetables contribute significantly to both local diets and ethno-medicine in developing countries, especially Nigeria [1]. The current global trend in the utilization of natural plant remedies has undoubtedly created an enormous need for database on the properties and uses of medicinal plants. This interesting paradigm shift is attributable to the widespread belief of their safety and dependability as compared to costly synthetic drugs, many of which have adverse side effects. Fortunately, Africa, indeed Nigeria is covered by large acres of land dominated by forest. Pitiably, the rate of disappearance occasioned by over-exploitation, deforestation, urbanization and industrialization is rather disturbing. Giving the high premium attached to the forest and its products, which include therapeutic precursors, nutriceuticals and raw materials for diverse industries, it becomes imperative to conserve and domesticate them. This will undoubtedly offer great array of opportunities for the sustainability of production and preservation of the products, which will add value and expand market.

P. biglobosa is an important multipurpose tree [2]. Virtually, all its parts are used therapeutically against diarrhoea, ulcers, pneumonia, burns, coughs, and jaundice [3,4]. The pulp contains higher cellulose and sucrose but less ascorbic acid than the cotyledons [5]. The seeds of *P. biglobosa* on fermentation are used in cooking stew and soup. The sweet yellow pulp contains 60% sugar when ripe and the seeds contain 30% protein as well as vitamins and minerals [2]. On the other hand, the hypoglycemic, hypolipidemic, antioxidative and anti-inflammatory properties of aqueous and ethanolic extracts of *Gongronema latifolium* have been reported [6,7,8,9]. However, [7, 10] reported its use as spice and vegetable. Various researchers revealed that it contains essential oils, saponins and pregnanes among others [9,11,12].

The need to conserve and domesticate these extinction-threatened spice plants though crucial, conscious effort to screen their bioactive compounds, which could be exploited through their identification, isolation and purification and also evaluate their effects on the haematological parameters become very pertinent.

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MATERIALS AND METHODS

Preparation of plant extract

The fermented seeds of *P. biglobossa* and leaves of *G. latifolium* were sun-dried and ground into powder with electric blender (Model: 4250 Braun, Germany). They were soaked in 98% absolute alcohol for two weeks. They were then filtered and the filtrates concentrated *in vacuo* using a rotary evaporator (Labo rota 3000 Resona, Edwards's model). Thereafter each extract was transferred into a round bottom flask and allowed to evaporate completely under pressure at 50˚C. Each extract was stored in a refrigerator until required. The concentration of the extract was gravimetrically determined [13].

Phytochemical screening of *P. biglobossa and Gonglonema latifolium*

The methods of [14, 15, 16] were used in the analyses of the various bioactive compounds in the test materials. Salkowski test was used in the analysis of Alkaloids and glycosides while saponins were identified through frothing test. The presence of phlobatanins, anthraquinones, flavonoids, tannins polyphenol and reducing compounds were also identified

Experimental animals

Sixty-four healthy matured male albino rats weighing between 120- 180 grams were used in the present study. They were housed in cages under standard laboratory conditions of temperature range of 25-29˚C and 12h light/dark cycle throughout the experimental periods. The rats were left to acclimatize for two weeks with water and feed *ad libitum*. Four experimental groups of eight rats each per extract were used for the study in 2x4 factorial experimental layouts using completely randomized design. Group 1 rats served as control and received 1ml of normal saline and normal chow. Rats in group 2 received 200 mg/kg BW while rats in groups 3 and 4 received 300mg/kg BW and 400 mg/kg BW of each extract, respectively for 60 days through oral gavage. Blood samples from the rats in the treatment groups and controls were collected through cardiac puncture after chloroform anaesthesia. This was then used for the analyses.

Estimation of haematological parameters

Blood samples from the different groups were diluted to 1:200 with Hayem's fluid for the preservation the corpuscles. The method of [17] was adopted in the estimation of red blood cell count while white blood cell count was done according to the methods of [18]. The method (using Sahli's haemoglobinometer) was employed for estimation of hemoglobin (Hb) content of the blood while packed cell volume (PCV) was done using the macro-haematocrit method of [17]. Differential white blood cell count was carried out using Leishman's stain. Additionally, the computations of absolute values were done as follows:

Mean corpuscular volume (MCV):
MCV (millimicron) = PCV% x 10 / RBC count (x million per mm3)

Mean corpuscular hemoglobin (MCH):
MCH (picogram) =Hb g/dl x 10 / RBC count (x million per mm3)

Mean corpuscular hemoglobin concentration (MCHC):
(MCHC picogram) = Hb g/dl x 100 / PCV %

RESULTS

Phytochemistry of *P. boglobosa* and *G. latifolium*

The result on the bioactive compounds revealed that alkaloids and glycosides were moderately present in ethanol extract of *G. latifolium* compared to the trace presence in *P. biglobosa*. Tannins and flavonoids were present in trace amount in *G. latifolium* but however, completely absent in *P. biglobosa*. Additionally, reducing compounds were present in trace amount in both plants while polyphenols was present in appreciable amount in *G. latifolium* but rather moderately present compared to their level in *P. biglobosa*. However, other phytochemicals such as saponins, phlobatanins, anthraquinones and hydroxymethyl anthraquinones were not identified in the two extracts (Table 1).

Table 1: Bioactive components of leaf extract of *G. latifolium* and seed extract of *P. biglobossa*

Chemical constituents	seed extract of *P. biglobossa*	Leaf extract of *G. latifolium*
Alkaloids	+	++
Glycosides	+	++
Saponins	-	-
Tannins	-	+
Flavonoids	-	+
Reducing compounds	+	+
Polyphenol	+++	++
Phlobatanins	-	-
Anthraquinones	-	-
Hydroxymethyl anthraquinones	-	-

+ , trace amount
++, moderate amount
+++, appreciable amount
-, complete absence

Haematological parameters

The effect of *P. boglobosa* and *G. latifolium* extracts on haematological parameters showed that there were significant differences ($P < 0.05$) in the treated rats (Table 1) except in the packed cell volume (PCV) that showed no significant difference ($P > 0.05$). The result revealed that rats treated with *P. biglobosa* extract caused the reduction of platelets as the concentration of the extract increased while it increased when the rats were treated with *G. latifolium* extract at 200mg/Kg and 300mg/Kg but however, reduced in rats administered with 400mg/kg of the extract. For the WBC, there was increase, especially when rats were treated with 400mg/kg of *P. biglobosa* extract but rats treated with 200mg/kg and 300mg/kg of *G. latifolium* extract had increase in white blood cells. The red blood cells (RBC) and haemoglobin concentration (Hb) increased with increase in the concentration of the extracts, the spice plant notwithstanding.

Results obtained for neutrophils of the treated rats showed that *P. biglobosa* extract caused its increase but at 400mg/kg the level of neutrophils declined while *G. latifolium* extract reduced it in a dose-dependent manner. The reverse was the case for lymphocytes as they reduced on treating rats with *P. biglobosa* extract but increased when *G. latifolium* extract was administered to the rats. However, result obtained on the basophils showed that when rats were treated with *P. biglobosa* extract there was significant increased ($P < 0.05$), which turned nil on treating with 300mg/kg and 400mg/kg of the extract. *G. latifolium* extract treatment caused a significant reduction (Table 1).

The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) after the treatment of rats with *P. biglobosa* and *G. latifolium* extracts showed significant differences ($P < 0.001$). The result revealed that these parameters increased in a dose-dependent fashion but the highest dose seems to reduce the level (Figs. 1-3). Generally, however, all the results obtained showed that the drugs were more effective when 200mg/kg and 300mg/kg were administered to the rats, though there were couples of deviations.

Table 2 : Effect of *P. biglobossa* (Dawa dawa) and *G. latifolium* (Utazi) extracts on haematological parameters in albino rats

Parameters	*P. biglobossa* (mg/kg)				*G. latifolium* (mg/kg)			
	0	**200**	**300**	**400**	**0**	**200**	**300**	**400**
PCV (%)	40.18a±0.71	39.28a±0.42	37.85a±0.6	30.48a±1.13	40.18a±0.71	30.8a±0.47	38.70a±0.52	45.20a±2.13
Hb (g/dl)	11.6d±0.44	11.8d±0.98	12.7f±0.17	8.68b±0.29	11.6d±0.44	12.05de±0.44	10.95c±0.44	7.73a±0.52
RBC (10^9/L)	6.70d±0.08	6.81e±0.1	7.74f±0.32	4.55b±0.1	6.70d±0.08	6.60d±0.15	6.21c±0.15	3.82a±0.31
WBC (10^7/L)	14.60a±0.49	19.9d±0.61	20.8e±0.37	26.2f±3.75	14.6a±0.49	17.05b±0.34	18.8c±0.60	19.9d±0.35
Platelets (10^9L^{-1})	1000.0f±3.16	874.25e±9.6	818.25d±8.05	607.25a±2.6	1000.0f±3.16	1186.25g±17.2	1583.0h±25.3	709.75b±16.07
Neutrophils (%)	11.0a±1.41	33.25e±3.59	44.25g±3.0	29.75c±1.70	11.0a±1.41	31.25d±1.5	29.0c±1.83	19.75b±1.70
Lymphocytes (%)	87.25h±2.5	65.75e±5.91	61.5c±1.29	49.5a±1.29	87.25h±2.5	63.25d±6.63	68.75f±7.11	77.75g±2.75
Monocytes (%)	2.50a±0.58	4.75b±0.95	5.0b±0.82	5.50bc±1.29	2.50a±0.58	3.0a±0.82	5.25b±0.95	6.0c±0.82
Eosinophils (%)	1.25a±0.5	1.25a±0.5	2.25b±0.5	2.50bc±0.58	1.25a±0.5	2.0b±0.82	1.25a±0.5	1.50a±0.58
Basophils (%)	0.75±0.01	0.56b±0.02	0.00a±0.0	0.00a±0.0	0.75bc±0.01	0.45b±0.5	0.00a±0.0	0.00a±0.0

* Means followed with the same letter along horizontal array indicate no significant difference ($P > 0.05$) from each other
PCV = Packed cell volume; Hb= Haemoglobin concentration; RBC = Red blood cell; WBC = White blood cell

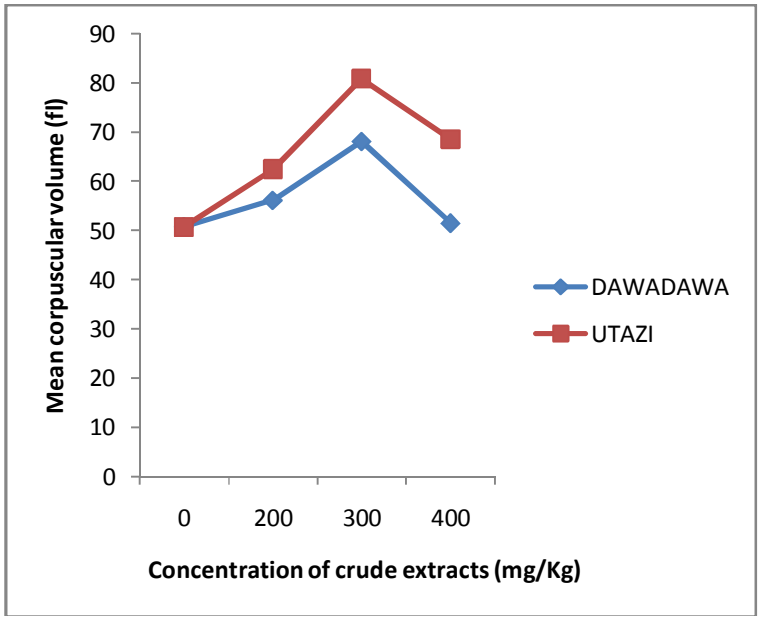

Fig.1 : Effect of crude extracts of two spice plants on the mean corpuscular volume

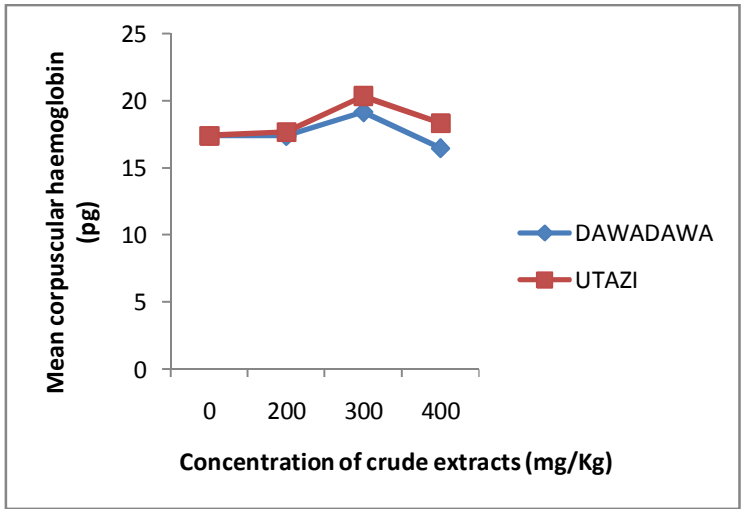

Fig.2 : Effect of crude extracts of two spice plants on the mean corpuscular haemoglobin

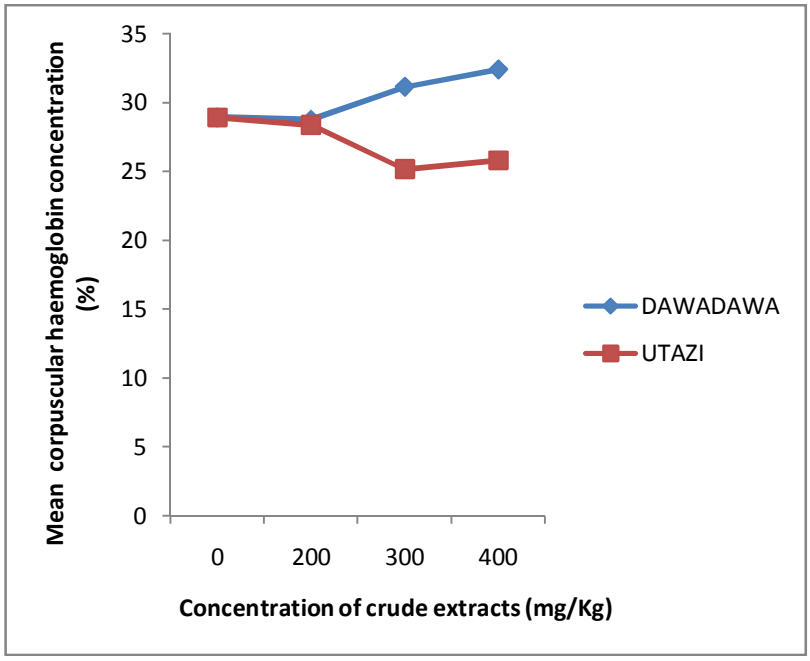

Fig. 3: Effect of crude extracts of two spice plants on the mean corpuscular haemoglobin concentration

DISCUSSION

Plants are known to contain a variety of secondary metabolites. These secondary metabolites or bioactive compounds produce definite physiological actions on the human system. According to [19], approximately 25 percent of all prescribed medicines today are substances derived from plants. Interestingly, many of these phytochemicals have been discovered and even isolated from a variety of medicinal plants. Regrettably, however, not many of them have been exploited for clinical use [20].

It is obvious from our phytochemical results (Table 1) that the ethanolic seed extract of *P. biglobossa* and leaf extract of *G. latifolium* contain similar bioactive compounds though in varying proportions except flavonoids and tannins that were completely absent in the seed extract of *P. biglobosa*. This result corroborates the report of [21] on the leaf meal of *G. latifolium* and [22] on the leaf extract of *P. biglobosa*. It is important to comment that the presence or absence of any particular bioactive compounds is fundamentally depends on the solvent of extraction and the plant part used for the extraction. Conversely, [23, 24, 25] and reported that *P. biglobosa* contains saponins, glycosides, tannins and other phenolics with trace quantity of alkaloids. [22] differed slightly, asserting that there is the absence of alkaloids. Our present is in total divergence to their submission, especially the presence of saponins and tannins and the complete absence of alkaloids.

Suspended in the blood are a number of formed elements otherwise known as blood cells – erythrocytes, leucocytes and thrombocytes. These cells importantly exist at fairly constant levels, which suggest the existence of feedback mechanism for the cells [26]. It is interesting to observe that the administration of *P. biglobosa* and *G. latifolium* extracts did not cause any significant effect ($P > 0.05$) on the packed cell volume (PCV). This according to [27] if in the affirmative, would have imply that the rats were anaemic, which inevitably could have resulted in the alteration of other physiological processes, including assimilation and utilization of nutrients.

[28] observed that saponins, which are implicated to be highly toxic when injected into the blood stream, caused haemolysis of the red blood cells and eventually destroys them. There was significant reduction in the red blood cell count (RBC) after the administration of the extracts. Surprisingly, there was complete absent of saponins in the extracts to have attributed the reduction of RBC. This therefore suggests that other bioactive compounds other than saponins might be responsible for the reduction. Suffice it to say that it is rather doubtful to assume that [28] injected purified form of saponins. Expectedly, it could be erroneous to make this assertion given that there are always synergistic interactions of these bioactive compounds present in the medicinal plants.

[29] reported that leaf extract of *G. latifolium* contains β- carotene (6.80mg/100g), Vitamins E, C and A (3.71mg/100g, 15mg/100g and 40.82mg/100g, respectively) and mineral elements, which stimulate the synthesis of

haemoglobin, thus leading to their increase in the blood. The phytochemical screening revealed a trace amount of flavonoids in the leaf extract of *G. latifolium* but was completely absent in the seed extract of *P. biglobosa*. [29] observed that the presence of phytsterols and flavonoids in the leaf extract of *G. latifolium* might possibly interfere with the process of WBC synthesis. Unexpectedly, *P. biglobosa* seed extract caused more significant increase ($P < 0.05$) in the WBC when compared with the rats treated with leaf extract of *G. latifolium* (Table 1). Though confounding and unclear the mechanism of action given the absence of flavonoids in extract of *P. biglobosa*, it is an unwavering indication that there are other intrinsic factors in these plants contributing integrally to either enhancing or impeding the biosynthetic pathways leading to their production. This might probably be the case in our present result.

[30] reported that anti-oxidant phytochemicals play a protective role on the lymphocytes and also decrease their destruction in the blood. The presence of high anti-oxidative factors in the leaf extract of *G. latifolium* [7, 21, 31,32,33] might be the underlying factors in the significant increase in haemoglobin concentration and the WBC, especially at the highest dose of 400mg/kg. The biological activities of flavonoids include action against allergies, inflammation, free radicals, hepatoxins [34]. Flavonoids implicated as anti-oxidant maintain the haem iron in its ferrous form, which obviously is associated with the production of defective methaemoglobin, thus enhancing erythropoiesis. [35] and [36] independently, commented the relationship between anti-oxidant activity and haemoglobin quality. They compared it to the action of ascorbic acid as a free radical scavenger, which increased significantly the haemoglobin level in children suffering from sickle cell anaemia. [37] attributed the effect on haematological parameters to complex formation between flavonoids and reactive metals such as iron, zinc, copper, etc., which [38] suggested as the remote cause of increase in haemoglobin synthesis and erythropoiesis.

Glycosides have anti-inflammatory property and thus have vital effect on inflammatory processes of some pathological states [10]. This was reported to exert significant effect on the WBC. Contrary to our result, [39] reported little or no effect of *G. latifolium* leaf extract on the RBC and the haemoglobin content of the blood but caused an increase in total WBC. This they attributed to the probable increase in monocytes and eosinophils in the blood. Our result corroborates with [39] at this point. Results obtained in this study for monocytes and eosinophils were significantly higher ($P < 0.05$) in the test groups compared to control, which interestingly agrees with the report of [26]. The treatment of the rats with extracts of *P. biglobosa* and *G. latifolium* caused significant reduction on the basophils though their function are yet unclear, probably helping in the defense of the integrity of the body. For the platelets, seed extract of *P. biglobosa* caused their reduction while the leaf extract increased their level, especially when 200mg/kg and 300mg/kg were administered to rats. Increasing the dose of *G. latifolium* extract to 400mg/kg caused significant reduction in their production, implying that it might be toxic at the concentration. It should be understood that seeds of *P. biglobosa* might still contain anti-nutritive factors though fermented. It may not be out of place therefore to attribute these differentials in effects to the possible residual of these factors in the rat. According to [27] the differential actions could be blamed partly on the variants of bioactive compounds and partly on their proportion received by the treated rats. Result on MCV, MCH and MCHC differed significantly, which did not agree with the report of [39].

Conclusion

Generally, our results indicate that the two spice plants can enhance the production of blood cells, especially at the doses of 200mg/kg and 300mg/kg, respectively though seed extract of *P. biglobosa* was seemingly more toxic than the leaf extract of *G. latifolium*. Implicitly, therefore, the conservation and domestication of these extinctions-threatened spice plants becomes crucial given the additional benefits inherent in them, their use as spices, notwithstanding.

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