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Full Length Research Paper

Phytochemical and antimicrobial screening of extracts of *Aquilaria agallocha* Roxb.

Manasi Dash[1]*, Jayanta Kumar Patra[2], Prasanna Priyadarshini Panda[2]

[1]Trident School of Biotech Sciences, TACT, Bhubaneswar, Orissa-751024, India.
[2]P. G. Department of Biotechnology and Bioinformatics, Academy of Management and Information Technology, Bhubaneswar, Orissa-751009, India.

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***Aquilaria agallocha* Roxb. is an endangered economic plant used for production of agar wood worldwide. The aqueous and methanol extracts along with dry powder of leaf and bark of the plant was screened for the presence of phytochemicals. Also they were tested for antibacterial activity against pathogenic bacteria such as *Shigella flexneri*, *Bacillus brevis*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. The result indicates the presence of alkaloids, anthroquinones, triterpenoids, tannins, fixed oils and fats and glycosides in methanol extracts whereas saponins, fixed oils and fats, alkaloids and triterpenoids were found in the aqueous extracts. The highest alkaloid content was in the aqueous extract of the bark (0.06%). The saponin content was found to be high in the leaf powder (0.169%). The bark powder contained 0.067% glycosides while the leaf powder had 0.036%. The leaf powder had the highest amount of carbohydrates (19.42 mg/g dry weight), protein (24.37 mg/g DW) and amino acids (12.1 mg/g DW). The methanol extract of the leaf gave the highest zone of inhibition against *B. subtilis* (19 mm). All other extracts showed moderate zones of inhibition (14 - 18 mm) against all the bacteria tested. The present study has proved the usefulness of agarwood tree for medicinal purposes. The presence of phytochemicals indicates its potential as a source of useful drugs.**

Key words: *Aquilaria agallocha*, phytochemicals, antibacterial activity.

INTRODUCTION

Today natural products derived from plants are being tested for presence of new drugs with new modes of pharmacological action. A special feature of higher plants is their capacity to produce a large number of secondary metabolites (Castello et al., 2002). Recent studies are involved in the identification and isolation of new therapeutic compounds of medicinal importance from higher plants for specific diseases (Erturk et al., 2006; Mohanta et al., 2007).

Knowledge of the chemical constituents of plant is helpful in the discovery of therapeutic agent as well as new sources of economic materials like oil and gums. The most important bioactive constituents of these plants are alkaloids, tannins, flavonoids and phenolic compounds (Kumar et al., 2007). In India large number of plant species had been screened for their pharmacological properties but still a vast wealth of endangered species are unexplored.

Aquilaria is a genus in the family Thymeleceae and class Magnoliopsida. It is native to southwest Asia. This tree occurs particularly in rain forest of Indonesia, Thailand, Cambodia, Laoes, Malaysia, Northern India, Philipines and Borneo. Studies revealed that agarwood has remarkable anticancer activity (Gunasekera et al., 1981). The benzene extracts of the plant have central nervous system antidepression activities (Okugawa et al., 1993). Rise in demand for agarwood resulted in irrational cutting of the tree trunk for extraction of the chemical. This has resulted in the tree becoming endangered. There is no detail systematic documentation of presence and type of phytochemicals in agarwood. Hence the present study aimed at an evaluation of the presence of different phytochemicals along with antibacterial activity of methanol soluble, water soluble extracts and dry powder obtained from bark and leaf of *Aquilaria agallocha.*

*Corresponding author. E mail: manasi_dash@yahoo.com, jayantakpatra@yahoo.co.in.

Table 1. Phytochemical composition of different extracts from leaf and bark of *A. agallocha*.

Phytochemical	MSB	WSB	BP	MSL	WSL	LP
Alkaloid	+++	+	+	++	++	+
Saponin	++	+++	+++	+	+	+++
Tannin	++	++	++	++	+	+++
Anthroquinone	+++	+	++	+++	+	++
Fixed oil & fats	++	+	++	+	++	++
Glycoside	++	+	++	++	++	++
Triterpinoid	+++	+++	++	++	++	++

+ = Low, ++ = moderate, +++ = high, MSB = methanol soluble bark extract, WSB = aqueous bark extract, BP = bark powder, MSL = methanol soluble leaf extract, WSL = aqueous leaf extract, LP = leaf powder.

MATERIALS AND METHODS

Plants materials

The leaves and bark of Agur (*A. agallocha*) utilised in this investigation were collected from Satsangha Vihar, Bhubaneswar. The specimen was identified by Prof. Trinath Moharana, Head (Retired), Department of Horticulture, Orissa University of Agriculture Technology, Bhubaneswar, Orissa. India.

Preparation of extracts

The bark and leaves of the plant were shade dried for 15 days and then pulverized into fine powder using pestle and mortar. 25 g of fine powder was added to a soxhlet apparatus along with a solvent methanol or water for extraction of chemicals. The liquid extracts were evaporated to dryness by vacuum distillation and stored at 4°C for further analysis (Mohanta et al., 2007). Percentage yield was calculated from the dry extract powder.

Preliminary phytochemical analysis

A qualitative phytochemical test to detect the presence of alkaloid, tannin, saponin, flavonoid, glycoside and phenol and quantitative estimation of alkaloids, saponin and glycosides were carried out using standard procedures as described by Sofowara, (1993), Trease and Evans (1989), Harborne (1973), Mohanta et al. (2007), Kumar et al. (2007) and Edeoga et al. (2005). Total carbohydrate content was determined by phenol sulphuric acid method and amino acid content by ninhydrin method (Sadasivam and Manickam (2005). Protein content was estimated by Lowry's method (Lowry et al., 1951).

Antimicrobial activity

All bacterial strains mainly *S. flexneri*, *B. brevis*, *P. aeruginosa* and *B. subtilis* were obtained from the P.G. Department of Biotechnology, North Orissa University, Baripada, Orissa. All the cultures were maintained in Nutrient Broth media at 37°C. Agar cup plate method (ACPM) of Mohanta et al. (2007) and Rath et al. (2002) were carried out to establish the antibacterial activity of the methanol and aqueous extracts against the test pathogens. Nutrients agar (NA) plates were prepared as per manufacturer instructions. Overnight nutrient broth culture of the test organisms was seeded over the NA plates using sterile cotton swab so as to make lawn culture. Wells of 6 mm diameter were punched over the agar plates using sterile gel puncher (cork borer). The bottom of the well was sealed by pouring 10 – 20 µl (1 – 2 drops) of molten NA into the scooped well by the sterile micropipette. 100 µl (50 mg/ml) of extract were poured into the wells. The plates were incubated at 37°C for 24 h. The zone of the clearance around each well after the incubation period, confirms the antimicrobial activity of the respective extract. Each experiment was carried out in triplicate. The clear zones formed around each well were measured and average diameter of the inhibition zone (excluding inhibition zone by DMSO) was measured and expressed in millimeter. It was used to determine the antibacterial activity of extracts. Standard gentamycin (10 µg/disc) was used as control.

Statistical analysis

All the experiments were done in three replicates with each replication consisting of three test tubes for quantitative analysis. The results were analyzed using ANOVA one way at $p \leq 0.05$.

RESULTS AND DISCUSSION

The percentage yield of methanol soluble extract and aqueous soluble extract of *A. agallocha* bark and leaf was 9.101, 5.389% and 8.285, 4.571% respectively. The phytochemical screening and quantitative estimation of the percentage crude yields of chemical constituents of the *A. agallocha* showed that the methanol soluble leaf extract (MSLE) contains high amount of carbohydrate and anthroquinone while protein, amino acid, alkaloid, tannin, glycoside and terpenoid occur in moderate amount (Table 1). The methanol soluble bark extract (MSBE) contain amino acid, alkaloid, anthroquinone and terpenoid in high concentration whereas saponin, tannin, glycoside, fixed oil and fat were present in lesser amounts. The water soluble extracts of both bark and leaf (WSBE and WSLE respectively) were rich in amino acids, saponins and terpenoids but poor in carbohydrates, proteins, alkaloids, tannins, anthroquinone, glycosides, fixed oils and fats (Table 1).

The analysis of variance indicated significant difference in alkaloid content for leaf and bark extracts as well as dry powder. Quantitative estimation indicated that WSBE contain high alkaloid content of 0.06% (Figure 1A) followed by 0.05% in leaf dry powder (LDP) and 0.038% in bark dry powder (BDP). Reports indicate that naturally

Table 2. Quantity of different biochemical present in different extracts of *A.agallocha*.

Sample	Carbohydrate content (mg/g)	Protein Content (mg/g)	Amino acid content (mg/g)
MSB	9.6	15.5	5.3
WSB	14.2	23.3	8.1
MSL	9.8	22.8	6.4
WSL	16.1	16.9	10.05
BP	14.325	16.4	9.6625
LP	19.425	24.375	12.7125
Mean	**13.908**	**19.879**	**8.7042**
C.V [E]	**3.53**	**5.32**	**5.83**
C.D [P=0.05]	**1.72**	**3.70**	**3.62**

MSB = Methanol soluble bark extract, WSB = aqueous bark extract, BP = bark powder, SL = methanol soluble leaf extract, WSL = aqueous leaf extract, LP = leaf powder, $C.V_{[E]}$ = coefficient of variance due to error, C.D. = critical differentiation.

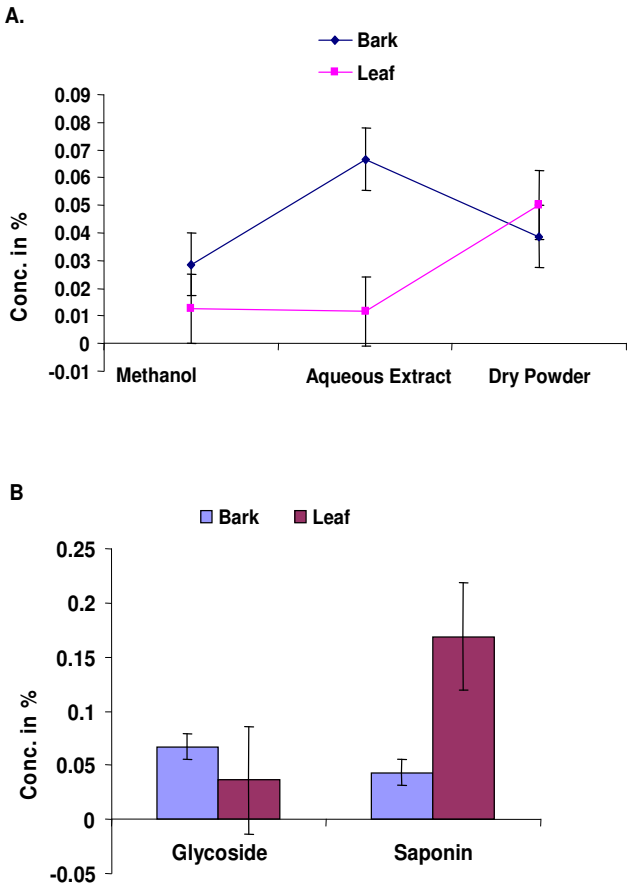

Figure 1. Phytochemical content in Agur plant extracts. **A.** Alkaloid content in methanol extract, aqueous extract and dry powder, **B.** Glycoside and saponin content in bark and leaf powder, bars represent standard error.

occurring alkaloids and their synthetic derivatives have analgesic, antisplasmodic and bactericidal activities (Okwu and Okwu, 2004). They exhibit marked physiological activity when administered to animals. Classes of alkaloids are among the major powerful poisons known. Apart from being poisonous, some alkaloids are known to be useful in correcting renal disorders (Konkwara, 1976).

The saponin content was found to be high in LDP (0.169%) as indicated in Figure 1B. The BDP contains 0.043% saponin. Saponin has the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Sodipo et al., 2000; Okwu, 2004). These properties bestow high medicinal activities on the extracts. It has also been shown that saponins are active antifungal agents (Sodipo et al., 1991). This therefore supports the earlier finding that extracts of the plants used in the present work may be useful in the chemotherapy of mycotic infections. The BDP contains 0.067% glycoside while LDP contain 0.036% (Figure 1B).

Several phenolic compounds like tannins present in the cells of plants are inhibitors of many hydrolytic enzymes such as proteolytic macerating enzymes used by plant pathogens. Other compounds like saponins also have antifungal properties (Aboaba and Efuwape, 2001; Mohanta et al., 2007). Many plants contain non-toxic glycosides that can get hydrolyzed to release phenolics that are toxic to microbial pathogens (Aboaba and Efuwape, 2001). Therefore the compounds detected may be responsible for the antibacterial activity of extracts of *A. agallocha.*

The analysis of variance (Table 2) indicated significant difference for carbohydrate content in the extract of *A. agallocha*. The LDP contains the highest amount of carbohydrate (19.42 mg/g dry weight (DW)). The MSBE and MSLE extract have the least amount of carbohydrate.

High amount of protein (24.3 mg/g DW) was found to be present in LDP and lowest protein content of 15.5 mg/g

Table 3. Inhibition zone in mm by different extracts of *A. agallocha*.

Strain	MSL	MSB	WSL	WSB	Gentamycin
S. fleximenia	-	-	18	15	23
B. brevis	-	-	-	-	22
P.aeroginosa	-	-	15	14	-
B. subtilis	19	-	-	15	19

- = no inhibition, MSB = methanol soluble bark extract, WSB = aqueous soluble bark extract, MSL = methanol soluble leaf extract, WSL = aqueous leaf extract.

DW was observed in MSBE (Table 2). The dry powder had high amount of protein (40.77 mg/g DW) as indicated in Table 2, compared to bark extract (38.8 mg/g DW) and leaf extract (39.7 mg/g DW). The amino acid content showed significant mean difference (Table 2). High amount of amino acid was found to be present in LDP (12.71 mg/g DW) followed by WSLE (10.05 mg/g DW).

The antibacterial activity of the methanol and aqueous extracts of leaf and bark of *A. agallocha* were studied by agar well method. It was observed (Table 3) that aqueous extract of both leaf and bark produced clear inhibition zones in *S. flexneri* and *P. aeruginosa*. Methanol extract of leaf showed inhibitory effect against *B. subtilis* whereas the methanol extract of the bark did not show any inhibition effect. Antimicrobial properties of substances are desirable tools in the control of harmful microorganisms especially in the treatment of infectious diseases and in food spoilage. The active components usually interfere with growth and metabolism of microorganisms and prevent them from contamination (Aboaba et al., 2001; Mohanta et al., 2007). The present study indicates the usefulness of agarwood tree for medicinal purposes. The presence of phytochemicals indicates its potential as a source of useful drugs.

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