HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original article http://dx.doi.org/10.1016/j.apjtb.2015.02.002

In vivo antiplasmodial and *in vitro* antioxidant properties of stem bark extracts of *Haematostaphis barteri*

Johnson Nyarko Boampong, Akua Afryie Karikari, Elvis Ofori Ameyaw*

Department of Biomedical and Forensic Sciences, University of Cape Coast, Cape Coast, Ghana

ARTICLE INFO

Article history:
Received 18 Dec 2014
Received in revised form 21 Jan 2015
Accepted 20 Feb 2015
Available online 3 June 2015

Keywords:
Malaria
Parasitemia
Haematostaphis barteri
Antioxidants
Phytochemicals

ABSTRACT

Objective: To evaluate the antimalarial and antioxidant properties of stem bark extracts of *Haematostaphis barteri* (*H. barteri*).

Methods: The prophylactic activity of the plant was performed by dosing mice with sulfadoxine-pyrimethamine (1.2 mg/kg), aqueous extract (30, 100, 300 mg/kg) and dichloromethane/methanol (D/M) (30, 100, 300 mg/kg) extracts of *H. barteri* for 3 days. On the 4th day, the mice were inoculated with *Plasmodium berghei*. The parasite density was estimated for each mouse 72 h post-parasite inoculation. The curative activity of the plant was also performed by inoculating mice with *Plasmodium berghei*. Three days later, they were treated with artemether-lumefantrine (4 mg/kg), aqueous and D/M extracts of *H. barteri* stem bark for 5 days. The *in vitro* antioxidant property of the aqueous extract was determined by using the reducing power, nitric oxide and total antioxidant capacity assays.

Results: The aqueous extract exerted significant ($P < 0.05$) curative and prophylactic antimalarial activities. The D/M extract exhibited significant curative ($P < 0.05$) but not prophylactic antiplasmodial effect. The aqueous extract exhibited *in vitro* antioxidant property with IC_{50}'s of (0.930 ± 0.021) mg/mL, (0.800 ± 0.001) mg/mL and (0.22 ± 0.05) mg/mL in the total antioxidant capacity, reducing power and nitric oxide assays. Histological assessment of the liver of aqueous and D/M treated animals did not reveal any sign of toxicity.

Conclusions: *H. barteri* is not toxic which exerted significant curative antiplasmodial effects but the prophylactic property was however fraction dependent. The mechanism of the antiplasmodial activity of *H. barteri* may partly be mediated by its antioxidant property.

1. Introduction

Malaria is still regarded as a major global infectious disease in the 21st century, with a high pediatric mortality toll in the developing world [1]. In Africa, malaria is one of the diseases causing the most morbidity and mortality. The number of Africans who die as a result of malaria each year is estimated at 800 000 people of which a greater proportion is children aged below 5 years [2]. This is coupled with the emergence and spread of parasite resistance to well-established antimalarial drugs and mosquito vectors resistant to insecticides. It is therefore evident that newer agents with improved efficacy and toxicity should be developed. Medicinal plants presently constitute a popular source of potential antimalarial agents. About 30% of drugs for the management of diseases are obtained from nature [2]. In this regard, *Haematostaphis barteri* (*H. barteri*), commonly known as ''blood plum'', from the Anacardiaceae family was studied for its antimalarial activity. *H. barteri* is found in the northern region of Ghana. Different ethnic groups use it for a number of reasons. Notable among its uses include the leaves and bark infusion employed in the treatment of malaria, hepatitis and sleeping sickness [3].

2. Materials and methods

2.1. Collection and identification of plant material

The stem bark of *H. barteri* was collected from Wechiua in the northern region of Ghana in the months of November to

*Corresponding author: Dr. Elvis Ofori Ameyaw, Department of Biomedical and Forensic Sciences, University of Cape Coast, Cape Coast, Ghana.
Tel: +233 279128631
E-mail: elvisameyaw@gmail.com
Peer review under responsibility of Hainan Medical University.

December, 2013. It was identified and authenticated by a botanist at the University of Cape Coast Herbarium and a voucher specimen was deposited at the herbarium.

2.2. Preparation of plant extract

Preparation of the aqueous stem bark of *H. barteri* was done by washing thoroughly the stem bark with tap water and sun-dried. The dried stem bark was pulverized into fine powder and 30.98 g of the powder was weighed and soaked in boiled water for 3 days. The mixture was filtered and freeze dried. To obtain the dichloromethane/methanol (D/M) fraction, the plant sample was extracted with D/M (1:1) solvent mixture. The extract was concentrated under reduced pressure and dried on a water bath. The dried material was then weighed, dissolved in water and used for this study.

2.3. Screening for secondary metabolites

The aqueous and D/M extracts were screened for the presence of phytochemicals using standard procedures described elsewhere [4–6].

2.4. Animals and husbandry

Institute for Cancer Research mice bred in the Animal House of the School of Biological Sciences, University of Cape Coast weighing between 20 and 30 g were used for the antimalarial studies. The animals were housed in stainless steel cages (34 cm × 47 cm × 18 cm) with soft wood shavings as bedding, fed with normal commercial pellet diet (Agricare Ltd, Kumasi, Ghana), given water *ad libitum* and maintained under ambient laboratory conditions. 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 (Department of Health and Human Services publication no. 85-23, 1985, revised 1996). All ethical protocols used for the study were approved by the Departmental Ethics Committee.

2.5. Drugs and chemicals

Artemether-lumefantrine (A-L) used for the curative antimalarial test was obtained from Ajanta Pharma Ltd. (Mumbai, India). Sulfadoxine/pyrimethamine (SP) was also obtained from Maxheal Laboratories Pvt Ltd. (Sachin, India). Ascorbic acid, ammonium molybdate, disodium hydrogen phosphate, sodium dihydrogen phosphate, ferric chloride, potassium ferricyanide, sodium bicarbonate, sodium carbonate and sulphuric acid were obtained from British Drug Houses, Poole, England.

2.6. Curative antiplasmodial effect of stem bark extracts of H. barteri

Mice were divided into eight groups and each inoculated with 1×10^6 *Plasmodium berghei* (*P. berghei*). About 72 h later, parasitaemia was determined in all the mice followed by the administration of the extracts. The various groups of mice were treated with aqueous (30, 100 and 300 mg/kg; *p.o.*) and D/M (30, 100 and 300 mg/kg; *p.o.*) and A-L (1.14:6.9 mg/kg, *p.o.* for 5 days. The negative control group was treated with normal saline. On Day 12, two animals from each group were randomly selected and sacrificed. Their livers were harvested, fixed in 4% phosphate-buffered paraformaldehyde, and embedded in paraffin. Sections were stained with hematoxylin and eosin, and fixed on glass slides for microscopic examination to estimate parasite density [7].

2.7. Prophylactic effect of stem bark extracts of H. barteri on P. berghei infection

The prophylactic antimalarial properties of the extracts were determined by treating mice with aqueous (30, 100 and 300 mg/kg), D/M (30, 100 and 300 mg/kg), SP (1.2 mg/kg) and normal saline. The mice were treated daily for three consecutive days. On Day 4, all the mice were infected with 1×10^6 *P. berghei*. About 72 h later, blood smears were prepared from the tail of the mice for the determination of parasite density [7].

2.8. In vitro antioxidant assays

2.8.1. Total antioxidant capacity

The antioxidant capacity or power of the aqueous extract was evaluated by the phosphomolybdenum reduction method with slight modifications [8]. The reagent solution prepared contained ammonium molybdate (4 mmol/L), sodium phosphate (28 mmol/L) and sulfuric acid (0.06 mol/L) mixed in 1:1:1 ratio respectively. Accurately 0.3 mL of the various concentrations of the ascorbic acid (20–200 μg/mL) and aqueous (20–200 μg/mL) were mixed with 3 mL of the reagent solution. The mixture was incubated for 90 min at 95 °C after which the absorbance of the green phosphomolybdenum complex formed was measured at 695 nm against a reagent blank (0.3 mL distilled water and 0.3 mL reagent solution). The results were expressed as ascorbic acid equivalents.

2.8.2. Reducing power capacity

The reducing power of aqueous was carried out using the potassium ferricyanide method by mixing 1 mL of aqueous and *n*-propyl gallate (20–200 μg/mL) with 2.5 mL phosphate buffer (0.2 mol/L, pH 6.6) and 2.5 mL potassium ferricyanide. The mixture was then incubated at 50 °C for 20 min. A volume of 2.5 mL of trichloroacetic acid was added to this mixture, which was then centrifuged at 3000 r/min for 30 min. Finally, 2.5 mL of the supernatant solution was collected and mixed with 2.5 mL of distilled water and 0.5 mL ferric chloride and absorbance was measured at 700 nm [9].

2.8.3. Nitric oxide radical scavenging activity

The nitric oxide radical scavenging activity of the extract was determined by mixing 2 mL of 10 mmol/L sodium nitropruside prepared in 0.025 mol/L of phosphate buffer (pH 7.4) with 0.5 mL of ascorbic acid or aqueous at concentrations of 20–200 μg/mL [10]. The mixture was incubated at 25 °C for 150 min. A volume of 0.5 mL of the incubated solution was mixed with 0.5 mL of Griess reagent. The mixture was incubated at room temperature for 30 min. The absorbance of the mixture was measured at 540 nm against a phosphate buffer blank.

2.9. Analysis of data

GraphPad Prism for Windows version 5 (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses and P value <0.05 was considered statistically significant. All data were expressed as mean ± SE (duplicate measurement). The column graph was subjected to One-way ANOVA with Turkey's *post hoc* test.

3. Results

3.1. Preliminary phytochemistry of the extracts

The aqueous and D/M extracts both contained triterpenoids and flavonoids. Aqueous in addition contained glycosides and saponins. Tannins were also presented in the D/M extract (Table 1).

Table 1
Constituents of stem bark extracts of *H. barteri*.

Secondary metabolites tested	Aqueous extract	D/M extract
Alkaloids	–	+
Anthraquinones	–	–
Flavonoids	+	+
Glycosides	+	–
Saponins	+	–
Steroids	–	–
Tannins	–	+
Triterpenoids	+	+

+: Present; –: Absent.

Table 2
Summary of the effect of the stem bark extracts on the survival of mice.

Treatments	Survival days
Aqueous 30 mg/kg	14.80 ± 3.20
Aqueous 100 mg/kg	14.60 ± 2.29
Aqueous 300 mg/kg	20.00 ± 1.30
D/M 30 mg/kg	13.60 ± 5.70
D/M 100 mg/kg	14.80 ± 4.01
D/M 300 mg/kg	16.80 ± 3.50
Control	10.20 ± 2.00
A-L (4 mg/kg)	24.80 ± 5.20

3.2. Daily curative activities and safety profile of stem bark extracts of H. barteri on P. berghei-induced malaria

The aqueous and D/M extracts produced significant ($P < 0.05$) curative antiplasmodial effect starting on Day 5–8 (Figure 1) compared to the saline treated control group. The longest survival times were achieved at the highest doses of both aqueous and D/M treated groups as 20.00 ± 2.30 and 16.80 ± 3.50 respectively (Table 2). There was no observed induced toxicity by both extracts in the liver sections (Figure 2).

3.3. Prophylactic effects of stem bark extracts of H. barteri on P. berghei-induced malaria

The D/M (30–300 mg/kg) extract did not produce significant prophylactic activity against *P. berghei* (Figure 3). On the other

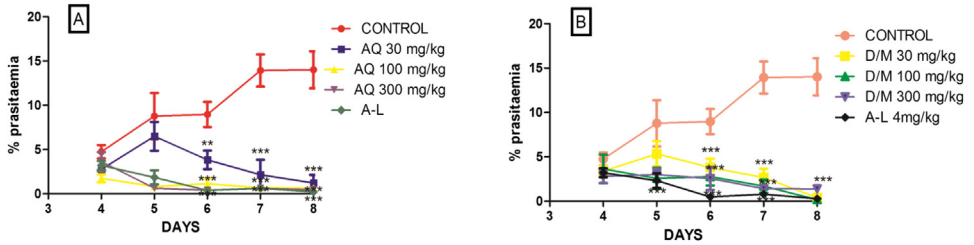

Figure 1. Daily curative activity of the aqueous, D/M extracts of *H. barteri* stem bark and A-L (4 mg/kg) on *P. berghei*-induced malaria. Data is presented as means ± SEM. **: $P < 0.01$; ***: $P < 0.001$, compared to vehicle-treated group (Two-way ANOVA followed by Bonferroni's *post hoc* test). AQ: Aqueous.

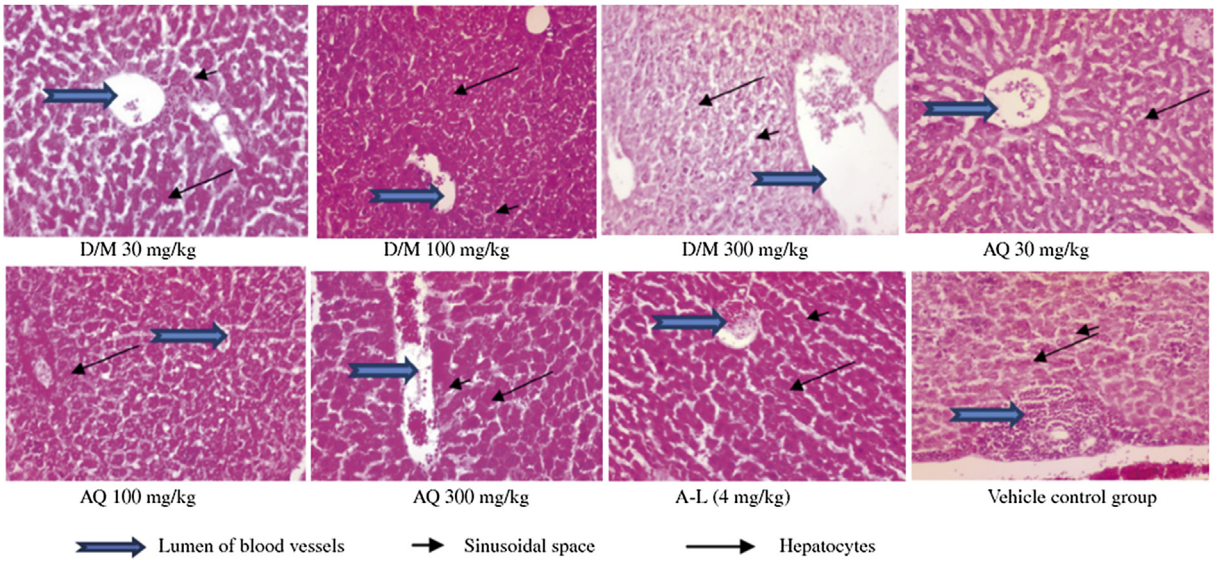

Figure 2. Histology of liver sections of control.
H. barteri (30–300 mg/kg) and A-L (4 mg/kg) were used for treating animals. AQ: Aqueous.

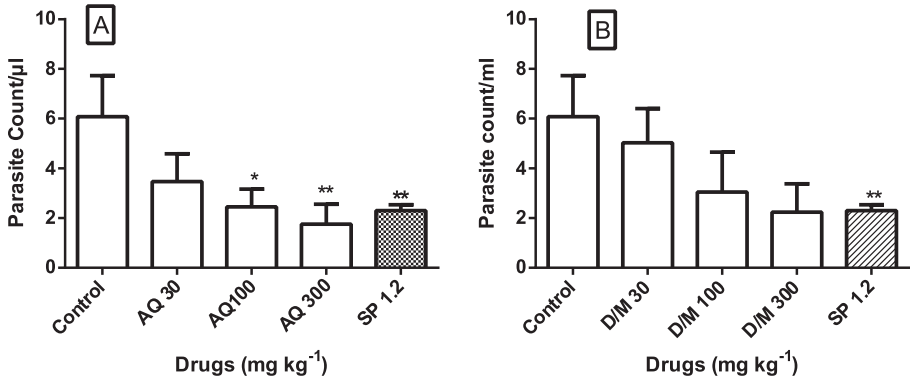

Figure 3. Prophylactic effect of aqueous (30–300 mg/kg), D/M (30–300 mg/kg) extracts of *H. barteri* on *P. berghei*-induced malaria. Data is presented as mean ± SEM. *: $P < 0.05$; **: $P < 0.01$, compared to vehicle treated groups (One way ANOVA followed by Tukey's *post hoc* test). AQ: Aqueous.

hand, the aqueous (30–300 mg/kg) produced significant ($P < 0.000\ 1$) prophylactic activity against *P. berghei in vivo* at all the three doses tested as seen as a significant reduction of the parasite count compared to the vehicle-treated group (Figure 3). Similarly, SP showed significant prophylactic activity ($P < 0.01$).

3.4. Total antioxidant capacity

The aqueous extract produced concentration-dependent antioxidant activity. The total antioxidant capacity of aqueous was (42.5 ± 2.1) mg ascorbic acid equivalents/g extract estimated from the standard curve of ascorbic acid [y (absorbance) = $9.539x - 0.034$; $r^2 = 0.989\ 9$].

3.5. Ferric-reducing antioxidant power

The aqueous extract and *n*-propyl gallate exerted a concentration-dependent antioxidant property in the ferric-reducing power assay. The potency of aqueous extract was 6.7 times less potent than the standard.

3.6. Nitric oxide radical scavenging activity

The aqueous extract was potent in scavenging the unstable nitric oxide species. The potency of aqueous was 8.1 times less potent than ascorbic acid.

4. Discussion

Malaria is an inflammatory cytokine-driven disease that often results in mortality due to sequestered parasitized red cells preventing sufficient oxygen access to where it is needed together with mitochondria inability to generate enough adenosine triphosphate to maintain normal cellular function [11]. *P. berghei* parasite is used in predicting treatment outcomes of any suspected antimalarial agent due to its high sensitivity to chloroquine, making it the appropriate parasite for this study [12]. In this study, three doses of the aqueous and D/M stem bark extracts of *H. barteri* exerted curative antimalarial activities against the blood stage of *P. berghei*. This implies that the active principles for the observed antimalarial effects are present in both the aqueous and D/M fractions. It is proposed that the presence of alkaloids, tannins, triterpenoids, flavonoids, glycosides and saponins in both fractions could be responsible for the observed curative antiplasmodial activities of the two extracts but further experiments are needed to confirm this hypothesis. Some alkaloids that act against malaria parasites inhibit protein synthesis by intercalating with DNA of the parasite [13,14]. In addition, saponin, flavonoids and tannins have been suggested to act as primary antioxidants or free radical scavengers that can counteract the oxidative damage induced by the malaria parasite [15]. Numerous studies have implicated oxidative stress in several pathological conditions such as stroke, diabetes, Parkinson's disease and so on. Apart from their detrimental effects, free radicals are also involved in cellular signaling and as carriers for iron requirement needed by parasites for survival in a host. Iron is a crucial nutrient for survival and replication of microorganisms such as *Plasmodium* parasites. Thus, the ability of an antioxidant to chelate/sequester iron needed by the *Plasmodium* parasite will adversely affect the survival of the parasite in a host. Flavonoids in particular have the ability to chelate iron and they have been suggested that such compounds with the ability to sequester iron should be employed in treatment of infections [16]. Both the aqueous and D/M extracts contained flavonoids, which possibly contributed to the antiplasmodial effect observed in the mice.

The aqueous extract also exhibited prophylactic antimalarial activity contrary to D/M extract. Despite the fact that the pharmacokinetic properties of the extracts have not yet been determined, it is likely that the D/M extracts is excreted rapidly resulting in low blood concentration of the extract before parasite inoculation. It is also possible that the selective presence of glycosides and terpenoids in the aqueous extract may be responsible for its prophylactic activity. Glycosides were however absent in D/M extract. Some glycosides and terpenoids attack the broadest age range of parasites, from the tiniest rings that have recently invaded erythrocytes to more mature stages of parasites such as developing trophozoites and schizonts [17]. Their relatively broad stage-specificity of action has been reported to extend to the ability to impede development of gametocytes [17]. The aqueous and D/M extracts did not produce toxicity in liver of the *P. berghei*-infected mice. The extracts may be safe for malaria treatment.

The data provide evidence that stem bark extracts of *H. barteri* possess antiplasmodial activity which may partly be mediated by its antioxidant property.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors are grateful to Albert Kwabena Atta Larbi, Roberta Naa Barkey Ayiku, Georgina Isabella Djameh and Ebenezer Asmah for their technical support.

References

[1] Veiga MI, Ferreira PE, Jörnhagen L, Malmberg M, Kone A, Schmidt BA, et al. Novel polymorphisms in *Plasmodium falciparum* ABC transporter genes are associated with major ACT antimalarial drug resistance. *PLoS One* 2011; **6**: e20212.

[2] Sanon S, Gansana A, Ouattara LP, Traore A, Ouedraogo IN, Tiono A, et al. *In vitro* antiplasmodial and cytotoxic properties of some medicinal plants from western Burkina Faso. *Afr J Lab Med* 2013; **2**: 1-7; http://dx.doi.org/10.4102/ajlm.v2i1.81.

[3] Amri E, Kisangau DP. Ethnomedicinal study of plants used in villages around Kimboza forest reserve in Morogoro, Tanzania. *J Ethnobiol Ethnomed* 2012; **8**: 1.

[4] Sutar RC, Kasture SB, Kalaichelvan VK. Fingerprinting analysis of the phytosterols from *Holoptelea integrifolia* (roxb.) planch leaves using high-performance thin layer chromatography analysis. *Asian J Pharm Clin Res* 2014; **7**: 160-4.

[5] Seth R, Sarin R. Analysis of the phytochemical content and antimicrobial activity of *Jatropha gossypifolia* L. *Arch Appl Sci Res* 2010; **2**: 285-91.

[6] Kujur RS, Singh V, Ram M, Yadava HN, Singh KK, Kumari S, et al. Antidiabetic activity and phytochemical screening of crude extract of *Stevia rebaudiana* in alloxan-induced diabetic rats. *Pharmacogn Res* 2010; **2**: 258-63.

[7] Boampong JN, Ameyaw EO, Kyei S, Aboagye B, Asare K, Afoakwah R, et al. *In vivo* antimalarial activity of stem bark extracts of *Plumeria alba* against *Plasmodium berghei* in imprinting control region mice. *Rep Parasitol* 2013; **3**: 19-25.

[8] Costa LS, Fidelis GP, Cordeiro SL, Oliveira RM, Sabry DA, Câmara RB, et al. Biological activities of sulfated polysaccharides from tropical seaweeds. *Biomed Pharmacother* 2010; **64**: 21-8.

[9] Bougatef A, Nedjar-Arroume N, Manni L, Ravallec R, Barkia A, Guillochon D, et al. Purification and identification of novel antioxidant peptides from enzymatic hydrolysates of sardinelle (*Sardinellaaurita*) by-products proteins. *Food Chem* 2010; **118**: 559-65.

[10] Balakrishnan N, Panda AB, Raj NR, Shrivastava A, Prathani R. The evaluation of nitric oxide scavenging activity of *Acalypha indica* Linn. root. *Asian J Res Chem* 2009; **2**: 148-50.

[11] Clark IA, Alleva LM, Vissel B. The roles of TNF in brain dysfunction and disease. *Pharmacol Ther* 2010; **128**: 519-48.

[12] Salomea C, Onyishi VI, Momoh MA, Onunkwo GC. Formulation and evaluation of ethanolic extract of *Cryptolepis sanguinolenta* root tablets. *Innovare J Ayurvedic Sci* 2014; **2**: 10-3.

[13] Okokon JE, Ettebong E, Antia BS. *In vivo* antimalarial activity of ethanolic leaf extract of *Stachytarpheta cayennensis*. *Indian J Pharmacol* 2008; **40**: 111-3.

[14] Christian AG, Mfon AG, Dick EA, David-Oku E, Linus AJ, Chukwuma EB. Antimalarial potency of the leaf extract of *Aspilia africana* (Pers.) C.D. Adams. *Asian Pac J Trop Med* 2012; **5**: 126-9.

[15] Gautam R, Jachak SM, Saklani A. Anti-inflammatory effect of *Ajuga bracteosa* Wall Ex Benth. mediated through cyclooxygenase (COX) inhibition. *J Ethnopharmacol* 2011; **133**: 928-30.

[16] Chime SA, Ugwuoke EC, Onyishi IV, Brown SA, Onunkwo GC. Formulation and evaluation of *Alstonia boonei* stem bark powder tablets. *Indian J Pharm Sci* 2013; **75**: 226-30.

[17] Yoo HJ, Kang HJ, Song YS, Park EH, Lim CJ. Anti-angiogenic, antinociceptive and anti-inflammatory activities of *Lonicera japonica* extract. *J Pharm Pharmacol* 2008; **60**: 779-86.