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Ethanolic Extract of Ground *Vernonia Amygdalina* Stem Exhibited Potent Antibacterial Activity and Improved Hematological Bio-Functional Parameters in Normal and Monosodium Glutamate-Intoxicated Rats

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ABSTRACT: Herein, *Vernonia amygdalina* stem ethanolic extract, VASEE, was elucidated for its antibacterial activity *in vitro* by disk-diffusion technique and, *in vivo* effect on some hematological parameters of normal and monosodium glutamate, MSG-intoxicated rats. For *in vivo study*, 20 male albino rats assigned into five groups (A, B, C, D, and E) were, for 14 days, respectively administered MSG (8000 mg/kg body weight, bw), VASEE (200 mg/kg bw), Control (Vital feed and tap water), MSG (8000 mg/kg bw + VASEE, 200 mg/kg bw), and MSG (8000 mg/kg bw + VASEE, 400 mg/kg bw). *In vitro*, the VASEE-related activity against the tested bacterial pathogens was significant ($P < 0.05$), dose dependent and comparable to that by the standard antibacterial drug, Ciprofloxacin. *In* vivo, VASEE compared to control and MSG groups improved ($P < 0.05$) and, notably at the highest tested dose modulated the monosodium glutamate intoxication-related effect on, the studied hematology of rats. Thus, VASEE exhibited potent activity against the tested bacterial pathogens, improved, and potentially modulated MSG-intoxication-related effect on, the rats' hematological functions. The study underscored a promising antibacterial application of the extract of hitherto wasted bitter leaf stem that could offer novel therapeutic benefits on the hematology of especially MSG-intoxicated rats, warranting further studies.

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In Nigeria, bitter leaf (*Vernonia amygdalina)* stem is not considered edible and is rarely used except for replanting, hence may add to the increasing sources of municipal solid waste. And, as the bitter leaf stem is not readily biodegradable, it could pose waste disposal problem and attendant environmental and health risk (Egbuonu, *et al.*, 2015; Egbuonu, 2015a,b,c,d; Egbuonu and Osuji, 2016; Egbuonu, *et al.*, 2016). Studies to enhance the utilization of such solid waste especially for novel bioactivity against chemical toxicants usually and intentionally added to foods are therefore warranted (Makris, 2007). *Vernonia amygdalina*, commonly called bitter leaf, is a medium sized shrub grown in the tropics (Achuba, 2018). And, among the Nigerian population it has different common names, including *Onugbu* (in Igbo). The leaves, that commonly serve as vegetable for traditional soup delicacy, are with bioactivities useful in ethno-medication against varied ailments including malaria, diabetes, cancer, infertility and ailments related to microbes and impaired hematological properties owing to a variety of flavonoid and bitter sesquiterpene lactones contents (Kadir, *et al.*, 2018; Achuba, 2018). Monosodium glutamate (MSG) is a common food addictive but with safety issues (Wijayasekara and Wansapala, 2017; Zeratsky, 2020; Veni, 2010). And, it could be abused inadvertently warranting the need for antidotes, including any readily available indigenous plant-part-waste-sourced material. Recent studies indicated possibility of plants and plant-parts-based antidotes for chemical toxicity in animals (Achuba, 2018; Okpoghono, *et al.*, 2018), scientific basis for ethno-medicinal uses (Egbuonu, 2015) and even improved keeping quality of plants-based antidotes (Egbuonu, *et al.*, 2014a; Egbuonu, *et al.*, 2014b). Earlier, extract of *Vernonia amygdalina* leaf elicited antibacterial activity against many pathogens (Cos *et al.*, 2002) and increased packed cell volume, neutrophil and white blood cells count with consequent hemolytic reduction (Kola, 2007; Kadir, *et al.*, 2018). Also, several research reports established the safety of bitter leaf administration either alone or together with known toxicants (Kadiri, 2017; Abebe and Gebru, 2015; Lolodi and Eriyamremu, 2013). However, antibacterial activity of *Vernonia amygdalina* stem extract or its influence on the hematology of MSG-burdened animals, to the authors' knowledge, has not been reported. Dietary

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intervention even from hitherto plant part waste including the *Vernonia amygdalina* stem could be relevant source for toxic chemical antidote (Ezeanyika and Egbuonu, 2011). Hence, the present study was undertaken with the aim to determine the antibacterial activity of bitter leaf (*Vernonia amygdalina*) stem extract and the effect of the stem extract on some hematological parameters of normal and monosodium glutamate-intoxicated rats. The objectives for achieving the stated study aim included determination of the (a) activity (expressed as the mean diameter of inhibition zones, mm) of ground Vernonia amygdalina stem ethanolic extract, VASEE, on *Escherica coli* (*E. coli*), *Neisseria gonorrhoeae* (*N. gonorrhoeae*), *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeroginosa* (*P. aeroginosa*), *Salmonella typhi* (*S. typhi*) and *Streptococcus pneumonia* (*S. pneumonia* pathogens and (b) effect of ground Vernonia amygdalina stem extract, VASEE on packed cell volume (PCV), hemoglobin (HB) and red blood cell (RBC) contents of normal and monosodium glutamate-intoxicated rats. In a recent similar study, bitter leaf stem extract exhibited potent antifungal activity without altering serum electrolytes in MSG-intoxicated rats' (Nwankwo, *et al.*, 2019). In particular, changes in hematological properties highly predicted animal toxicity (Chike, *et al.*, 2018) and hematological parameters, including red blood cells (RBC) count, hemoglobin (HB) concentration and packed cell volume (PCV), determined herein served as important indices of physiological and pathological status in animal studies (Iwueke, *et al.*, 2018; Egbuonu, *et al.*, 2019).

MATERIALS AND METHODS

Materials: Monosodium glutamate, MSG, (99.9 %) was purchased from *Ubani* foodstuff market in Umuahia, Abia state. Nigeria. All the chemicals, including absolute ethanol (99 %), picric acid and chloroform, used were of analytical grade and products of Sigma Aldrich, St. Louis, USA.

Fresh stems of bitter leaf (*Vernonia* amygdalina) were collected in the month of May, 2015 from locals in Ikwuano Local Government Area, Abia state, Nigeria and authenticated as that of biter leaf (*Vernonia* amygdalina) by a Taxonomist in the Department of Plant Science and Biotechnology, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike.

A total of 20 adult male Wistar rats which weighed between 120 g – 160 g were procured from the animal house of the Department of Zoology, University of Nigeria, Nsukka. The animals were harbored in the animal housing unit of the Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, in clean metabolic cages at room temperature and under standard laboratory condition of 12 hour light/dark cycle. The rats had free access to pellet feed (Vital Feed) and clean tap water during acclimatization for two weeks.

*Methodology***:** The bitter leaf stems were rinsed with clean water, cut into smaller pieces and air dried for a period of one week. The dried stems were ground using a pulverizing machine in the Department of Soil Science, National Root Crops Research Institute, Umudike. From the obtained ground bitter leaf stem, 400 g of the powder was soaked in 300 ml of absolute ethanol for a period of 48 hours. The resulting mixture was then filtered using the Whattman's filter paper. The filtrate obtained in a beaker of known weight was placed on a water bath at temperature of 40 °C. After evaporation of the ethanol, approximately 80 g thick brown semisolid paste of the extract (1.5 g yield) was obtained. Refrigeration of extract was maintained at 2 – 8 °C until used.

Escherica coli (*E. coli*), *Neisseria gonorrhoeae* (*N. gonorrhoeae*), *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeroginosa* (*P. aeroginosa*), *Salmonella typhi* (*S. typhi*) and *Streptococcus pneumonia* (*S. pneumonia*) used in this study were clinical isolates obtained from the Department of Pharmaceutical Microbiology Laboratory, College of Health Sciences, University of Nigeria teaching Hospital (UNTH) Enugu, Nigeria. They were characterized with respect to their morphological, biochemical and antibiotic susceptibility. Antibacterial activity of different VASEE concentrations in 10 mm discs was tested by the disk-diffusion technique as in Kigigha and Atuzie (2010). In brief, antibiotic discs (10 mm) were prepared from Whattman No. 1 filter paper using cock borer and autoclaved at 0 ^{o}C for sterility. Subsequently, the discs were respectively impregnated with varying concentrations of the VASEE (125, 250, 500, 1000 and 2000 mg/ml) or 10 mg/ml of Ciprofloxacin control, and (in triplicates) placed on well-spaced separate nutrient agar plates to avoid overlapping of the zones of inhibition. The plates were then incubated at 37 °C for 72 h. The antibacterial activity was expressed as the mean diameter of inhibition zones (mm).

Experimental animal design: After acclimatization, the rats were weighed and randomly assigned to five groups, A,B,C,D and E that respectively received MSG only, the extract, VASEE, only, feed and clean tap water as others, MSG plus low dose (200 mg/kg bw) of the extract, VASEE and MSG plus high dose

(400 mg/kg bw) of the extract, VASEE. Accurately weighed quantity of hydro-alcoholic (98 % ethanol) extract of *V. amygdalina* stem, VASEE, was suspended in distilled water and was administered orally to the experimental animals.

Freshly prepared MSG solution (using distilled water as solvent) was administered orally with the aid of a gavage according to previous studies (Mariyamma, *et al.*, 2009; Egbuonu and oriji, 2017; Egbuonu and Ekwuribe, 2017; Mbah and Egbuonu, 2017a,b; Egbuonu and Ejike, 2017; Mbah, *et al.*, 2018; Mbah, *et al.*, 2019; Anuforo, *et al.*, 2020; Obidike and Egbuonu, 2020). Treatment was by daily intubation and lasted for 14 days. The animal study was carried out following approval from the departmental ethical committee which was in strict adherence to the National research council guide for the care and use of laboratory animals (National Research Council, NRC, 2011).

All the rats in the different groups were sacrificed by mild stunning following overnight fast after 14 days. Thereafter, the respective rat was dissected and blood collected from the heart. As described earlier (Egbuonu and Oparah, 2017), the red blood cell count was determined by haemocytometric method which involved diluting 1:200 blood specimen with RBC diluting fluid (Sodium Citrate) and counting the red blood cells under high power (40×) objective by using a counting chamber. The number of cells was calculated and reported as the concentration of red blood cells of whole blood ($\times 10^{12}$/L).

The hemoglobin concentration was determined using cyanomethaglobin method as described by Ochei and Kolhatkar (2008). This was based on the principle that hemoglobin when mixed with Drabkin's solution (that contains potassium ferricyanide, potassium cyanide and potassium dihydrogen phosphate) the ferricyanide form methaemoglobin is converted to cyanmethaemoglobin by the cyanide and the cyanmethaemoglobin produces a colour which is measured colorimetrically in g/dl. The packed cell volume, PCV was estimated as described by Ochei and Kolhatkar (2008). Blood sample was taken with a capillary tube, cleaned and sealed with plasticine. The filled tubes were placed in the microhematocrit centrifuge, spun at 12,000 rotor *pe*r minute (rpm) for 5 minutes and placed in a specially designed scale. PCV (%) was read as percentage content in a whole blood using a hematocrit reader (Hawskey, England) and as calculated thus:

$$\text{PCV (\%)} = \frac{\text{Packed RBC column height}}{\text{Total blood volume height}} \times 100$$

Where PVC = packed cell volume,

Statistical analysis: Data were subjected to analysis of variance (ANOVA) and least significance difference (LSD) at 95 % confidence level using statistical package for social sciences, SPSS, statistical software package, version 20. Mean results for triplicate determinations of bacterial activity, and animal sample size, n = 4 rats were expressed as Mean ± Standard error of mean (SEM). Difference in mean was accepted as statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

The extract had a significant ($P<0.05$) and dose dependent activity against the tested bacterial pathogens. Maximum activity on all tested organisms was observed at 2000 mg/ml and was comparable to the respective activity elicited by the standard antibacterial drug, Ciprofloxacin (Table 1, Figure 1 and Figure 2). Results of earlier antibacterial studies of *V. amygdalina* leaf showed variable degree of antibacterial activities (Ali, *et al.*, 2019).

The result of this study conformed to reported earlier studies that ethanol extract of *V. amygdalina* leaf elicited activity against *S. aureus* (Ogundare, 2011; Zubairu, *et al.*, 2019), *E. coli* and *S. typhi* (Zubairu, *et al.*, 2019), indicating the potency also of *V. amygdalina* stem against these pathogens. The present result, however, contradicted that of Ogundare (2011) who reported inactivity of *V. amygdalina* leaf extract against *E. coli*, indicating selective potency of *V. amygdalina* stem over *V. amygdalina* leaf against *E. coli* (and apparent preference of *V. amygdalina* stem over the leaf in managing *E. coli*-related ailments).

Table 1: Activity (expressed as the mean diameter of inhibition zones, mm) of ground Vernonia amygdalina stem ethanolic extract, VASEE, on E. coli, N. gonorrhoeae, S. aureus and P. aeroginosa pathogens

Pathogen (mm)	VASEE 2000 mg/ml	VASEE 1000 mg/ml	VASEE 500 mg/ml	VASEE 250 mg/ml	VASEE 125 mg/ml	Ciplofloxacin 10 mg/ml
E. coli	50.33±0.88	24.00±0.58	11.00±0.58	5.33±0.33	1.00±0.58	68.00±1.15
N. gonorrhoeae	48.00±1.15	23.00±0.58	11.00±0.58	4.00±0.58	0.67±0.67	64.67±0.88
S. aureus	45.00±0.58	21.00±0.58	10.67±0.33	4.00±0.58	0.33±0.33	54.00±1.15
P. aeroginosa	31.67±0.88	16.00±0.58	8.00±0.58	2.67±0.33	1.33±0.33	37.33±0.88

Result Values are expressed as Mean ± standard error of mean (SEM) for sample size, n = 4 rats. Significant difference in mean was accepted at P < 0.05

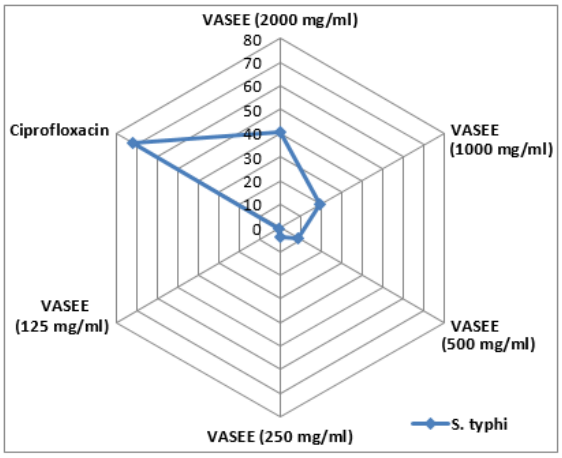

Fig 1: Activity (mm) of ground Vernonia amygdalina stem ethanolic extract, VASEE, on S. typhi pathogen. *Result Values are expressed as Mean for sample size, n = 4 rats. Significant difference in mean was accepted at P < 0.05*

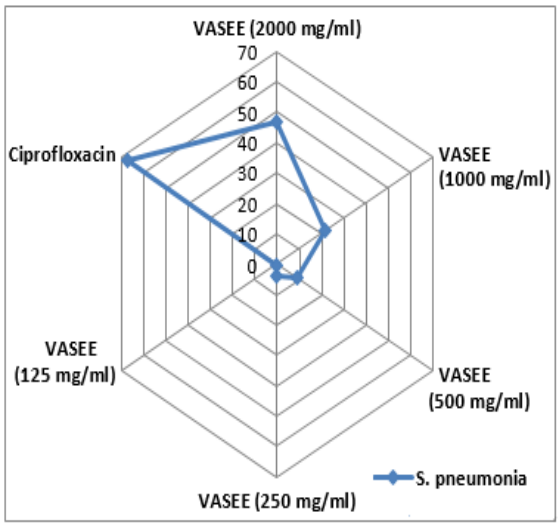

Fig 2: Activity (mm) of ground Vernonia amygdalina stem ethanolic extract, VASEE, on S. pneumonia pathogen. Result Values are expressed as Mean for sample size, n = 4 rats. Significant difference in mean was accepted at P < 0.05

Thus VASEE exhibited potent activity against the tested bacterial pathogens which comprises Gram-positive and Gram-negative bacteria, suggesting that *V. amygdalina* stem extract (VASEE) could be beneficial if incorporated in the development of novel herbal formulation for preventing broad spectrum bacterial infections (Ali, *et al.*, 2019). Furthermore, *in* vivo the extract (at 200 mg/kg bw) as compared to the control and MSG groups improved (P<0.05) and, notably at the highest tested dose modulated the monosodium glutamate intoxication-related effect on, the studied hematological parameters of rats (Table 2). Results of earlier antibacterial studies of *V. amygdalina* leaf showed variable degree of antibacterial activities (Ali, *et al.*, 2019). The result of this study conformed to reported earlier studies that ethanol extract of *V. amygdalina* leaf elicited activity against *S. aureus* (Ogundare, 2011; Zubairu, *et al.*, 2019), *E. coli* and *S. typhi* (Zubairu, *et al.*, 2019), indicating the potency also of *V. amygdalina* stem against these pathogens. The present result, however, contradicted that of Ogundare (2011) who reported inactivity of *V. amygdalina* leaf extract against *E. coli*, indicating selective potency of *V. amygdalina* stem over *V. amygdalina* leaf against *E. coli* (and apparent preference of *V. amygdalina* stem over the leaf in managing *E. coli*-related ailments). Thus VASEE exhibited potent activity against the tested bacterial pathogens which comprises Gram-positive and Gram-negative bacteria, suggesting that *V. amygdalina* stem extract (VASEE) could be beneficial if incorporated in the development of novel herbal formulation for preventing broad spectrum bacterial infections (Ali, *et al.*, 2019). Furthermore, *in* vivo the extract (at 200 mg/kg bw) as compared to the control and MSG groups improved (P<0.05) and, notably at the highest tested dose modulated the monosodium glutamate intoxication-related effect on, the studied hematological parameters of rats (Table 2).

Table 2. Effect of ground Vernonia amygdalina stem extract, VASEE on packed cell volume (PCV), hemoglobin (HB) and red blood cell (RBC) contents of normal and monosodium glutamate-intoxicated rats

Groups	PCV (%)	HB (g/dl)	RBC (× 10^{12}/L)
MSG (8000 mg/kg bw)	58.75±0.75	30.08±0.75	252.50±4.79
VASEE (200 mg/kg bw)	64.75±0.48	34.45±0.48	290.00±5.77
Normal control	58.75±0.48	22.40±0.48	245.00±2.89
MSG + VASEE (200mg/kg bw)	60.00±0.82	30.85±0.82	255.00±5.00
MSG + VASEE (400mg/kg bw)	62.50±0.96	34.90±0.96	270.00±5.77

Result Values are expressed as Mean ± standard error of mean (SEM) for sample size, n = 4 rats. Significant difference in mean was accepted at P < 0.05

Red blood cell and hemoglobin function in the efficient transport of oxygen into and carbon dioxide out of, the body and in concert with PCV (the percentage of RBC in blood) indicates oxygen – carbon dioxide balance status (Egbuonu, *et al.*, 2019; Oyedeji and Bolarinwa, 2013; Ugwuene, 2011). Furthermore, although non-significant influence on rats' hemoglobin and RBC levels but slight reduction in packed cell volume (PCV) concentration, were interpreted as absence of adverse effect on the rats'

hematological properties (Iwueke, *et al.*, 2018); Agiang, *et al.* (2017) associated possession of some anti-anemic properties following improved/elevated RBC, Hb and PCV. Thus, the significant elevation in the levels of RBC, HB and PCV of the rats following exposure to bitter leaf stem extract as reported herein could be indicating improved gaseous (oxygen and carbon dioxide) transport leading to favorable oxygen - carbon dioxide balance, and improved anti-anemic properties in the rats. This may further suggest that *V. amygdalina* stem extract could confer overall non-adversity and could potentially modulate MSG-intoxication-related effect, on the rats' hematological functions. The study therefore underscored a promising application of the extract of hitherto wasted bitter leaf stem in novel drug development that could offer novel therapeutic benefits on the hematology of especially MSG-intoxicated rats, warranting further studies.

Conclusion: The crude extract of bitter leaf stem, VASEE exhibited potent activity against the tested bacterial pathogens, improved, and potentially modulated MSG-intoxication-related effect on, the rats' hematological functions. The study underscored a promising antibacterial application of the extract of hitherto wasted bitter leaf stem that could offer novel therapeutic benefits on the hematology of especially MSG-intoxicated rats. Furthermore, the study could be a stepping stone to potential novel drug development, warranting further studies.

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