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Phytochemical investigation and *in-vitro* anti-oxidant activity of the non- polar extract of agarwood

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ABSTRACT

The famous Oudh is taking from the agarwood tree .The current investigation deals with the identification of potent chemical compounds present in the petroleum ether extract of agarwood. In-vitro anti-oxidant study was also initiated for the validation of plant and also for the volatile matter of the Oudh. The work revealed that variety of potent molecule like flavonoids was present in the bark and also the plant possesses significant anti-oxidant value with a EC-50 of 60mg/ml.

Keywords: Oudh, Agarwood, Anti-Oxidant activity, Nitric Oxide assay

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INTRODUCTION

Plants have been utilised as a natural source of medicinal compounds since thousands of years[1]. Agarwood, also known as oud, oodh or agar is a dark resinous heartwood that forms in Aquilaria and Gyrinopstrees when they become infected with a type of mould[2]. Prior to infection,the heartwood is relatively light and pale colored; however, as the infection progresses, the tree produces adark aromatic resin in response to the attack, which results in a very dense, dark, embedded heartwood. The resin embedded wood is commonly called gaharu, jinko, aloeswood, agarwood or Oudh and is value in many cultures for its distinctive fragrance and thus is used for incense and perfumes.

One of the main reasons for the relative rarity and high cost of agarwood is the depletion of the wild resource[3]. Since 1995 *Aqularia malaccensis*, the primary source has been listed in Appendix II by the convention on International Trade in Endangered species Wild Fauna and Flora. In 2004, all Aqularia species were listed in Appendix II; however, a number of countries have outstanding reservations regarding that listing.[4]

MATERIALS AND METHODS

COLLECTION AND AUTHENTIFICATION OF SAMPLE

The samples were collected from natural resources from the Malappuram District and authenticated from the Taxonomy Department of Uwin Life Science, Malappuram. The sample specimen was stored in Uwin Life Science, Malappuram. The collected specimens were then coarsely powdered[5].

EXTRACTION

The dried and powdered samples were extracted separately by using Petroleum ether. The extraction was carried out by refluxing method and can be used to check antioxidant activity of the samples[6].

PRELIMINARY PHYTOCHEMICAL SCREENING

1. Test for Alkaloids

a) Dragendroff'stest

8g of Bi(NO3)3.5H2O was dissolved in 20 ml of HNO3 and 2.72g of KI in 400ml of water. These were mixed and allow to stand when KNO3 crystals out. The supernatant was decanted off and made up to 100ml with distilled water. The alkaloids were regenerated from the precipitate by treating with sodium carbonate followed by extraction of the liberated base with ether. To 50ml of alcoholic solution of extract was added to 2ml of HCl. To this acidic medium 1ml of reagent was added. An orange red precipitate produces immediately indicates the presence of alkaloids.

b)Wagner's test (Iodine-Potassium iodide solution)

1.2 gm of Iodine and 2gm of H2SO4 and the solution was diluted to 100ml. 10ml of alcoholic extract was acidified by adding 1.5% v/v of HCl and a few drops of Wagner's reagent. Formation of yellow or brown precipitate confirmed the presence of alkaloids.

2. Test for Glycosides

A small amount of alcoholic extract was dissolved in 1ml of water and the aqueous NaOH solution was dissolved in 1ml of water and the aqueous NaOH solution was added. Formation of yellow color indicates the presence of glycosides.

4. Test for Flavanoids

In a test tube containing 0.5ml of alcoholic extract, 5-10 drops of dilute Hcl and small piece of ZnCl or magnesium were added and the solution was boiled for a few minutes. In the presence of flavanoids, reddish pink or dirty brown color was produced.

6.Test for Steroid

Salkowski test

To 2ml of chloroform extract, 1ml of concentrated sulphuric acid was added carefully along the sides of the test tubes. A red color was produced in the chloroform layer in the presence of steroids[7].

NITRIC OXIDE ASSAY

Nitric oxide radical scavenging assay

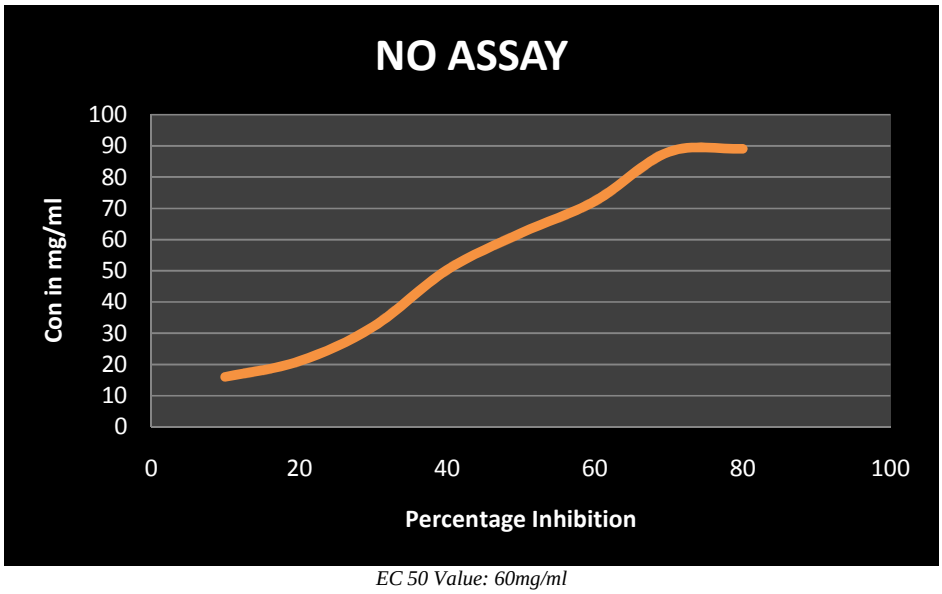
Sodium nitro prusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with O2 to produce nitrite ions, which can be measured at 540nm spectrophotometrically in the presence of Griess reagent.

Procedure: 5mg of extract was dissolved and mad up to 10ml with methanol. The sample was completely soluble. 50µl of 10mM sodium nitro prusside and 50µl test solution of various concentrations are illuminated using fluorescence light at room temperature for 150 minutes. Following incubation, 125µl of Griess reagent was added and incubated for 30 minutes at room temperature. The absorbance was measured at 546nm.(Griess reagent: 1% sulphanilic acid, 2% phosphoric acid and 0.1% N-1- napthyl ethylene diamine dihydrochloride).[8]

RESULTS

Class of Compounds	Results
Alkaloids	+
Flavanoids	+
Glycosides	_
Steroids	_
Carbohydrates	+
Amino acids	_

Nitric Oxide Assay



EC 50 Value: 60mg/ml

CONCLUSION

The present investigation revealed the presence of carbohydrates flavonoids and alkaloids in the non-polar extract of the agar wood. Most of the volatile compounds are non-polar in nature so the phytochemical screening of the petroleum ether extract revealed the class of compound present in the volatile constituent of the agar wood, which is being used as a costly perfume. The biological efficacies of compounds are also determined by using the nitric oxide scavenging assay. The study showed that the extract also possess significant free radical scavenging activity.

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