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EVALUATION OF ANTIMALARIAL, ANTI-INFLAMMATORY AND ANTIPYRETIC ACTIVITIES OF LEAVES EXTRACTS OF *HAEMATOSTAPHIS BARTERI*

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ABSTRACT

Haematostaphis barteri, known as blood plum found in Upper West region of Ghana. It is used locally for treating malaria, hepatitis, and swollen body parts. The curative property of dicloromethane:methanol (D/M) and aqueous (AQ) extracts were investigated by inoculating mice with $1x10^6$*P. berghei*. On the fourth day, the mice were treated orally with AQ (30-300 mg/kg), D/M (30-300 mg/kg), saline and Artemether-Lumifantrine (1.14:6.9 mg/kg) for 5 days. In the prophylactic assay, AQ, D/M and 1.2 mg/kg sulfadoxine/pyremethamine (SP) were orally administered to the mice for three consecutive days before inoculating them with *P. berghei.* Parasite density was measured after 72 h. The anti-inflammatory activity of AQ was determined by inducing inflammation in hind paws of rats by intra plantar injection of 100 µl of 1% of carrageenan, histamine and serotonin before oral treatments with AQ (30-300 mg/kg), saline and 10 mg/kg diclofenac. The effects of the treatments on inflammation were measured with digital calipers. Pyrexia was also induced in rats with 0.135 g/kg, i.p. yeast. The rats were treated orally with AQ and 25 mg/kg paracetamol 18 h later. Rectal temperature was taken for three hours. AQ and D/M produced significant ($P<0.05$) curative antimalarial activity. In the prophylactic test however, only AQ significantly reduced the parasite density. AQ significantly ($p<0.05$) inhibited carrageenan, histamine and serotonin-induced inflammation as well as pyrexia induced with yeast. *H.barteri* possesses antimalarial, anti-inflammatory and antipyretic properties. The anti-inflammatory properties involved the inhibition of inflammatory the mediators: histamine and serotonin.

Key Words: *Haematostaphis barteri*, Inflammation, Pyrexia, glycosides, Malaria, Carrageenan, Histamine, Serotonin.

INTRODUCTION

Malaria, an inflammatory disease is one of the most serious pathogenic diseases in endemic areas of the world, particularly in Africa, Asia, and Latin America (Ravikumar *et al*., 2012; Sha'a *et al*., 2011). About 300 to 500 million new cases are diagnosed each year and approximately 1.5 million people die of the disease with about 800 000 deaths occurring in African children less than five years (Ramazani *et al.,* 2010; Zofou *et al*., 2011). Malaria is characterized by systemic inflammation, pain and fever (Boampong *et al*., 2013).Inflammation is associated with localized increases in the number of leukocytes and a variety of complex mediator molecules (Gupta *et al*., 2006). Inflammation is associated with virtually all diseases (Amponsah *et al*., 2013)and it serves as a beneficial host response to foreign invaders and necrotic tissue, but it is itself capable of causing tissue damage (Groeneveld *et al*., 2001).Although inflammation occurs innately, it can also produce some systemic effects. Fever (Pyrexia) is one of the commonest manifestations of the acute-phase response, especially when inflammation is caused by infections such as malaria (Groeneveld *et al*., 2010).

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Malaria often leads to miscarriage in pregnant women at the early stage of pregnancy (Bulter, 1997; Raimi, 2010). The problem is further compounded by the upsurge in the resistant strains of the parasite against conventional antimalarial drugs such as chloroquine, quinine, and recently, artemisinin derivatives (Sha'a *et al*., 2011; Zofou *et al*., 2011). Thus, the continuous search for novel and more effective antimalarial compounds especially from medicinal plants extracts is of utmost importance in combating malaria infection (Asase *et al*., 2005; Ramazani *et al*., 2010; Ravikumar *et al*., 2012). In Africa, the use of indigenous plants play an important role in the traditional methods of malaria treatment by providing good sources for novel antiplasmodial and anti-inflammatory compounds (Chukwujekwe *et al*.,2009Gupta *et al*., 2006). The therapeutic properties ascribed to most of these plants are linked to the phytochemical compounds contained in them. Phytochemicals such as alkaloids, glycosides, phenols, saponins, triterpenoids, flavonoids, etc. have been suggested to possess antimalarial property (Zofou *et al*., 2011; Ravikumar *et al*., 2012).*Haematostaphis barteri* also known as blood plum is a member of anacardiaceae family(Tadzabia *et al*., 2013). In Ghana, *H. barteri* is mostly found in the Brifo and Wale communities in the Upper West region where the leaves are boiled in the treatment of malaria (Imoro*et al*., 2013). The stem bark is used to treat hepatitis while others take a decoction along with some other plants for the treatment of sleeping sickness. The roots of *H. barteri* are used in the treatment of swollen body parts (Imoro Abukari, Khan Aikins, & Eledi, (2013). Preliminary phytochemical sceening of ethanol leaves extract of *H. barteri* showed the presence alkaloids, tannins, saponins and flavonoids (Tadzabia *et al*., 2013).Although *H. barteri* leaves are being used locally in the treatment of malaria, its antiplasmodial, anti-inflammatory and antipyretic activities have not been established.

MATERIALS AND METHODS

Collection and identification of plant material

The leaves of *Haematostaphis barteri* was collected from Wechiua in the Northern Region of Ghana in the months of December, 2012- January, 2014and authenticated by Mr. Agyarkwah, a botanist in the School of Biological Sciences. The leaves were dried under shade for 7 days. A voucher specimen (BIO/BMS/162) has been kept in the School of Biological Sciences' herbarium for reference.

Preparation of aqueous and dichloromethane: methanol leaves extracts of Haematostaphis barteri

The dried leaves of *Haematostaphis barteri* were pulverized with an electric mill. Two hundred grams of the powdered leaves were extracted with 600 ml of distilled waterand maintained at 80^0C for 24 h. The filtrate was evaporated and lypholized by freeze drying. The yield of the lypholized freeze dried aqueous extract of *H. Barteri* (AQ) was 64.44%.Similarly 200g of the powder was soaked in 2L of the dichloromethane and methanol D/M solvent [1:1] for 48 h in room temperature and then filtered. Filtrate obtained was evaporated into crude extract (D/M) of yield 8.65% and maintained at 4^oC in a refrigerator.

Drugs and chemicals

The drugs and chemicals used in this research were artemether and lumefantrine (A- L) obtained from Ajanta Pharma Ltd (Mumbai, India), sulfadoxine/pyrimethamine (SP) obtained from Maxheal Labs Pvt Ltd (Sachin, India), diclofenac sodium and paracetamol, purchased from Troge, Hamburg, Germany. Carrageenan, serotonin and histamine were purchased from Sigma Aldrich Co Ltd. Irvine, UK. *Plasmodium berghei* NK65 was a gift from Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana.

Screening for secondary metabolites

The aqueous leaf extract was screened to ascertain the presence of phytochemicals using standard procedures described elsewhere (Glasl, 1983; Wagner &Bladt, 1996, Harborne, 1998).

Selection and maintenance of animals

Healthy adult albino rats of either sex, weighing between 200-250g and ICR mice (150-200 g) were obtained from the animal house facility of the Department of Biomedical and Forensic Sciences, University of Cape Coast (UCC). All the studies were performed in conformity with the guidance for care and standard experimental animals study ethical protocols. All procedures and techniques used in these studies were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals All ethical protocols used were approved by the Departmental Ethics Committee.

Curative antimalarial test

To assess the curative potential of the aqueous and D/M extracts of *H. barteri* on established *P. berghei*infection, forty mice were individually infected with $1x10^6$*P. berghei*on Day 0 and assigned to eight groups (n=5).After 72 hours, the animals were treated orally with 30, 100, and 300 mg/kg/day of aqueous extract (Groups 1–3); 30, 100, and 300 mg/kg/day of D/M extract (Groups 4–6); 1.14:6.9 mg/kg/day of A-L (standard drug; Group 7); and 10 ml/kg/day normal saline daily (control; Group 8) for 5 days. The parasite density was monitored daily for five days as described elsewhere (Boampong *et al*., 2013).

Prophylactic/Suppressive test

The method described by Peters (1984) was used to evaluate the prophylactic activity of the extracts. Briefly,

forty mice were randomly assigned to eight groups and each group was pre-treated orally with 30, 100, or 300 mg/kg/day of the aqueous extract (Groups 1–3) 30, 100, or 300mg/kg/day of the D/M extract (Groups 4–6), 1.2mg/kg/day SP (the reference drug; Group 7); or 10ml/kg/day normal saline (Group 8). The treatment was continued for 3 consecutive days. On the fourth day, all mice were infected with 1×10^6 *P. berghei*and 72 hours later, thick films were prepared from blood drawn from the tail of the mice. The parasite density and percentage chemo suppression for all the treatment groups were determined.

Carrageenan-induced rat paw oedema

The baseline paw diameters of the rats were measured with digital callipers. Acute inflammation was induced by injecting 100 μl of 1% solution of carrageenan into the plantar surface of rat left hind paw of all the five groups of animals. After 2.5 h, rats were treated orally with different doses of *Haematostaphis barteri* aqueous extract (30, 100 and 300 mg/kg, *p.o.*; groups 1-3), 10 mg/kg of diclofenac sodium (group 4) and normal saline (group 5). Prior to treatment, the paw diameter of rats were measured in all the animals to confirm inflammation. The paw diameter was measured hourly for three hours after the various treatments (Ganguly *et al*., 2013).

Histamine and serotonin induced rat paw oedema

Inflammation was induced by injection of 0.1 ml of 1% freshly prepared histamine and serotonin into plantar surface of the left hind paw of rats. After one hour, the rats were treated with the aqueous extract at doses of 30, 100, & 300 mg/kg*, p.o*. The positive control groups were treated orally with 0.35 mg/kg of chlorpheniramine and 28.5 mg/kg granisetron in the histamine and serotonin tests respectively while the negative control group received distilled water. The paw volume was measured in a similar manner to that of carrageenan induced paw edema model (Gupta *et al*., 2006).

Yeast (Saccharomyces cerevisiae)-induced pyrexia

The baseline rectal temperatures of the rats were measured using a digital thermometer. Pyrexia was induced by subcutaneous injection of (0.135 g/kg, i.p.) baker's yeast suspension. Animals were fasted and 18 h later, the rectal temperature of each animal was recorded by a digital thermometer. Rats were treated with *Haematostaphisbarteri* extract at doses of 30, 100 & 300mg/kg, *p.o.* The standard group received 25 mg/kg *p.o.*paracetamol while the control group received normal saline. During the first, second and third hours after treatment, rectal temperatures of the rats were recorded (Gupta *et al*., 2006).

Data Analysis

GraphPad Prism 5 was used to analyse the results. Differences between the means were analysed with one-way ANOVA for the column graphs and two-way ANOVA for the time-course curves. All values were expressed as mean±SEM. In all tests, the criterion for statistical significance was $P < 0.05$.

RESULTS

Phytochemical screening of the leaves of H. barteri

The aqueous leaf extract contained glycosides and saponins. Alkaloids, anthraquinones, flavonoids, steroids, tannins and triterpenoids were absent (table 1). The D/M extract contained Alkaloids, anthraquinones, steroids, tannins and triterpenoids.

Table 1. Phytochemical Screening of Leaf Extract of *Haematostaphis Barteri*

Secondary metabolites tested	AQ	D/M
Alkaloids	-	+
Anthraquinones	-	+
Flavonoids	-	-
Glycosides	+	-
Saponins	+	-
Steroids	-	+
Tannins	-	+
Triterpenoids	-	+

Notes: + =present; - =absent.

Curative and prophylactic activities of H. barteri extracts

The aqueous reduced the parasite density dose-dependently with a significant ($P<0.05$) effect at the highest dose of 300 mg/kg (fig. 1). Again, the aqueous extract produced significant prophylactic activity at the highest dose (fig. 2). The D/M extract reduced the parasite density albeit not significant in both the curative and prophylactic tests (fig 1&2). A-L and SP produced significant antiplasmodial activities in the curative and prophylactic tests respectively.

The effects of aqueous extract H. barterion carageenan-induced paw oedema

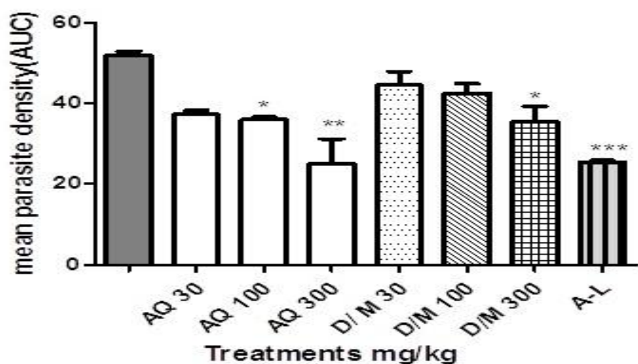
Intraplantar injection of carrageenan into hind paw of rats produced an increase in paw oedema beginning the first hour after the injection (Fig. 3a). Oral administration of AQ (30, 100 & 300mg/kg) showed significant inhibition in paw oedema ($p<0.05$) as compared to the control group.At the second hour of treatment the 100 and 300mg/kg doses exhibited a significant decrease in paw oedema ($p<0.01$) (Fig. 3a) but this effect was not dose dependent (fig. 3b). Diclofenac significantly blocked the carrageenan-induced inflammation.

The effect of aqueous extract of H. barterion histamine and serotonin induced paw oedema and yeast-induced pyrexia.

The aqueous extract of *H. bartri* significantly reduced the inflammation induced by histamine at the

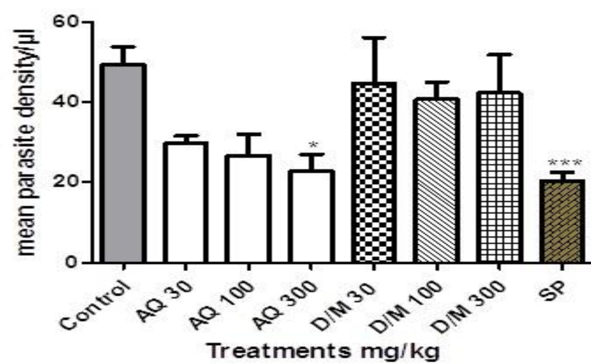
highest dose of 300 mg/kg (fig. 4a). The inflammation induced by serotonin was however significantly reduced at the lowest dose employed (fig. 4b). Pyrexia induced with yeast was also significantly reduced at the 300 mg/kg dose level (fig. 5).

Fig 1. Effect ofaqueous (AQ) extract of *Haematostaphis barteri* and dichloromethane/ methanol extract (D/M) of *H. barteri* presented as AUC from the time course-curves on *Plasmodium berghei* infection in mice.



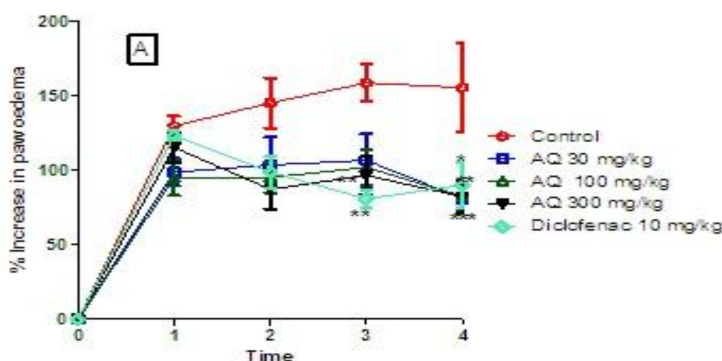
*Data is presented as mean ± SEM, (n=5). ***p<0.001, ** p<0.01, *p<0.05; one-way ANOVA followed by Tukey's post hoc test.*

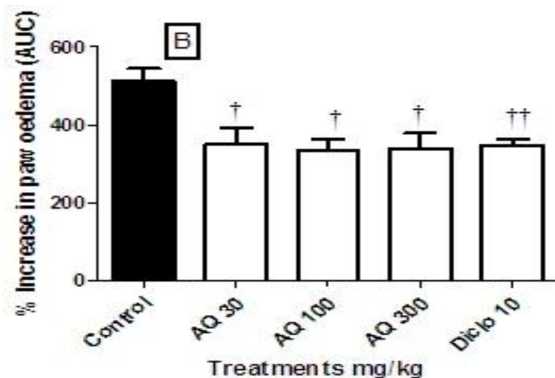
Fig 2. Prophylactic effect of aqueous extract of *Haematostaphis* dichloromethane/ methanol extract of *Haematostaphisbarteri* on residual malaria infection of *Plasmodium berghei*in mice



*Data is presented as mean ± SEM, (n=5). ***p<0.001, *p<0.05; one-way ANOVA followed by Tukey's post hoc test.*

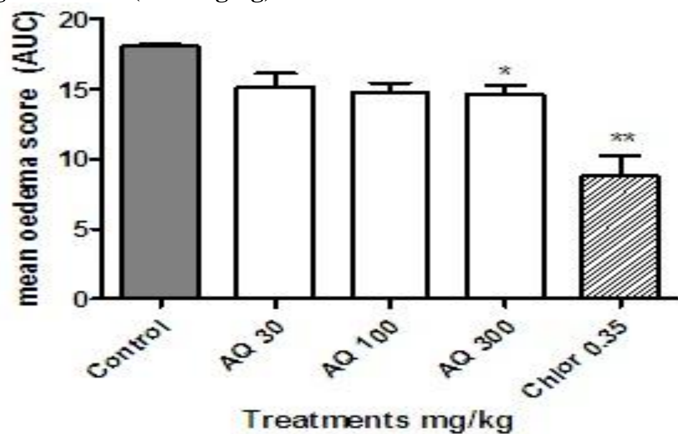
Fig 3. The effect of AQ (30,100 & 300 mg/kg) on the time course curve (A) and the area under the curve (AUC) (B) of carrageenan- induced inflammation in rats

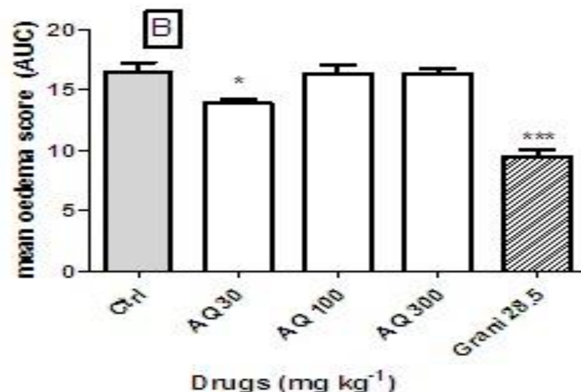




*Data is presented as mean ± SEM, (n=5). *p<0.05, **p<0.01, *** p<0.001; one-way ANOVA followed by Tukey's post hoc test. ††p<0.015, †p<0.05; two-way ANOVA followed by Bonferonni's post hoc test.*

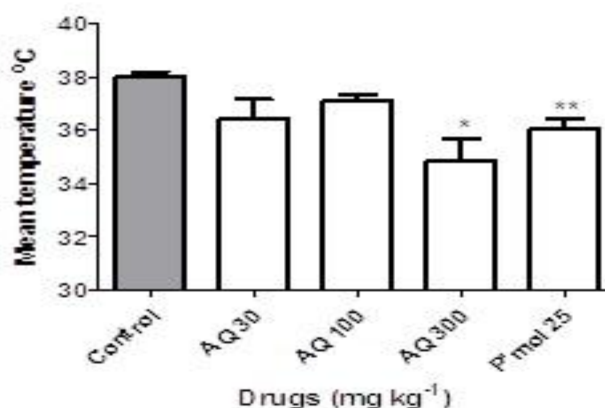
Fig 4. Effect of AQ (30, 100, & 300 mg/kg) and chlorpheniramine (0.35 mg/kg) on (A) histamine- and (B) AQ and granisetron (28.5 mg/kg) on Serotonin-induced inflammation presented as AUC of the time course-curves.





*Data is presented as mean ±SEM. (n=5). *p< 0.05, one-way ANOVA followed by Tukey's post hoc test.*

Fig 5. Effect of AQ (30-300 mg/kg) and 25 mg/kg paracetamol (P'mol) on yeast-induced pyrexia



Data is represented as mean ± SEM. (n=5). *p< 0.05, **p<0.01, one way ANOVA followed by Tukey's post *hoc* test.

DISCUSSION

The aqueous and D/M extracts of *H. barteri* exerted curative activities against *P. berghei*infection in the mice, although the D/M extract was not effective prophylactically. This suggests that *Haematostaphisbarteri* leaves extract can suppress parasite growth if given orally for curative purposes in higher doses as well as prophylactic use if given in the aqueous form. The curative effect of *H. barteri*may to some extent be ascribed to the presence of phytochemicals such alkaloids, anthraquinones, glycosides, saponins, steroids, tannins and triterpenoids. These phytochemicals have indeed demonstrated antimalarial properties (Philipson & Wright, 1990; David *et al*., 2004; Okokon *et al*., 2001 Vasanth *et al*., 1990; Odugbemi and Akinsulire, 2007). The prophylactic activity of the extract may be attributed to Saponins and glycosides in the aqueous extract. Interestingly, some glycosides and terpernoids have been identified to attack the broad stage of malaria infection thereby acting as both curative and prophylactic agents (Olajide *et al*., 2000). It is possible that the aqueous extract contained this broad-stage acting glycosides thereby acting as both curative and prophylactic agent. The terpenoids in the D/M extract may be devoid of this broad-stage antiplasmodial effect and studies are underway to investigate this claim. It is however possible that other phytochemicals not detected by the screening method employed may contribute to the observed antiplasmodial effect of the extracts.

Malaria infection is a complicated syndrome involving inflammatory responses and pyrexia and host-derived factors (Depinay *et al*., 2011). It is therefore likely that the antimalarial action of the extracts may be accompanied by anti-inflammatory and anti-pyretic activities. Because the aqueous extract appeared to be more promising than the D/M extract in treating and preventing malaria, it was selected for further studies in the inflammatory and pyrexia models. The aqueous extract exhibited anti-inflammatory and antipyretic activities making it a promising candidate for antimalarial drug development. Carrageenan has been used for years in testing the anti-inflammatory properties of drugs because of its non-antigenic and apparent non-systemic effects (Ganguly *et al*., 2013). Carrageenan- induced inflammation is believed to have a biphasic action, where the first phase (normally from 0-2hrs) is attributed to the release of histamine, serotonin and increased synthesis of prostaglandins (Adamu *et al*., 2013; Ganguly *et al*., 2013). The second phase is associated with the release of bradykinin and prostaglandins (Gupta *et al*., 2003). There was marked anti-inflammatory activity of the aqueous leaf extract of *H. barteri*in this model. The anti-inflammatory activity of the extract in this model can be attributed to a likely inhibitory effect on the synthesis and/or release of histamine and/or serotonin because histamine and serotonin are the principal inflammatory mediators in the first phase of inflammation in this model. To evalute the possible mechanism(s) of the anti-inflammatory effect of the aqueous extract of *H. barteri*, the notable inflammation mediators in the carageenan-induced inflammation model, histamine and serotonin were employed.The extract also blocked the inflammation produced byhistamine and serotonin. This confirms that the anti-inflammatory activity of the *H. Barteri* involves the inhibition of histamine and serotonin. Phytochemicals like saponins and glycosides in the aqueous leaf extract could be responsible for the observed anti-inflammatory activity of the aqueous extract. Glycosides have been reported to inhibit inflammation by suppressing the actions of TNF-alpha and interferon-gamma (Korkina *et al*., 2003). The pharmacological activities of saponins in plants, such as their anti-inflammatory, analgesic and antipyretic effects have been well documented. Saponins may cause suppression of pro-inflammation mediators such as PGE_2, iNOS and TNF-alpha (Hostettmann& Marston, 1995; Yaun *et al*., 2006).

Yeast induced pyrexia introduces exogenous pyrogens when injected systemically into experimental animals. These exogenous pyrogens have been shown to

induce the production of pro-inflammatory cytokines, such as interleukins 1β and 6 (IL-1β, IL-6), interferon (INF-α) and tumor necrosis factor (TNF), which enter the hypothalamic circulation and stimulate the release of local prostaglandins and hence reset the hypothalamic thermal set point (Dalal& Zhukovsky, 2006; Ganguly *et al*., 2013). In the yeast induced pyrexia model, the aqueous extract exhibited antipyretic activity. Since fever is vastly mediated by prostaglandin release, it is possible that the antipyretic activity of the extract may be due to inhibition of cyclooxygenase pathway to reduce prostaglandin synthesis and/or effect,

CONCLUSION

In conclusion *Haematostaphisbarteri*possess antimalarial, anti-inflammatory and antipyretic activities making it a promising candidate for antimalarial drug development.

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