RESEARCH ARTICLE

Antioxidant activity of ethyl acetate extract of *Aquilaria agallocha* on nitrite-induced methaemoglobin formation

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Aquilaria agallocha Roxb, family, *Thymelaeaceae*, is an evergreen plant of India, China and Tibet, commonly described as aloe wood or agarwood. Traditionally, the bark, root and heartwood are used for their medicinal properties as a folk medicine to treat inflammation, arthritis, vomiting, cardiac disorders, cough, asthma, leprosy, anorexia, headache and gout. The present study was carried out to investigate the antioxidant activity of ethyl acetate extract of *Aquilaria agallocha* (EAA). EAA was tested *in vitro* at different concentrations for inhibitory effect on nitrite-induced oxidation of haemoglobin in human blood haemolysate. Results indicate a strong antioxidant effect of EAA in a concentration range of 500-3500 µg/ml. However pro-oxidant activity was observed at higher concentrations of these compounds.

Key words: Antioxidant, *Aquilaria agallocha*, methaemoglobin, nitrite-radical

INTRODUCTION

Aquilaria agallocha (Thymelaceceae) Roxb is a native evergreen plant of India, China and Tibet. It is commonly described as aloe wood or agarwood. Traditionally, the bark, root and heartwood are used for their medicinal properties. It is reported to contain sesquiterpenes.[1,2] Agarwood is highly charged with resinous matter and contains 48% of alcohol soluble matter. After saponification of the alcoholic extract, benzyl acetone, an unidentified ketone, molecular formula, C14H20O2, a sesquiterpene alcohol, and some acids (including hydrocinnamic acid) are obtained. The sesquiterpene alcohol possesses the characteristic odour of the wood.[3] The plant extract of *Aquilaria agallocha* was reported to possesses antihistaminic properties and hence it may be used for the treatment of anaphylactic reactions.[4] The plant is traditionally used to treat inflammation, arthritis, vomiting, cardiac disorders, cough, asthma, leprosy and anorexia.[5] Since the plant has been used in folk medicines in the treatment of headache, inflammation, gout and arthritis,[6,7] an investigation was undertaken to explore the antioxidant activity of ethyl acetate extract of the heartwood of *Aquilaria agallocha* (EAA).

Haemoglobin is subjected to severe oxidant stress. When haemoglobin binds to molecular oxygen, there is an accompanied risk of superoxide formation along with the oxidation of haemoglobin to methaemoglobin.[8] There are several inherent antioxidant defence mechanisms, which prevent methaemoglobin formation. Superoxide dismutase, catalase, ascorbic acid and glutathione peroxidase constitute a few of these endogenous antioxidants.[9] In spite of this, oxidation of haemoglobin to methaemoglobin occurs in response to a variety of chemical stimuli, which include drugs like primaquine and dapsone and environmental pollutants like nitrogen dioxide. The endogenous antioxidant defence system present in our body maintains the level of methaemoglobin within 1%. Many antioxidants such as ascorbic acid, uric acid, 3-ribosyluric acid and glutathione have been found to protect haemoglobin in two stages, viz. a slow stage followed by a rapid autocatalytic stage involving superoxide anion, hydrogen peroxide and nitrogen dioxide. Curcumin, an established free-radical scavenger, protects haemoglobin against nitrite-induced oxidation.[10,11]

The chief objective of this work was to identify new uses for *Aquilaria agallocha*, a drug currently in use. An investigation was undertaken to study the effect of EAA at different concentrations, on nitrite-induced oxidation of haemoglobin in blood haemolysate to methaemoglobin.

MATERIALS AND METHODS

Plant Material

Fresh heartwood of *Aquilaria agallocha* obtained from a commercial source in Pune, was identified and authenticated by Dr. H.B. Singh, Head, Raw materials Herbarium and Museum Division, New Delhi, India. Specimen voucher is preserved in the Department of Pharmacognosy, Sinhgad College of Pharmacy, Pune.

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Preparation of Extract

The heartwoods were dried under shade and powdered by mechanical grinder. The powdered heartwood (100 g) was extracted with ethyl acetate (60-80°C) using Soxhlet apparatus for 72 h. The extract was concentrated under vacuum and dried at room temperature. The extract (5.8 g) thus obtained was resinous in nature and of brownish black colour. Various qualitative tests were performed for EAA to confirm the chemical constituents *viz*., triterpenoids, tannins, phenolic compounds and glycosides.

Preparation of Hemolysate

Blood samples were centrifuged (2500 rpm for 20 min) to remove the plasma and buff coat of white cells. Erythrocytes thus obtained were washed three times with phosphated-buffered saline and were haemolysed by suspending them in 20 volumes of 0.5%w/v sodium chloride solution for 30 min. The haemolysate thus obtained was then centrifuged at 2500 rpm for 1 h to remove membrane and cell debris. The resulting solution was diluted with 0.9%w/v sodium chloride solution to yield a final concentration of oxyhaemoglobin (0.5, at 577 nm) suitable for spectrophotometric analysis.

Procedure of Experiment

Haemolysate prepared, as per the procedure mentioned above, was incubated at different concentrations of test compounds (viz. 500 µg/ml, 1000 µg/ml, 1500 µg/ml, 2000 µg/ml, 2500 µg/ml, 3000 µg/ml and 3500 µg/ml) for different time intervals (0 to 10 min). Curcumin was used at a concentration of 20 µm. Compounds were added to the reaction mixture by dissolving them in ethanol. Controls were prepared with an equivalent amount of ethanol. Haemoglobin exhibits maximum absorption at 577 nm and 560 nm while methaemoglobin absorbs at 631nm. Formation of methaemoglobin (induced by sodium nitrite at a final concentration of 300 mM) was estimated by monitoring the absorbance at 631 nm using Jasco UV 430 double beam spectrophotometer. Day to day variations were significant and results are representative samples drawn from a number of trials.

Preliminary investigations indicate that EAA inhibits nitrite-induced oxidation of haemoglobin. Fig. 1 shows a dose-dependant decrease in absorbance at 631 nm in the haemolysate treated with EAA. The EAA gave a significant inhibition at concentration 500 µg/ml. The results are consistent at different time intervals tested *viz*., 0-10 min. However, curcumin showed a more significant activity even at lower concentration levels than EAA. (The results are not included because the concentration used was not comparative. Curcumin concentration was kept low in the range of 20 µm because of solubility problems).

RESULTS

EAA in different concentrations was tested against standard drug curcumin. Results indicate inhibitory role of EAA in a concentration range from 500-3000 µg/ml. The pro-oxidant activity of EAA was higher at higher concentration.

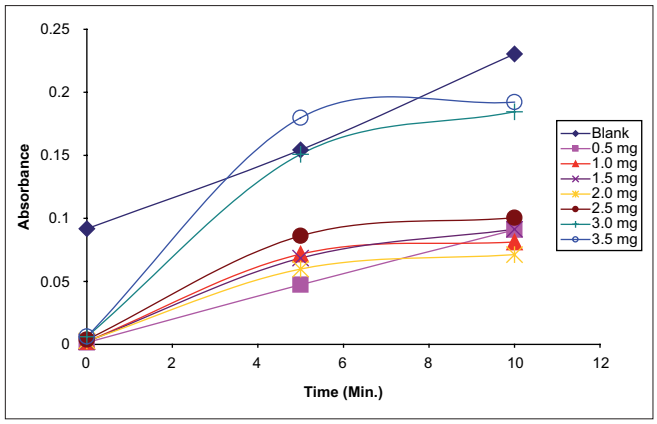

Figure 1: Inhibition of nitrite-induced oxidation of hemoglobin by EAA

DISCUSSION

Aquilaria agallocha is used to treat inflammation in folk medicine. The purpose of the study was to establish scientific evidences for the usage of this plant as an antioxidant. The EAA was studied for its inhibitory effects on methaemoglobin, oxidation product of haemoglobin, produced by treatment with sodium nitrite. The antioxidant effect of EAA was tested at different concentrations. It was observed that EAA showed antioxidant activity at lower concentration range. However, the action was reversed at higher concentrations.

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