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Hydroethanolic stem bark extract of *Vernonia amygdalina* Del. (Asteraceae) suppresses yeast-induced pyrexia and *Plasmodium berghei* malaria in murine models

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Vernonia amygdalina **has been widely utilized in Ghana and other West African states for numerous parasitic infections, diabetes and some inflammatory conditions. In this study we evaluated the antipyretic and antimalarial properties as well as *in vitro* antioxidant activities of an ethanolic stem bark extract of *V. amygdalina* (VAE). The antipyretic effect of VAE was assessed using Baker's yeast-induced pyrexia and the antimalarial activities of plant extract against *Plasmodium berghei*-infected mice in the Peters 4-day suppressive test. The antioxidant activities of the stem bark extract were determined by DPPH radical scavenging and total phenol content assay. Phytochemical screening was carried out using standard methods. *V. amygdalina* (100-600 mg kg^{-1}) dose related decreased the Baker's yeast-induced fever in young rats. Also, it exhibited a significant ($F_{(5, 24)} = 91.35$, $P < 0.0001$) anti-plasmodial activity in the mouse model. It caused a percentage suppression of 81.80 ± 3.76 at 600 mg $kg^{-1}$and a relative antioxidant activity in the DPPH radical scavenging assay with an IC_{50} of 146.4 ± 2.31 µgml^{-1} and a total phenol content of 345.7 ± 4.56 mg g^{-1} of gallic acid equivalence. Preliminary phytochemical screening showed that the extract contained tannins, reducing sugars, flavonoids, terpenoids and alkaloids. *V. amygdalina* hydroethanolic stem bark extract evoked promising antipyretic, anti-plasmodial and antioxidant effects.**

Key words: *Vernonia amygdalina,* pyrexia, malaria, antioxidants, Baker's Yeast, *Plasmodium berghei.*

INTRODUCTION

The use of plant medicine has been in existence since centuries ago. It was the only remedy available for

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diseases but how and where they were being used was lost in pre-history. In recent time, the use of plant medicines is resurfacing and growing at a very fast rate globally especially in the developing countries including Ghana (Boadu and Asase, 2017). This is because of their easy accessibility and relatively cheap compared to the orthodox medicine. In addition, the majority of the people in developing countries still resort to medicinal plants or herbal remedies for their health care needs. The function of free radicals in the pathophysiology of many diseases is responsible for the recent attention this chemical group of substances is receiving in drug research. The creation of reactive oxygen and nitrogen species has been linked with cellular and metabolic injury, cardiovascular and degenerative diseases, cancer, ageing, diabetes mellitus, infectious diseases, neurologic, and inflammatory conditions (Peng et al., 2009)**.** Antioxidants serve as guards against oxidative damage, preserve adequate immunity and restore or maintain homeostasis (Victor et al*.,* 2004). The commonest sources of these agents are fruits and vegetables and many phytochemicals from plants especially the phenolics and flavonoids (Zou et al*.,* 2004). Herbal antipyretic and antimalarial medicines are preferred over the synthetic agents due to the fact that they are believed to have fewer or no side effects, they are easily accessible, and acceptable because of their rich traditional heritage in the traditional healing systems (Sultana et al*.*, 2015). It is therefore rationally sound to search for new antipyretic and antimalarial agents with antioxidant properties from plant sources.

Vernonia amygdalina is a small shrub, member of the Asteraceae family and grows in especially the tropical regions of Africa. In folk-medicine the plant is used as an antipyretic, analgesic, antidiabetic, anthelmintic, anti-inflammatory, antibacterial, antiparasitic, astringent, diaphoretic, anticancer and purgative (Adaramoye, 2008; Adesanoye and Farombi, 2010; Afolabi et al*.*, 2012; Erukainure et al*.*, 2019; Ruth et al*.*, 2017; Shewo and Girma, 2017). This research was designed to assign scientific evidence to the ethnobotanical uses of the stem of *V. amygdalina* as an antioxidant and in the treatment of pyrexia and malaria in murine models.

MATERIALS AND METHODS

Plants collection

The stem bark of *V. amygdalina*was gathered from the wild at Asakraka-Kwahu, in the Eastern region of Ghana. The plant material was authenticated at the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences (FPPS), KNUST, Kumasi. The fresh plant materials were cleaned and dried at a temperature of about 25°C for 10 days.

Extract preparation

Using a hammer mill, the dried plant sample, was milled into coarse powder. The milled sample was extracted with 70% ethanol and then filtered. The filtrate was concentrated at 40°C using a rotary evaporator. The concentrated extract was further air dried to obtain a more solid extract and kept in a desiccator for further use.

Phytochemical screening of *V. amygdalina* stems bark

The hydro-ethanol extract of the stem bark of *V. amygdalina* (VAE) was screened for the presence/or absence of secondary metabolites following standard laboratory methods for phytochemical screening as reported by Ayensu and Quartey, (2015) and Evans (2009).

Experimental animals

Plasmodium berghei NK 65 was obtained from Noguchi Memorial Institute for Medical Research. ICR mice (17–25g) and Sprague-Dawley rats (90-120 g) being made of both sexes were obtained from the animal house of FPPS, KNUST, Kumasi. They were sustained on standard animal pellets and water *as desired*. The College of Health Sciences Animal Ethics committee, KNUST, Kumasi, gave permission and approval to the researchers for the animal studies. During the period of adaptation, the murine were subjected to natural conditions of lighting and handled according to approved standard protocols for the use of laboratory animals as permitted by the laboratory committee of Animal husbandry – Pharmacology Department, FPPS, Kumasi-Ghana. All the experimental guidelines applied in this work were in line with the National Institute of Health Guidelines for Care and use of Laboratory Animals (Directive 2010/63/EU, Animal Care and Use Committee, 1998). All the methods used were certified by the Departmental Ethics Committee.

Evaluation of anti-plasmodial effect of VAE (Suppressive model)

The chemo-suppressive effects of VAE was evaluated as reported by Iyiola et al*.* (2011). The rodents were in no particular order grouped into six (n = 5). All mice were infected with intraperitoneal injection of 0.2 ml of saline suspension of 1.0×10^7 parasitized erythrocytes. Group 1 (negative control) was given normal saline (5 ml/kg, *p.o.*); Group 2 (positive control) was given the standard drug artemether at parenteral dose of 4 mg/kg, *i.p*. The remaining groups were treated orally as illustrated in Figure 1.

The extracts, normal saline and artesunate were administered once daily for four consecutive days. A day after the completion of the treatment, the tail blood was collected from each mouse, and thin films of the samples were stained with Giemsa. The thin films were viewed at 100× magnifications under the microscope and the average percentage parasitemia and percentage of parasite suppression were calculated using equations 1 and 2 respectively (Penna-Coutinho et al*.*, 2011):

$$\%\ Parasitemia = \frac{Number\ of\ infected\ RBC's}{Total\ number\ of\ RBC's}\ x\ 100 \quad (1)$$

$$\%\ Suppresion = \frac{Parasitemia\ in\ Control\ group - Parasitemia\ in\ Test\ group}{Parasitemia\ in\ Control\ group} \quad (2)$$

Anti-pyretic effect of VAE (Yeast model)

The antipyretic effect of VAE was evaluated using standard procedure as reported by Tomazetti et al*.* (2005). Sprague-Dawley rats were selected in no particular order and put into six groups of five animals each. The murine were denied feed but allowed free

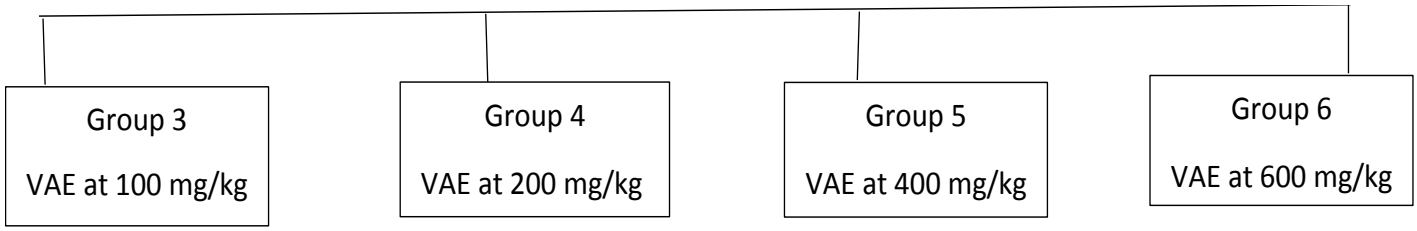

Figure 1. Treatment of rodent groups.

access to water. The baseline rectal temperature was taken before the administration of 0.135 mg/kg*i.p* yeast solution. The anal temperature of each animal in their respective group was measured 3 h post pyrexia induction and rats which showed a rise in temperature of at least +1°F (0.6°C) of the baseline were used for the study. The murine with fever were placed into six groups (n= 5) and were treated with three doses of VAE (100, 200, 400 and 600 mg/kg), 200 mg/kg of paracetamol, or 10 ml/kg of normal saline solution (the control), orally, 2 h after the yeast-induced pyrexia. Rectal temperature was measured after every hour for up to 5 h after administering the drugs. Drug solutions and suspensions were prepared such that no dose exceeded 0.5 ml orally or intraperitoneally. The average rectal temperature was taken for extract and standard drug groups and compared to that of the control group. A time course for the change in temperature was obtained.

In-vitro antioxidant effect of VAE

Evaluation of DPPH radical scavenging activity of VAE

The free radical scavenging activity was determined as described by Govindarajanet al, (2003). 1 ml each of the different concentrations of the extract (500- 62.5 µg/ml) was added to a methanolic solution of DPPH (20 mg/L) in a test tube. The reaction mixture was kept at 25°C for 30 min. The process was repeated for concentrations of ascorbic acid with concentrations (500 -6.25 µg/ml). The absorbance of the remaining DPPH was evaluated at 517 nm in UV-visible spectrophotometer. The DPPH radical scavenging activity was estimated according to Equation 3:

$$\%\ DPPH\ scavenging\ activity = 1 - \frac{Abs\ sample}{Abs\ control}\ x\ 100 \quad (3)$$

Where Abs sample and Abs control are absorbances of sample and control respectively. The concentration of sample required to scavenge 50% of DPPH is expressed as EC_{50}.

Evaluation of the total phenol content of VAE

The total phenolic content of the extract was determined by the Folin–Ciocalteu method. Test tubes containing different concentrations of the extract (500-62.5 µg/ml) were made up to 3 ml with distilled water and mixed thoroughly with 0.5ml of Folin–Ciocalteu reagent for 3 min, followed by the addition of 2mL of 20% (w/v) sodium carbonate solution. The mixture was incubated for 60 min in the dark and the absorbance was measured at 760 nm. The process was repeated for concentrations of gallic acid (50 -6.25 µg/ml). A blank solution was prepared by adding every other solution but without the extract/gallic acid. The total phenolic content was calculated from the gallic acid calibration curve and expressed as mg of gallic acid equivalent per g dry weight of extract.

Statistical analysis

The difference in total anti-plasmodial and the antipyretic score was determined using one-way ANOVA with Bonferoni's*post hoc* test using treatment data as the between-subject factor for data which were distributed normally.

RESULTS

Phytochemical screening of *V. amygdalina* stems bark

The phytochemical investigation of stem bark sample discovered the presence of tannins, flavonoids terpenoids, steroids, reducing sugars and traces of alkaloidal compounds with the most dominant being tannins and flavonoids (Table 1).

Evaluation of anti-plasmodial effect of VAE (Suppressive model)

The findings of this study showed that V*AE* exhibited a potent activity against *the* malaria parasite. The percentage parasitemia was significantly ($F_{(5, 24)} = 91.35$, P) < 0.0001 reduced in all the VAE (100-600 mg kg^{-1}) treated groups as compared with the untreated group. The highest percent suppression of the parasitemia at 600 $mgkg^{-1}$ body weight was 88.19 ± 3.67. The mice treated with the standard drug (Artemether 4mg/kg) also significantly ($F_{(5, 24)} = 103.9$, P < 0.0001) inhibited the parasitemia (Table 2).

Anti-pyretic effect of VAE (Yeast model)

VAE (100, 200, 400 and 600 mg kg^{-1}) given orally, dose-dependently and significantly ($F_{(25, 144)} = 2.22$; P = 0.0018) reduced the elevated rectal temperature (Figure 2a). Furthermore, VAE produced a significant ($F_{(5, 24)} = 10.01$; P<0.0001) and dose dependent decrease in total pyrexia (Figure 2b). Similarly, paracetamol (200 mg kg^{-1} *p.o.)* significantly ($F_{(25, 144)} = 2.22$; P = 0.0018) decreased the area under the curves of the time course curves compared to that of vehicle-treated group (Figure 2a).

Table 1. Qualitative phytochemical screening of VAE.

Test	Inference
Tannins	+
Glycosides	+
Saponins	+
Alkaloids	+
Flavonoids	+
Terpenoids	+
Steroids	-

Table 2. Anti-plasmodial activity of VAE against *P. berghei malaria* parasite in mice.

Sample	Dose (mg/kg)	Anti-plasmodial activity ± S.E.M	
		% parasitemia	% Suppression
Control	-	34.52 ± 1.03	0
VAE	100	27.51 ± .093**	20.30 ± 2.82**
	200	21.28 ± 1.59****	38.34 ± 4.62****
	400	15.84 ± 1.19****	54.11 ± 3.45****
	600	6.28 ± 1.30****	81.80 ± 3.76****
Artemether	4	4.08 ± 1.27****	88.19 ± 3.67****

The data presented as Mean ± S.E.M. The asterisks (**P < 0.01, ****P < 0.0001) compared with the control group (untreated) (1-way ANOVA completed with Bonferoni's multiple comparison test).

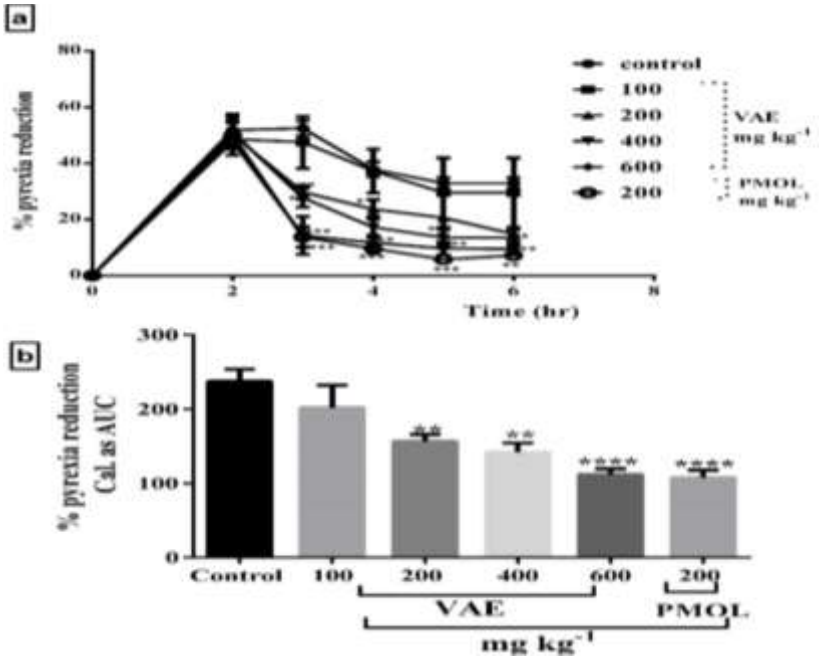

Figure 2. (a) Time course effect of VAE (100-600 mg kg^{-1}*p.o*) and Paracetamol (200 mg kg^{-1}*p.o*) on baker yeast-induced fever in young rats and the AUC (total pyrexia) (b) for variation of rectal temperature along time, compared to the vehicle group. Each point in (a) represents the Mean ± S.E.M (n = 5). *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P < 0.0001 compared to respective controls (two-way repeated measures ANOVA followed by Bonferroni's post hoc); each column in (b) represent the mean ± S.E.M. **P ≤ 0.05, ****P ≤ 0.0001 (one-way ANOVA followed by Dunnett's post hoc test).

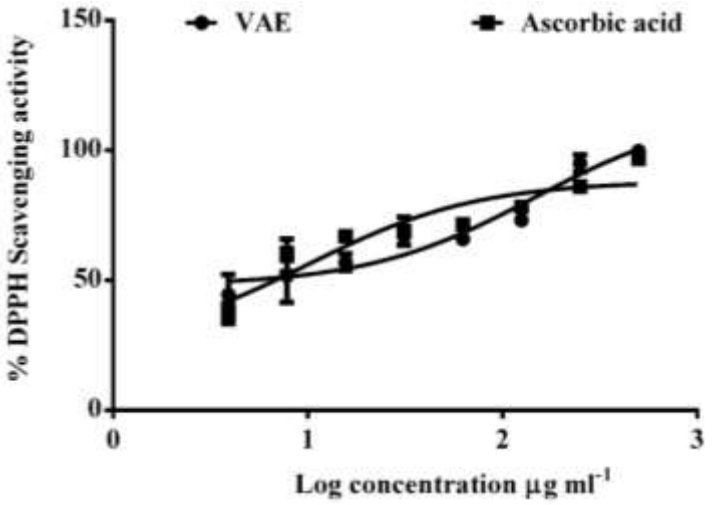

Figure 3. Free radical scavenging activity of VAE (31.13-500 µg ml^{-1}) against ascorbic acid (31.13-500 µg ml^{-1}) in the DPPH radical scavenging assay. Data represents Mean ± S.D.

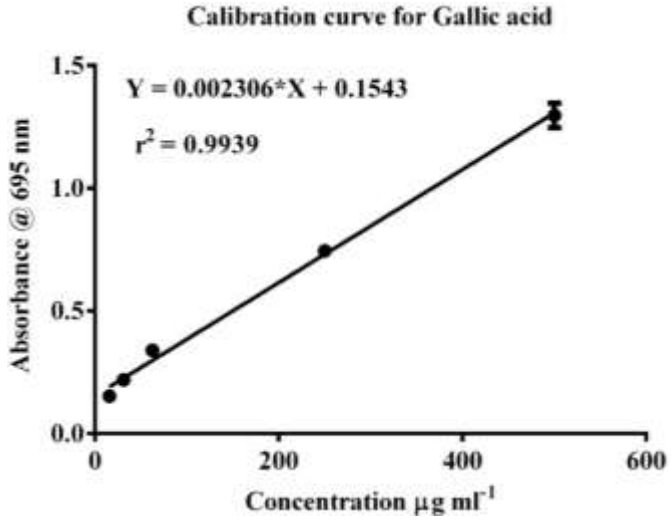

Figure 4. The calibration curve of Gallic acid (6.25-100 µg ml^{-1}).

In-vitro antioxidant effect of VAE

The *in-vitro* antioxidant capacity of VAE was evaluated using DPPH radical scavenging and the total phenol content assay which is extensively employed to detect antioxidant activity of plant extracts. In the DPPH free radical scavenging assay VAE and ascorbic acid showed an $IC_{50}$of 146.4 ± 2.31µgml^{-1} and 10.07 ± 1.09 µgml^{-1} respectively. Figure 3 shows free radical scavenging activity of VAE against ascorbic acid.From the Gallic acid calibration graph (Figure 4) VAE demonstrated a total phenolic content of 345.7 ± 4.56 mg g^{-1} extract equivalence of gallic acid upon interpolation.

DISCUSSION

The preliminary phytochemical analysis of the ethanolic stem bark extract of *V. amygdalina* showed the presence of tannins, flavonoids, alkaloids and hydrocyanide as previously reported by Eyong et al. (2011). Many phytochemicals such as triterpenes, flavonoids, alkaloids, steroids, tannins and glycosides have been found to be accountable for the many biological properties exhibited by extracts from plants (Fabricant and Farnsworth, 2001; Güçlü-Üstnüdağa and Mazza, 2007; Velu et al., 2018; Xiao 2015; Yuan et al., 2016). Phytochemicals from mostly the leaves and roots of *V. amygdalina* have numerous

pharmacological actions (Adaramoye, 2008; Ruth et al., 2017).These constituents could be accountable for the antioxidant, antipyretic and the antiplasmodial action of VAE.

The findings from our study showed that VAE exhibited an *in vivo* antiplasmodial activity that was evident from the chemo-suppression it produced during the Peters 4-day suppressive test. This test has been employed to evaluate the schizontocidal activity of extracts, fractions and other compounds against early *P. berghei* infection in mice (Berendt et al., 1994; Hunt and Grau, 2003).The hydro-ethanolic stem bark extract of *V. amygdalina* also exerted pronounce suppressive of the malaria parasite in mice. The observed antimalarial activity of VAE is consistent with the traditional use of the plant (mostly the leaves) as herbal medication against malaria (Agbodeka et al., 2016; Asnake et al., 2016; Njan et al., 2008; Simbo, 2010) and is indicative of its potential as an antimalarial agent.

VAE showed a potent antipyretic activity in Baker's yeast-induced pyrexia in murine model. Baker's yeast is a lipopolysaccharide which is a cell wall component of gram-negative bacteria. It is an exogenous pyrogen which binds to an immunological protein called lipopolysaccharide-binding protein (LBP). Previously, it was reported by Li et al. (2008) that prostaglandin E_2 (PGE_2) is a fever mediator in the brain, specifically in the preoptic area of the anterior hypothalamus. PGE_2 slows the rate of firing of warm sensitive neurons and results in increased body temperature. The set-point temperature of the body will remain high until PGE_2 is absent (Anochie and Ifesinachi, 2013; Gross, 2006; Walter et al., 2016). Antipyretic activity is commonly mentioned as a feature of agents which have an inhibitory effect on prostaglandin-biosynthesis and an indispensable part of proinflammatory markers in febrile response has been demonstrated (Aronoff and Neilson, 2001). Thus, it could be plausible to conclude that the VAE prevents the synthesis of prostaglandins. Hence, the hydroethanol stem bark extract of *V. amygdalina*may exert its antipyretic activity probably through the inhibition of prostaglandins synthesis.

In the current study the antioxidant effect of the extract was assessed using two different methods: Total phenol content and DPPH scavenging activity assay. Polyphenols are electron-rich compounds and capable of entering into efficient electron-donation reactions with oxidizing agents. Various plant phenols have been found to interfere with the oxidation of different biomolecules important for life (Han et al., 2007; Ivanova et al., 2005). Preceding the antioxidant assay, phytochemical screening to determine the presence of phenols (Tannins) in the extract was carried out. The results indicated that VAE had some plant phenols present. The total phenol content estimated for the extract through the Folin-Ciocalteu assay reveals the possibility of the stem bark extract to give out electrons to free radicals or reactive oxygen species as displayed by chain-breaking antioxidants such as propyl gallate, gallic acid, rutin and catechol (Roginsky, 2003). The extract produced a concentration dependent de-colorization of DPPH and methanol mixture. Therefore, it can be said that the extracts may have the capacity to directly interact with free radicals to produce less harmful products.

Conclusion

The findings of this novel research have presented evidence to support the use of *V. amygdalina* stem bark extract as an antioxidant, antipyretic and anti-plasmodial agent in traditional medicine practice.

CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

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