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HAEMATIC AND HEPATOPROTECTIVE POTENTIALS OF *HYPOESTES TRIFLORA* AQUEOUS LEAF EXTRACT IN GUINEA-PIGS

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ABSTRACT: Local traditional healers in the Great Lakes Region used to administer *Hypoestes triflora* (Roem) leaf decoctions to treat patients with anemia and liver diseases, and also to boost immunity in HIV patients. This study was designed to evaluate, in guinea-pigs, the antianemic and hepatoprotective potentials of this plant. Anemia was induced with phenylhydrazine hydrochloride and hepatotoxicity with paracetamol. Animals were divided into normal (N), control (C), test (T), and reference (R) groups. T group included ill-induced animals treated with *H. triflora* aqueous extracts and R ill-induced animals treated with an Iron solution for anemic animals or silymarin for hepatitis animals. Dosing was made as 1-day single dose or 7-days repeated dose. In anemic animals, the production rate of RBC was significantly ($p<0.001$) higher in the T group as compared to the C group. In paracetamol-induced hepatitis animals, the plant extract exhibited about 80-90% protective effect in T group as compared to the C group. The effects were comparable to or higher than R responses. *H. triflora* leaf extracts have both haematic and hepatoprotective potentials lending credence to its use by traditional healers to manage anemia, and hepatic disorders. The antianemic effect may support in part its use in HIV patients mostly affected in blood components deficiency.

INTRODUCTION: For decades, plants have been used as a primary source of medications to manage all kind of diseases, from single headache to deadly microbial infections and metabolic diseases, or as antipoison, and the practice is being firmly-rooted in Africa and Asia where more than 80% of people still rely on plants for all their health problems as they have limited economic access to modern drugs [1-4].

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A survey conducted throughout the South-Kivu province of the Democratic Republic of Congo (DRC) showed that aqueous extracts of *Hypoestes triflora*, locally called mageru, magara or mpindulo in Mashi, or pindula in Swahili, are administered by traditional healers to treat anemia, liver disorders, heart palpitations, amoebic dysentery and gastralgia, snakebites, food poisoning, and also to strengthen the immune system in people affected by HIV/AIDS.

Here, we are assessing, in an animal experiment, the ethnopharmacological claimed activities, before planning its standardization and use as improved traditional herbal medicine, particularly for antianemic and hepatoprotective effects. A few studies have been reported in the literature only on

the chemical composition **Fig. 1**, and its antihepatitis activity and data are conflicting [5, 6].

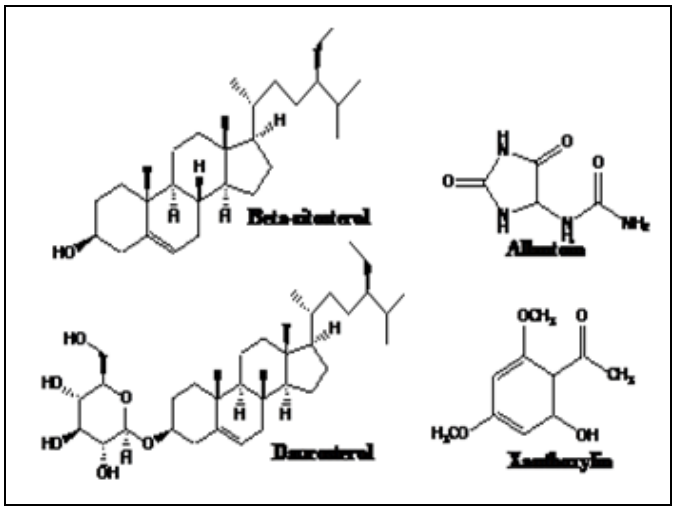

FIG. 1: CHEMICAL STRUCTURE OF COMPONENTS FOUND IN *H.TRIFLORA*

Anemic conditions and liver diseases are real public health problems, affecting all countries, whether developed or developing. According to the World Health Organization (WHO), anemia affects people of all ages, although the people at greater risk are the elderly, young women of child-bearing age and the infants. Globally, anemia affects 1.62 billion people (95% CI: 1.50–1.74 billion), which corresponds to 24.8% of the population (95% CI: 22.9–26.7%). The highest prevalence is in preschool-age children (47.4%, 95% CI: 45.7–49.1), and the lowest prevalence is in men (12.7%, 95% CI: 8.6–16.9%) [7, 8]. On the other hand, two billion people are infected with hepatitis B with 400 million chronic carriers including 60 million in Africa against 9 million people infected with hepatitis C or 1% of the European population [9].

In the Great Lake Region of Africa, a number of plants, like *Justicia flava*, *Manihot esculenta*, *Bidens pilosa*, *Hypoestes triflora*, are used by many people to manage anemic conditions [10]. Besides, the use of plant extracts for the treatment of toxic or viral-induced hepatic diseases is largely anchored in traditional medicines. Among a large range of plants used, we quote *Aspilia africana*, *Bidens pilosa*, *Hypoestes triflora*, *Anogeissus leiocarpus*, *Terminalia macroptera*, *C. vitelinum*, *Silybum marianum*, *Cynara scolymus*, and *Combretum micranthum* to name a few [11, 12]. Some of them have been studied and are now used in the manufacture of hepatoprotective drugs like *Silybum marianum* (Legalon^{R}) and *Combretum micranthum* (Hepatisane^{R}) [13, 14]. *Hypoestes* is a flowering plant genus with many dozens of species widely distributed throughout the tropical and subtropical lands around the Indian Ocean, and some adjacent regions.

The species belongs to the subfamily Acanthoideae of the acanthus family Acanthaceae, order Lamiales, class Asteropsida, sub-branch Rosophytina, and the branch of Bidens pilosa together with *Magnoliophyta*, *Thumbergia alata*, *Acanthus pubescens*, *Justicia flava*, and *Hygrophyla auriculata*. Therein, it is classified in the sub-tribe Justiciinae of tribe Ruellieae, making it a relative of such American genera as the mosaic plants (*Fittonia*), water-willows (*Justicia*) and wright-worts (*Carlowrightia*) [15]. The plant is a highly branched herb from China, India, Nepal, and East Africa, growing up to 1 m in height. The species growing in the Eastern part of the Democratic Republic of Congo is shown in **Fig. 2**.

FIG. 2: PHOTO OF SPECIES FOUND IN BUKAVU

MATERIALS AND METHODS:

Plant Material: The plant material used consisted of the aerial part of *Hypoestes triflora*, collected during the semi-rainy period in the municipality of Bagira in Bukavu city. The specimen was identified and conserved at the department of botany in the faculty of Sciences of the University Official of Bukavu (UOB). The leaves collected were dried under shade at room temperature for two weeks and then pulverized in a traditional mortar, sieved and stored in a plastic jar.

Preparation of the Plant Aqueous Extract: Aliquot of 10 g of the crude powder were decocted in 100 ml of distilled water for 30 min and then filtered. The filtrate was evaporated to nearest

dryness on a hot plate and then completed in the oven at 50 ºC. After evaporation of the filtrate to dryness, we obtained a residue representing about 23% of the weighted powder. This allowed calculating the dose of the extract to be given to animals. The plant crude extracts were reconstituted with distilled water and given orally to animals.

Animals: Guinea pigs of both sexes aged 4 to 6 months old and weighing 350 to 550 g were used. There were kept in the animal boundary of the Faculty of Medicine and Pharmacy UOB, prepared and handled according to the standards required for an experiment on laboratory animals [16].

Induction of Anemia and Treatment of Animals: Animals were divided into 4 groups, including normal (N), control (C), test (T), and reference (R) groups. N-group served to acquire baseline blood values; C-group was anemia-induced animals not treated, given only water 25ml/kg BW; T-group was anemia-induced animals treated with 50mg/kg *H. triflora* aqueous extracts, and R-group anemia-induced animals treated with Iron complex solution 50 mg/kg BW as reference medication. Anemia was induced with phenylhydrazine hydrochloride 30 mg/kg (sc) given in two consecutive days. Medications were daily administered orally for 30 days. At various days, about 1ml of blood was collected from each guinea-pig into bijou bottles with EDTA by puncturing the prominent leg vein with syringe needles. The 1 ml blood was used for haematological tests: Red Blood Count (RBC), Hemoglobin (Hb), and packed cell volume or hematocrit (PCV). Blood samples were collected before and after anemia induction.

Induction of Hepatotoxicity and Treatment of Animals: The experiment was conducted in two ways following a one-day single dose (1DSD) or 7 days repeated dose (7DRD) of extracts given before induction of hepatitis with paracetamol 500 mg/kg BW. In 1DSD groups, animals were divided into 6 sub-groups of 3 guinea-pigs each. The normal group (N) received only distilled water (25 ml/kg) and no intoxication; the control group (C) received distilled water (25ml/kg) followed by suspension of paracetamol 500 mg/kg; the test groups (T) received *H. triflora* extract 50 mg/kg or 100mg/kg before hepatitis-induction; the reference groups (R) received sylimarin Legalon^R solution 50 mg/kg or 100 mg/kg before intoxication. In 7DRD groups, we proceeded by the administration of water (N), extracts (T); and reference (R) for 7 days followed by intoxication with paracetamol 500 mg/kg the last day. After 24 h, the animals were sacrificed, blood was collected, centrifuged at 3000 rpm and the serum was used for the determination of transaminases (AST=SGOT) and (ALT=SGPT) and creatinine (SCr).

Laboratory Tests: Packed cell volume (PCV), red blood cell (RBC) count, and hemoglobin (Hb) were determined by classic laboratory methods (microhaematocrit method, Neubauer hemo-cytometer method, and cyanmethemoglobin method) using a spectrophotometer respectively [17]. SCR was measured by the Jaffe method and transaminases by Emekyn Kits in the medical laboratory at UOB teaching hospital.

Statistical Analysis: The means and standard deviations (SD) of the data were calculated. The results were analyzed by one-way analysis of variance (ANOVA) using Microsoft Excel 2010 where applicable, and the least significant difference was used to determine significant results (p-value< 0.05).

RESULTS:

Haematic Potential: The potential haematic effect of *H. triflora* in comparison to control and reference groups is shown in **Fig. 3**. Values are mean for n=3. Phenylhydrazine was given for two days. The treatment was started on Day1 after administration of the toxic. The baseline values in normal animals were 17.43 ± 0.72 g/100ml, 6.50 ± 0.06 10^6 cells, and 51.56 ± 1.31% for Hb, RBC, and PCV, respectively. For example the hemoglobin **Fig. 2a**, the administration of 50 mg/kg phenylhydrazine hydrochloride for two consecutive days caused a decrease measured on Day 3 from 17.4 g/100 ml to 7.2 g/mm^3 (58.6% reduction) in the untreated control (C-Hb), 9.6 g/100ml (44.8% reduction) in the group treated with extract (T-Hb), 9.7 g/100ml (44.8% reduction) in the group treated with Iron Complex (R-Hb). From day 7 to day 30, the regeneration of hemoglobin resumed slightly in the untreated control group (C-Hb) and significantly higher in the reference (R-Hb) and the test (T-Hb) groups.

The recovery reached 86% (15.8/17.4) of the baseline value for *H. triflora* while the control group remained in the anemic state (10.7/17.4). The profiles of PCV **Fig. 2c** and RBC count **Fig. 2b** resemble that of Hemoglobin. However, for RBC count, the capacity of regeneration is significantly higher ($p<0.001$) in T-RBC plant extract group (T-RBC) than reference Iron complex group (R-RBC).

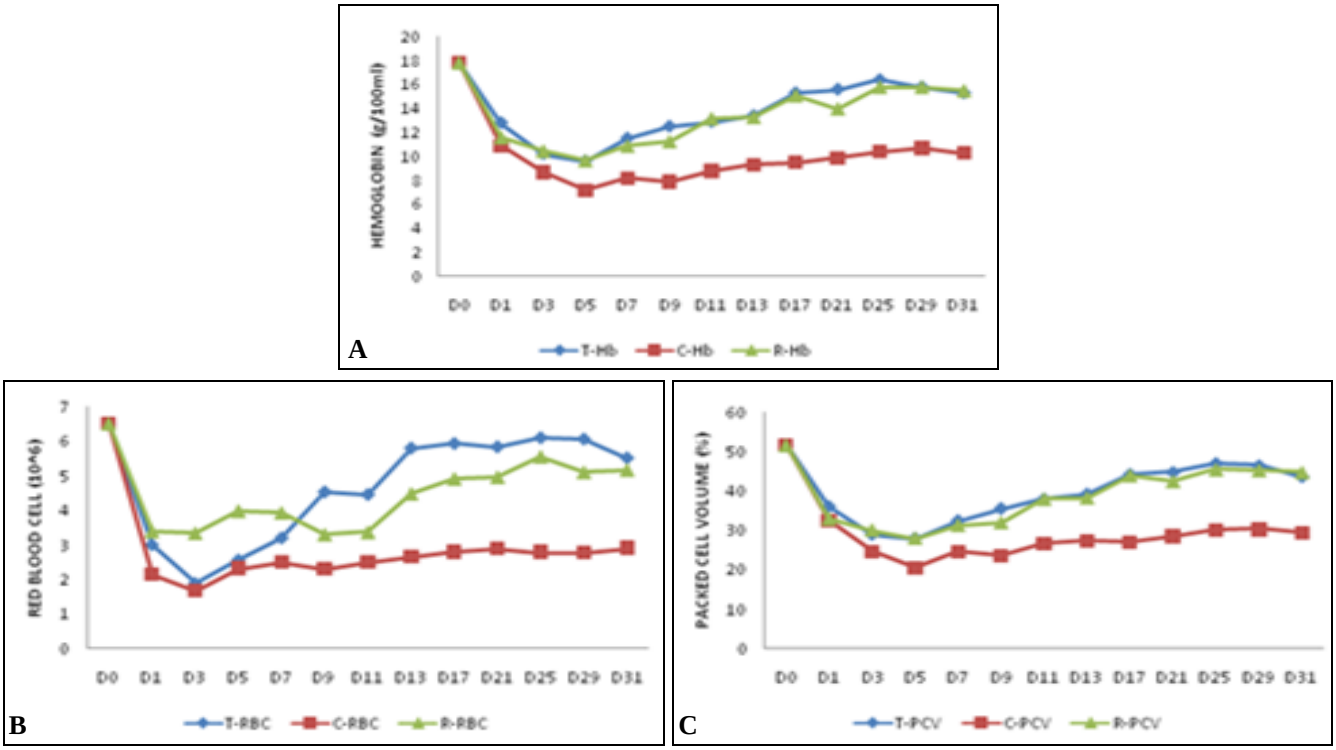

FIG. 3: HAEMATIC EFFECT OF *H. TRIFLORA EXTRACT* (T) COMPARED TO CONTROL (C) AND IRON SOLUTION (R)

Hepatoprotective Potential: The values of AST, ALT, and SCr measured in different groups for both 1DSD and 7DRD experimental groups are summarized in the **Table 1** showing mean ± SD values following intoxication with 500mg/kg paracetamol in control, test, and reference groups for 1DSD (AST^A, ALT^A) and 7DRD (AST^B, ALT^B) groups. The normal baseline values in 1DSD group averaged 19.1 ± 1.0 IU/L (19.2-22.5) for AST^A, 20.6 ± 1.8 IU/L (19.1-23.2) for ALT^A; and 0.80 ± 0.06 (0.73-0.91) mg/dL for SCr^A. The measured values for 7DRD group are slightly lower. In the control group, given at 500 mg/kg BW, paracetamol-induced hepatotoxicity monitored by increased values of AST (81.7 and 112.0IU/L) and ALT (100.1 and 96.8 IU/L) in 1DSD and 7DRD groups respectively, without much disruption of SCr (0.88 - 0.78 mg/dl). In animals protected with *H. triflora* extract 50 mg/kg or the reference Legalon^R 50 mg/kg, the values of AST^A (22.8; 29.3) or 31.3; 32.7) are significantly lower ($p<0.001$) compared to the positive controls (81.7). That gives the potential protective effect of *H. triflora* as about 82.1% (22.8/81.7) for AST^A, 80.7% (29.3/110.1) for ALT^A, while the reference Legalon (silymarin) assured 61.7% (31.3/81.7) for AST^A and 67.3% (32.7/81.7) for ALT^A respectively. The values of SCr are almost unchanged.

TABLE 1: LEVELS OF TRANSAMINASES AND CREATININE IN BLOOD

	Normal	**Positive**	***H. triflora extract* dose**		**Silymarin dose**	
Biomarker	Baseline	Control	50mg/kg	100mg/kg	50mg/kg	100mg/kg
AST^A UI/l*	19.1±1.0	81.7±5.4	22.8±2.8	15.2±3.5	31.3±3.9	23.4±3.7
ALT^A UI/l*	20.6±1.8	100.1±14.1	29.3±2.4	16.5±8.1	32.7±4.9	25.0±3.5
AST^B UI/l*	16.5±2.8	112.0±8.6	9.0±1.0	1.6±0.7	12.9±2.2	2.1±0.3
ALT^B UI/l*	18.4±1.8	96.8±7.3	12.6±1.6	4.8±1.1	15.6±1.7	4.1±1.4
SCr^A mg/dl	0.80±0.06	0.88±0.19	0.82±0.10	0.83±0.07	0.85±0.03	0.78±0.03
SCr^B mg/dl	0.72±0.03	0.78±0.10	0.73±0.09	0.89±0.08	0.63±0.06	1.05±0.07

When the animals received 50mg/kg dose repeated during 7 days, the values of transaminases AST[B] and ALT[B] fell down the normal baseline values for both the test extract and reference Legalon. The differences are better illustrated in Figure-4 expressed in percent increase or decrease from the respective baselines. *H. triflora* extract *(HT) and silymarin* (Legalon; LG) were given at 50, and 100 mg/kg BW; ASTa and ALTa correspond to the 1DSD group; ASTb and ALTb correspond to the 7DRD group. The percent change is calculated as 100(V2-V1)/V1 where V1 is baseline mean value in normal healthy animals and V2 the value obtained in treated ill-animals. For example, at 50mg/kg, the elevation of ASTa and ALTa values are about 19% (22.8/19.1) and 42% (29.3/20.6) high, while the values of ASTb and ALTb are reduced about -45% (9/16.5) and -31% (12.6/18.4) from baseline values. This also happened with one single dose of 100mg/kg of *H. triflora* extract.

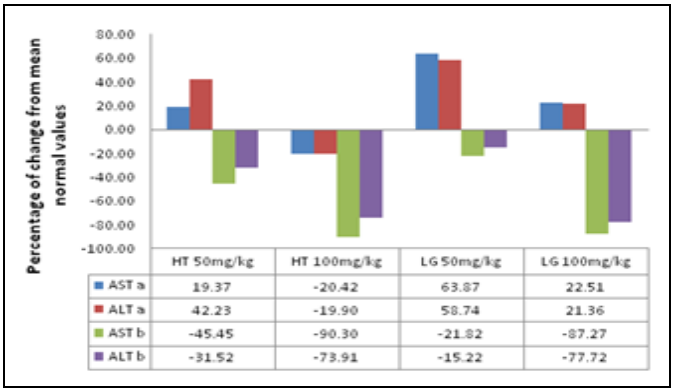

FIG. 4 HEPATIC BIOMARKERS CHANGE (HT = *H. TRIFLORA*; LG = LEGALON)

DISCUSSION:

Haematic or Antianemic Effect: Traditionally, anemia is one of the numerous ailments claimed to have been successfully treated with plant materials by traditional medicine practitioners, regardless of anemia type**.** It is well established that human being consumes a wide variety of local crops and vegetables, which contribute significantly to the improvement of human health in terms of disease prevention and therapy.

Many researchers have earlier used phenylhydrazine hydrochloride to induce anemia in rats and rabbits to measure the antianemic activity of various [18-21]. In those studies, anemia was observed after 3 to 6 days with subcutaneous injection of 30mg/kg BW.

In our study using guinea-pigs, anemia was evidenced in 3 days after injection of phenylhydrazine 30 mg/kg BW with a 61% reduction in RBC, 38% reduction in PCV, and 36% reduction in Hb concentration. The treatment of anemia-induced animals with aqueous leaf extract of *H. triflora* significantly stimulated the production of hemoglobin and regeneration of blood red cells as compared to untreated animals. A comparable effect was obtained in the animals treated given Iron solution. However, the plant extract exhibited a significantly higher efficacy in RBC than Iron salt, as shown in **Fig. 2b.** This may suggest that the plant has another mechanism of action alongside the presence of iron content.

As stated in the introduction section, local traditional healers use decoctions of this plant in HIV/AIDS patients. The haematic potential found in our experiment may support this use as it is known that anemia stands for one of the major complications in HIV/AIDS persons. At this stage, nothing allows us to rule out the possible direct antiretroviral activity in this plant.

Hepatoprotective Effect: Carbon tetrachloride and paracetamol (acetaminophen) are frequently used to induce hepatotoxicity *in-vivo* [2, 22-24]. We selected, in this study, paracetamol model since it is the most likely situation to occur in our environment where paracetamol rises as the first Over-The-Counter medication. The experiment showed that a single dose of 500 mg/kg was sufficient to disrupt liver function without affecting renal function, but with 1000 mg/kg, the renal function was also impaired. Thus, we fixed 500 mg/kg to minimize the impact of renal dysfunction.

When *H. triflora* extract was administered as a single 50 mg/kg BW, we obtained a significant reduction ($p<0.01$) in AST and ALT elevation among the intoxicated animals, compared with control untreated animals. The same profile was also observed with the reference drug Legalon[R] containing silymarin, giving a reduction between 61-82%. However, after administration of a single dose of 100mg/kg or 7 repeated 50mg/kg doses, the values of transaminases fell under the normal baseline values (e.g. from 17 to 9 IU/L for ALT). Normally, toxic substances increase transaminases while hepatoprotective substances would prevent it

[2]. But, when a substance inhibits liver metabolism, the capacity of the liver to produce transaminases may be affected as a result of the toxic effect. That means the safe therapeutic dosing of *H. triflora* or Silymarin should be taken less than 100 mg/kg once daily or less than 50 mg/kg in repeated doses. This drives attention to fixing actual safe regimens in human during the standardization phase.

Possible Active Compounds: In the present study, we did not search for the chemical composition of the plant. However, when aerial organs (leaves, stems) are soaked in water for about an hour, red color is released due to its rich composition in polyphenolic compounds and water-soluble minerals. Some screening done on Congolese species signaled the presence of zinc, manganese, iron, vitamin C, phenolic glycosides, irridoïdes, alkaloids, and diterpenes as main groups [11]. One previous study in Rwanda reported that premedication with water extract of the leaves prevented the prolongation of the barbiturate sleeping time associated with carbon tetrachloride-induced liver damage in mice, and the compound responsible for that hepatoprotective activity was presented as benzoic acid [6]. Another study from China isolated and identified six compounds from the acetone extract of aerial part, namely allantoin, beta-sitosterol, daucosterol, octadecanoic acid, xanthoxylin, and potassium nitrate based on their spectral properties as well as X-ray crystallographic analysis **Fig. 1** [5]. This study on Chinese species did not signal the presence of benzoic acid. The hepatoprotective active molecule like sylimarin has not been detected in *H. triflora* composition in the published papers. Nevertheless, some reported components may sustain evidence of some ethnopharmacological claimed activities.

The presence of polyphenolic flavonoids and eventually other new chemicals yet not identified could be responsible for the multiple pharmacological properties of this plant. The differences in antianemic potentials of plant extracts might be due to different present phytochemicals, particularly flavonoids that protect cells as powerful antioxidants in preventing or repairing the damage done to red cells by free radicals or highly reactive oxygen species. It has been reported that phenylhydrazine causes oxidative damage to red cells by increasing the formation of reactive oxygen species [25, 26]. The presence of minerals like zinc, iron could also contribute.

β-Sitosterol is one of several phytosterols with characteristic odor found in *Nigella sativa*, *Serenoa repens* (saw palmetto), *Pygeum africanum*, sea-buckthorn, wolfberries, *Mirabilis jalapa*, *Cannabis sativa*, *Urtica dioica* [27]. Alone or in combination with similar phytosterols, β-sitosterol reduces blood levels of cholesterol and is sometimes used in treating hypercholesterolemia. In Europe, β-sitosterol is used in herbal therapy, especially for benign prostatic hyperplasia [28]. Another study showed that Daucosterol, a beta-sitosterol glycoside is an immunomodulator and protected mice against disseminated candidiasis by the CD4+Th1 immune response [29].

It has been found that xanthoxylin, isolated from *xanthoxilum bungeanum*, increases melanin production, several dendrites, tyrosinase, and microphthalmia-associated transcription factor (MITF) expression in cultured B16F10 cells and also has a significant inhibitory effect on platelet aggregation [30-32].

Allantoin is an active ingredient in over-the-counter cosmetics as the moisturizing and keratolytic effect, promoting cell proliferation and wound healing [33].

CONCLUSION: Ethnopharmacological information gathered from traditional healers indicated that aqueous extracts of *Hypoestes triflora* are used to treat liver disorders, heart palpitations, anemia, amoebic dysentery and gastralgia, snakebites, food poisoning, and also to strengthen the immune system in people affected by HIV/AIDS. This study showed that *H. triflora* leaf extracts have antianemic and hepatoprotective potentials lending credence to traditional healers' claims. Still more studies are needed to determine the active compounds and how they actually work.

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CONFLICT OF INTEREST: Nil

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