

Study on Agarwood (*Aquilaria malaccensis*) to evaluate Antibacterial and Antioxidant activities of n-hexane, chloroform and ethyl acetate extracts



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Abstract

The present research aims to investigate the antibacterial and antioxidant activities of different leaf extracts of *Aquilaria malaccensis*. Antibacterial tests were done by Disc diffusion method using Kanamycin as standard. The chloroform extract showed good antibacterial activity against *Shigella boydii* and *Escherichia coli* with 19 mm and 17 mm zone of inhibition respectively. All other extracts showed mild to moderate antibacterial activity against other bacteria with 7 – 16 mm zone of inhibition. The antioxidant activity of the extracts was determined by using DPPH spectrophotometrically and ascorbic acid was used as standard. All three extracts showed significant antioxidant activities having IC_{50} value of 38.96 μ g/ml, 39.63 μ g/ml and 64.75 μ g/ml for ethyl acetate, n-hexane and chloroform extracts respectively.

Keywords: Agarwood, Antibacterial, Antioxidant activities, DPPH free radical scavenging

INTRODUCTION

Aquilaria malaccensis or Agarwood (Family : Thymelaeaceae) is a large evergreen tree growing over 15-30 m tall and distributes in Bangladesh, Bhutan, India, Indonesia, Iran (Islamic Republic of), Malaysia, Myanmar, Philippines, Singapore, Thailand. Agarwood has been used for medicinal purposes for thousands of years, and continues to be used in Ayurvedic, Tibetan and traditional East Asian medicine. Wood is heating, alterative tonic, carminative, laxative, stomachic, diuretic, aphrodisiac and febrifuge; useful in diseases of ear and skin, hiccup, leucoderma, chronic diarrhoea, bronchitis, asthma. The fragrant resinous substance is prescribed in gout and rheumatism. The bark is used for heart disease in Khagrachari. Besides these uses, various important pharmacological activities had been evaluated from Agarwood extract. Agarwood extract showed strong anticancer activity towards human colon cancer cells^[1]. Anti-hyperglycemic and antioxidant activity of the leaves of *A. malaccensis* had also been reported^[2,3]. Medicinal uses and biological properties of Agarwood manifest to the presence of many bioactive chemical constituents. Agarwood is rich in alkaloids, tannins, phenols, terpenoids, quinones

and flavonoids^[4]. Apart from these, many compounds as 4-phenyl-2-butanone; Jinkoh-eremol; Alpha-guaiene; Beta-agarofuran; Alpha-bulnesene; Kusunol; Selina-3,11-dien-9-one; Oxo-agarospirol; Guaia-1 (10), 11-dien-15,2-olide; 1,2,3-propanetriol, monoacetate; Phenyl- β -D-glucoside, n-hexadecanoic acid; Glycerine; Squalene; 2-propanone,1,3 dihydroxy; 3,7,11,15-Tetramethyl-2-Hexadecen-1-ol (Phytol); Dodecyl acrylate; 2,3-Dihydro-3,5-Dihydroxy-6-methyl-4H-pyran-4-on; 1-Tetradecanol; 6-ethyl-5-hydroxy-2,3n,7-trimethoxynaphthoquinone; 9,12,15-Octadecatrienoic acid, Octaethylene glycol monodecyl ether had also been isolated from the volatile oils of Agarwood^[5,6]. Nine endophytic fungal strains were isolated with three new compounds as 1,7-dihydroxy-3-methoxyanthraquinone, propyl p-methoxy phenyl ether and 6-methoxy-7-O-(p-methoxyphenyl)-coumarin having moderate antimicrobial activity^[7].

DRUGS AND CHEMICALS

DPPH (1, 1-diphenyl - 2-picryl hydrazyl) was obtained from Sigma Aldrich USA. Ascorbic acid was obtained from SD Fine Chem. Ltd, Biosar, India. Kanamycin was collected from Square Pharmaceuticals Ltd.,

Bangladesh. Vincristine sulfate was collected from Alfa Asear Ltd. USA.

INSTRUMENTATION

The antioxidant potentiality was assayed from the bleaching of purple colored methanol solution of DPPH radical by the plant extracts using UV-spectrophotometer (Model NO. 1501PC Shimadzu, Japan) at 517 nm.

Collection and identification of the plant

The fresh leaves of *A. malaccensis* were collected in May 2014 from Khagrachari district, Bangladesh and identified by Dr. M. A. Razzaque Shah, Tissue Culture Specialist, BRAC Plant Biotechnology Laboratory, Dhaka, Bangladesh.

Plant materials extraction and fractionation

The fresh leaf was collected, sun dried for seven days and ground. The dried powder of *A. malaccensis* leaf (200gm) was soaked in 600ml of methanol for 7 days and filtered through a cotton plug followed by Whatman filter paper number 1. The concentrated ethanolic extract of leaf (16 gm) was fractionated by the modified Kupchan partitioning method^[8] into n-hexane, chloroform and ethyl acetate. The subsequent evaporation of solvents afforded n-hexane (450 mg), chloroform (700 mg) and ethyl acetate (350 mg) from leaf extract.

Microbial strains and culture media

Antimicrobial activity was carried out against six Gram negative bacteria such as *Vibrio parahemolyticus*, *Vibrio mimicus*, *Pseudomonas aeruginosa*, *Shigella boydii*, *Escherichia coli*, *Salmonella paratyphi* and three Gram positive bacteria such as *Sarcina lutea*, *Bacillus megaterium* and *Bacillus subtilis*. These bacteria were chosen to be studied as they are important pathogens and also due to rapidly developed antibiotic resistance. The microorganisms were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh. For bacteria, the culture media was prepared by nutrient agar, reconstituting with distilled water according to specification (2.8% w/v).

Antibacterial screening by disc diffusion method

Antibacterial activities of various extracts of *A. malaccensis* were carried out *in vitro* by the standard disc diffusion method^[9]. In this method, solutions of known concentration (500 µg /disc) of the test samples were made by dissolving measured amount of the samples (50 mg) in 1 ml of methanol. Then sterile filter paper discs (5 mm diameters) were impregnated with known test substances and dried. The dried discs were placed on plates (Petri dishes, 120 mm diameters) containing a suitable medium (nutrient agar) seeded with the test organisms. Standard disc of kanamycin (30 µg/disc) and blank discs (impregnated with solvents followed by evaporation) were used as positive and negative control. These plates were kept at low temperature (4 °C) for 24 hours to allow maximum diffusion. The plates were then kept in an incubator (37 °C) for 24 hours to allow the growth of microorganisms. Antibacterial activity of the test samples was observed by growth inhibition of organisms forming clear, distinct zone surrounding the discs. The antibacterial activity was expressed in terms of millimeter by measuring the diameter of the zone of inhibition. The greater zone of inhibition indicates the greater activity of the test material against the test organism.

Antioxidant activity using DPPH free radical scavenging activity

The free radical scavenging activity (antioxidant capacity) of the plant extracts on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) were estimated by the method of Brand-Williams *et al.*,(1995)^[10]. During this experiment the test samples of the extracts of *A. malaccensis* at different concentrations were mixed with 3.0 ml of DPPH methanol solution. The antioxidant potentiality was assayed from the bleaching of purple colored methanol solution of DPPH radical by the plant extracts by UV- spectrophotometer at 517 nm. Ascorbic acid was used as a positive control. Percent scavenging of the DPPH free radical was measured using the following equation-

$$\% \text{ DPPH radical scavenging} = [1 - (As/Ac)] \times 100$$

Here, Ac = absorbance of control, As = absorbance of sample solution.

Then % inhibitions were plotted against respective concentrations used and from the graph IC₅₀ was

calculated. The lower IC₅₀ indicates higher radical scavenging activity and vice versa.

RESULT AND DISCUSSION

Antibacterial assay

Antibacterial activity of *A. malaccensis* leaves was evaluated by Disc diffusion method using Kanamycin as standard. The extracts possess anti bacterial activity with average zone of inhibition 6 -17mm against nine human pathogenic gram positive and gram negative bacteria. The chloroform extract showed highest zone of inhibition of 19mm, 17mm and 16mm against *S. boydii*, *E. coli* and *P. aeruginosa*. Ethyl acetate and n – hexane extracts also showed good antibacterial activity with 15 mm zone of inhibition against *V. mimicus* and *P. aeruginosa*. These extracts showed mild to moderate activity against other pathogenic bacteria. Among the all extracts the chloroform extract correlate the medicinal uses of Agarwood with the research study.

Antioxidant activity

Antioxidant activity of Agarwood leaves was evaluated by DPPH free radical scavenging activity using ascorbic as standard. The n-hexane, chloroform and ethyl acetate leaf extracts of Agarwood possess significant antioxidant activity with IC₅₀ value of 39.63µg/ml, 64.75µg/ml and 38.96µg/ml respectively whereas IC₅₀ value of ascorbic acid was 13.96µg/ml. As is observed in Figure 1, the extracts showed concentration dependant DPPH radical scavenging activity that verifies the previous chemical reports.

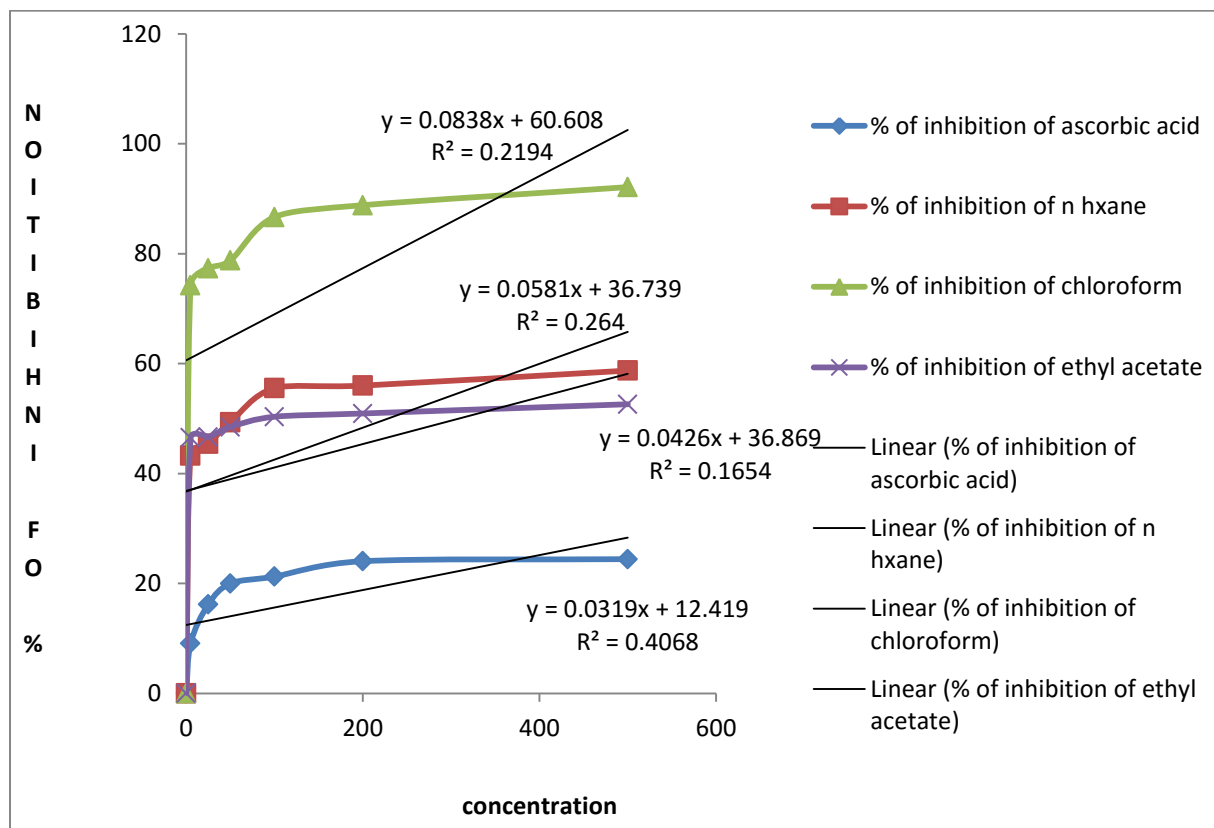
CONCLUSION

The present study reveals Agarwood as a potential source of antimicrobial agents and natural antioxidants. The chloroform extract showed the strongest antibacterial activities that pursue further characterization in different models to obtain bioactive compounds. All extracts potentiate the medicinal uses of Agarwood having strong antioxidant activity. Safe natural antioxidants are of great demand in recent years. So further studies may also be conducted active constituents.

Table 1: Antibacterial Screening of different extracts of *A. malaccensis*.

Group	Test Organisms	Diameter of Zone of Inhibition (mm)			
		n-hexane 500µg/ disc	Chloroform 500µg/ disc	Ethyl acetate 500µg/ disc	Kanamycin 30µg/ disc
Gram positive bacteria	<i>B. subtilis</i>	11	14	7	33
	<i>S. lutea</i>	7	14	8	29
	<i>B. megaterium</i>	9	12	8	30
Gram negative bacteria	<i>V. parahaemolyticus</i>	11	14	7	32
	<i>V. mimicus</i>	9	8	15	29
	<i>S. dysenteriae</i>	7	13	8	30
	<i>E. coli</i>	8	17	9	35
	<i>P. aeruginosa</i>	15	16	6	28
	<i>S. boydii</i>	11	19	10	34

Figure 1: Determination of IC₅₀ values for standard and n - hexane, chloroform and ethyl acetate extracts of *A. malaccensis* from linear correlation between of concentration versus percentage of scavenging of DPPH.



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