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Phytochemical Screening and Exploration of the Pharmacological Potentials of the Mangrove Plant *Excoecaria agallocha* Linn

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Abstract

This study focused on evaluating the methanolic extract of leaves of *Excoecaria agallocha* Linn., a mangrove tree of Euphorbiaceae family, for its phytochemical properties and pharmacological activities. The antioxidant assay was performed by using the DPPH free radical scavenging method, ferric reducing power assay, and by investigating total phenolic and total flavonoid content. The cytotoxic assessment of the extract was performed by using the brine shrimp lethality bioassay method. The phytochemical assessment of leaf extract indicated the existence of carbohydrates, reducing sugars, glycosides, tannins, alkaloids, steroids, terpenoids, and flavonoids. The quantitative antioxidant potentiality by DPPH free radical scavenging assay revealed that the extract indicated antioxidant activity with IC_{50} value of 65.75 µg/mL as compared to ascorbic acid (IC_{50} = 4.17 µg/mL). In the ferric reducing power assay, the extract exhibited an increasing trend of ferric reducing power activity with the increasing concentration. According to this study, the total flavonoid and total phenolic content of the extract were 77.28 ± 0.16 mg QE/g and 61.76 ± 0.15 mg GAE/g, respectively. In the brine shrimp lethality bioassay, the LC_{50} value for the extract was 26.05 µg/mL, which was compared to vincristine sulphate (LC_{50} = 1.54 µg/mL). The findings of this study exposed that different bioactive phytochemicals existed in the extract that showed different pharmacological activities. This research has served as background for further study to isolate, purify, and identify the responsible bioactive compounds for clinical use against various disease conditions.

Keywords

Excoecaria agallocha Linn., Phytochemical, Pharmacological Potential,

Cytotoxic, Antioxidant

1. Introduction

Sundarbans is the world's largest mangrove forest, situated in the southern part of Bangladesh [1]. Mangrove plants are naturally adapted to various tough and harsh environmental conditions such as low oxygen, sturdy winds, high salinity, and muddy anaerobic soil [2]. Due to these adaptive features, the secondary metabolites of mangrove plants are rich sources of valuable bioactive compounds. These secondary metabolites exhibit several significant biological actions [3]. Nowadays, the emergence of dreadful illnesses, including cancer, microbial infections, and neurological diseases, has become the biggest threat to global health. It is also concerning that cancer and microbial infections have become resistant to current medications. To treat these illnesses, a novel therapeutic component is therefore obviously required. It is commonly known that mangrove plants found in unique ecological niches, like the Sundarbans, have remarkable secondary metabolites such as alkaloids, flavonoids, phenols, and terpenoids that have therapeutic value against many alarming physical and neurological diseases [3].

Oxidative free radical chain reaction is one of the major causes of many physical and neurological diseases including cancer, Alzheimer's, and Parkinson's disease [4] [5]. Antioxidants can prevent the production of Reactive Oxygen Species (ROS), and thus neurodegeneration is prevented in Alzheimer's disease. Natural and synthetic antioxidant agents have the potential to minimize that oxidative damage, but synthetic antioxidants are allied with adverse effects and are prone to toxicities. For this reason, natural antioxidant alternatives derived from plant sources are highly recommended. Flavonoids and phenolic compounds show antioxidant properties and therefore are qualified to have an oxidative stress-lowering impact. There is a strong relationship between oxidative stress and cancer. Oxidative stress plays a vital role in the growth and uncontrolled cell proliferation of tumors. High proliferation of tumor cells is proportional to high ROS production, and antioxidants can protect against ROS production; thus, antioxidants are effective against cancer cells such as lung, breast, and prostate cancer [6]. The high content of flavonoids and phenols is related to the medicinal properties of phytochemicals, which are useful therapeutic agents to treat cancer and many infectious diseases.

To explore the richest source of mangrove plants in Bangladesh, we were motivated to select the mangrove plant *Excoecaria agallocha* Linn., which is an evergreen tree of the Euphorbiaceae family. The local name of this plant is Gewa [1]. This plant is distributed throughout the mangrove forest worldwide. The goal of the study was to screen the prominent biochemical components and explore the biological activities of the methanolic extract of leaves of *E. agallocha.*

2. Methods and Materials

2.1. Sample Collection and Extract Preparation

The leaves of *E. agallocha* Linn. (Family: Euphorbiaceae) were collected from the Andermanik range of Sundarbans. The time of collection was March 2023 during the daytime. The fresh leaves were collected from trees on the bank of the river. During collection, all types of contamination were strictly prohibited. They were identified by the experts of Bangladesh National Herbarium, Dhaka, Bangladesh, and tagged with the accession number DACB 114863.

To prepare the leaf extract, at first the leaves were thoroughly cleaned and subjected to drying without any direct sunlight. The dried leaves were finally ground by a grinder machine. Then, 2.5 kg of leaf powder of *E. agallocha* was macerated with 7.5 liters of distilled methanol for two weeks. To optimize efficient extraction, the mixture was shaken periodically. Following maceration, the extract was filtered using filter paper. After filtration, the solvent was evaporated by rotary evaporator. This cold maceration and filtration process was repeated thrice over a 7-day period. The final methanolic extract of leaves of *E. agallocha* was formed with a weight of 144.39 gm.

2.2. Phytochemical Assessment

The qualitative phytochemical assessment was conducted to identify the main group of chemical constituents of the extract using standard procedures [7]-[10]. This experiment was carried out for screening reducing sugar, combined reducing sugar, tannins, flavonoids, saponins, gum, steroids, alkaloids, glycosides, protein, terpenoids, and acidic compounds (**Table 1**).

2.3. Antioxidant Activity

Antioxidant potentiality of the extract was estimated by the DPPH free radical scavenging assay [11]. In this assay, ascorbic acid was used as the standard. Samples of different concentrations (1, 5, 10, 50, 100, 200, 500 μg/mL) were incubated with 0.004% DPPH solution for 30 minutes in a dark and dry place. After 30 minutes, absorbance data were taken by a UV-visible spectrophotometer at a 517 nm wavelength. To ensure accuracy, all measurements were executed in triplicate. By using the % inhibition vs. concentration graph (**Figure 1**), IC_{50} values for samples and standard were calculated [12].

$$\% \text{ inhibition} = [(\text{blank absorbance} - \text{sample absorbance})/\text{blank absorbance}] \times 100$$

2.4. Ferric Reducing Power Assay

The ferric reducing power assay is one of the methods to estimate the antioxidant capacity of substances [13]. Various concentrations of extract were added with 2.5 mL 0.2 M phosphate buffer (pH 6.6) and 2.5 mL 1% w/v potassium ferricyanide, followed by incubation at 50˚C for 20 minutes. Then, 2.5 mL 10% w/v trichloroacetic acid was added to the mixtures and subjected to centrifugation at 3000 rpm for 10 minutes. 2.5 mL supernatant was taken and mixed with 2.5 mL of distilled

water and added to 0.5 mL 0.1% w/v ferric chloride solution, followed by thorough mixing. Absorbance was measured at 700 nm wavelength by UV-visible spectrophotometer. Reducing power of the extract was assessed by comparison with standard ascorbic acid by plotting absorbance vs concentration (**Figure 2**). Increase of absorbance with the concentration was considered the parameter to ascertain reducing power.

2.5. Total Phenolic Content

Total phenolic content of the extract was assayed by the Folin-Ciocalteu (FC) method [14]. The standard calibration curve (**Figure 3**) was created using absorbance data resulting from the standard (Gallic Acid) concentrations (20, 40, 60, 80, 100 µg/mL). Five mL of 10% FC reagent and four mL of 7% $NaCO_3$ solution were added to each concentration tube and the final volume was made up to 10 mL. A blue-colored mixture was formed, which was incubated at 40˚C for 30 minutes in a hot water bath. The absorbance of the extract was measured at 593 nm wavelengths by a UV-visible spectrophotometer. The experiment was executed in triplicate. The result was expressed as milligrams of Gallic Acid Equivalent (GAE) per gram of extract.

2.6. Total Flavonoid Content

Total content of flavonoid in extract was evaluated by aluminum chloride colorimetric assay [15]. In this assay, quercetin was used as a standard. A simple description of this assay involves the addition of 0.7 mL, 5% w/v $NaNO_2$ into 1 mL of extract solution and various concentrations of standard. Then 10 mL methanol and 0.7 mL of 10% w/v $AlCl_3$ were added. One minute later, 5 mL of 1 M NaOH was added to the resultant solution followed by incubation at 37˚C for 30 minutes. The standard calibration curve (**Figure 4**) was constructed using absorbance data resulting from the quercetin standard concentrations measured at 415 nm wavelength. The experiment was executed in triplicate to ensure accuracy. The result was expressed as milligrams of Quercetin Equivalent (QE) per gram of extract [16].

2.7. Cytotoxic Activity

The cytotoxic activity test was assayed by brine shrimp lethality bioassay [17]. For this assay, vincristine sulphate was used as the standard. Through serial dilution, different concentrations of the sample and standard were prepared. Ten alive brine shrimp nauplii (*Artemia salina*) were added into each set of test tubes. After 24 hours, the three sets of test tubes were observed, and the number of surviving nauplii was counted and noted down. To ensure efficacy, the total procedure was conducted in triplicate. The LC_{50} value of the extract and standard was estimated by linear correlation found from the graph (**Figure 5**, **Figure 6**) by plotting percentage of mortality vs concentration.

% mortality = [(avg. no. of alive shrimp in control − avg. no. of alive shrimp in sample)/avg. no. of alive shrimp in control] × 100

3. Results

3.1. Phytochemical Assessment

The findings of the phytochemical assessment showed that the methanolic extract of *E. agallocha* contained various groups of bioactive components (Table 1).

Table 1. Result found from phytochemical assessment.

Components	Result
Reducing sugar	Present
Combined reducing sugar	Absent
Tannins	Present
Flavonoids	Present
Saponin	Absent
Gums	Present
Steroids	Present
Alkaloids	Present
Glycoside	Present
Proteins	Absent
Terpenoids	Present
Acidic compounds	Absent

3.2. DPPH Assay

The DPPH assay revealed that the extract exhibited prominent free radical scavenging potential. From the percentage of inhibition vs concentration curve (Figure 1), the obtained IC_{50} values of the sample and standard ascorbic acid were 65.75 µg/mL and 4.17 µg/mL, respectively.

3.3. Ferric Reducing Power Assay

From the findings of the ferric reducing power assay, the pattern of the diagram (Figure 2) gives a clear idea that both the extract and the standard follow an increasing trend of ferric reducing activity with increasing concentration.

3.4. Total Phenolic Content

The standard calibration curve (Figure 3) quantified the content of total phenols in the extract, which was measured in terms of Gallic Acid Equivalent (GAE). The standard curve equation was $y = 0.0051x + 0.007$, with a coefficient of determination $R^2 = 0.986$. The calculated total phenolic content was 61.76 ± 0.15 mg of Gallic Acid Equivalent (GAE) per gram of extract.

3.5. Total Flavonoid Content

The standard calibration curve with quercetin (Figure 4) was used to evaluate the

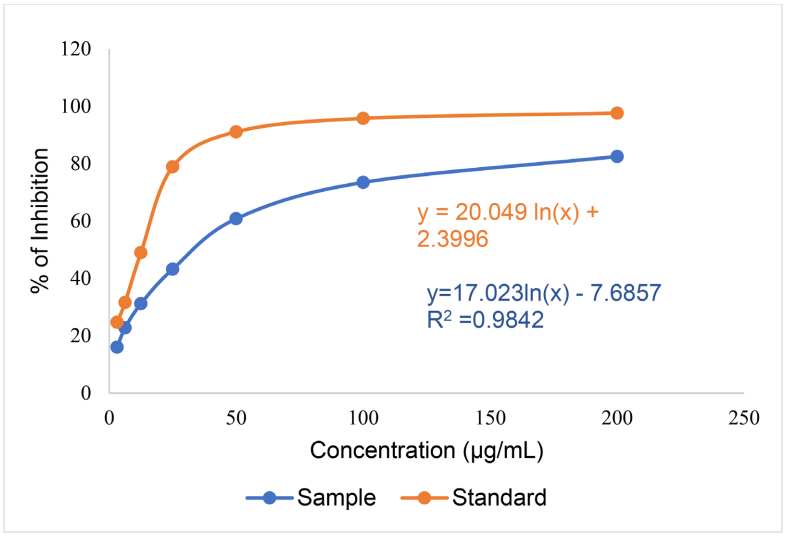

Figure 1. Percent inhibition vs concentration curve for extract and standard.

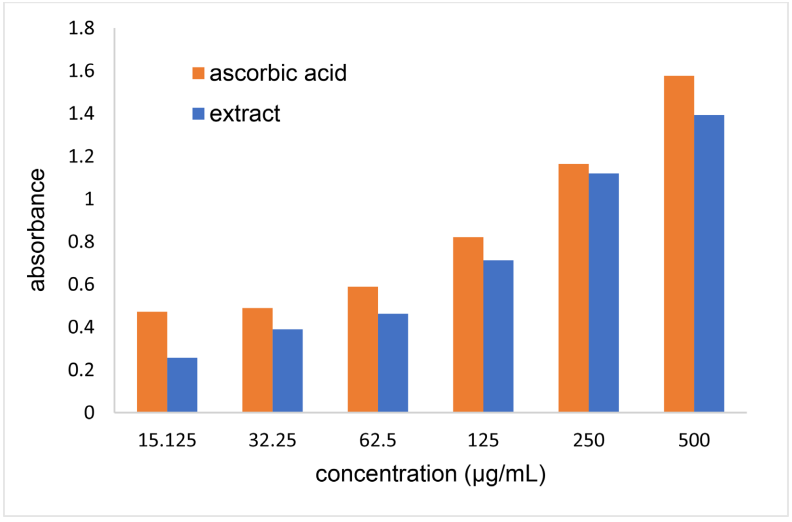

Figure 2. Absorbance vs concentration diagram of ascorbic acid and extract of *E. agallocha.*

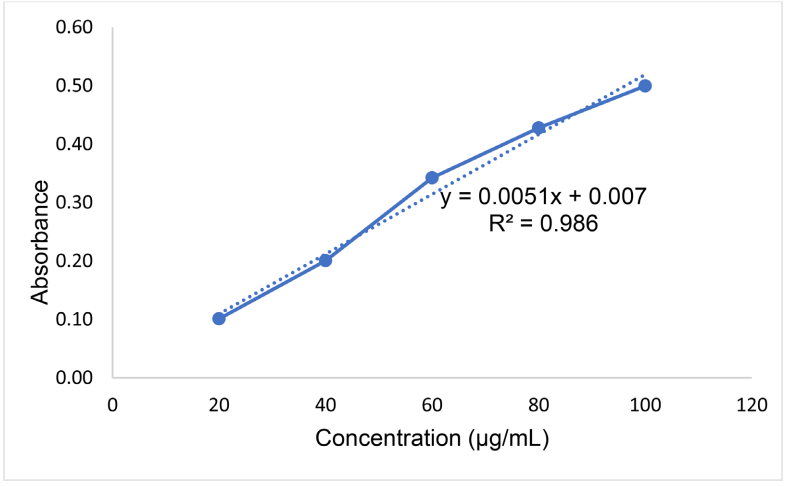

Figure 3. Standard calibration graph of gallic acid.

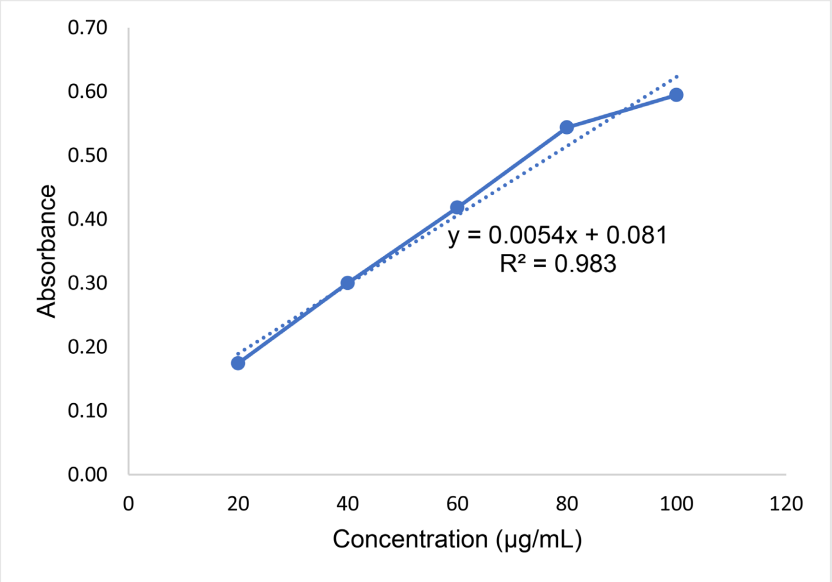

Figure 4. Standard calibration graph of Quercetin.

total flavonoid content of the extract. The standard curve equation was y = 0.0054x + 0.081 with a high coefficient of determination (R^2) of 0.983. This analysis showed that the total flavonoid content was 77.28 ± 0.16 mg of Quercetin Equivalent (QE) per gram of extract.

3.6. Cytotoxic Activity

In the brine shrimp lethality bioassay, the extract showed potential cytotoxic activity. From the percentage of mortality vs. concentration curve (**Figure 5**), the calculated LC_{50} value of the extract was 26.05 µg/mL, and from the percentage of mortality vs. concentration curve (**Figure 6**), the calculated LC_{50} value of vincristine sulphate was 1.54 µg/mL.

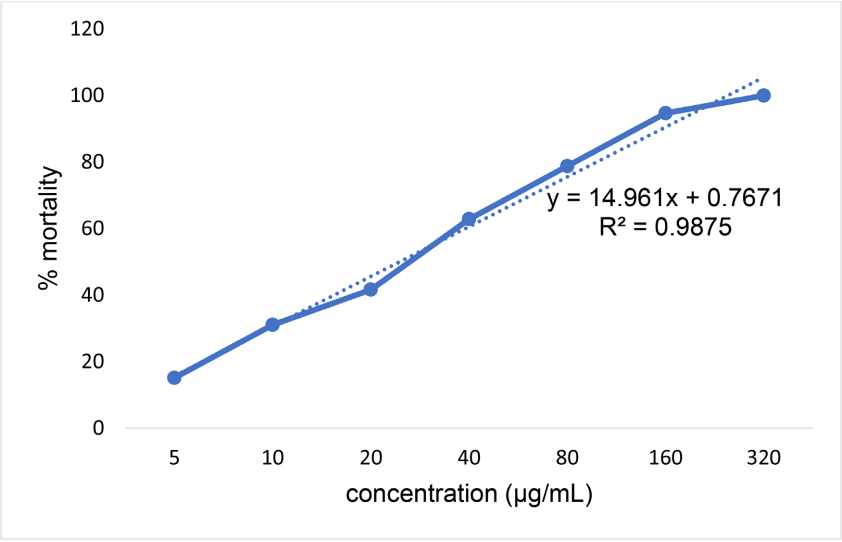

Figure 5. Percent of mortality vs. concentration curve for extract of *E. agallocha.*

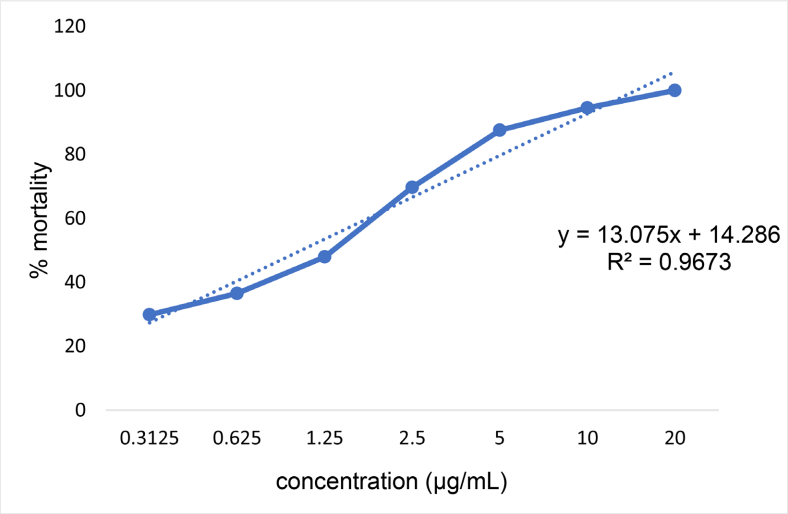

Figure 6. Percent of mortality vs concentration curve for Vincristine sulphate.

4. Discussion

This study was designed to assess the phytochemical evaluation and explore the pharmacological potentiality of the methanolic extract of leaves of the mangrove plant *E. agallocha,* which is highly distributed throughout the largest mangrove forest, Sundarbans, Bangladesh. The crude extract was subjected to analysis for its phytochemical constituents and various pharmacological activities.

Chemical group test revealed the existence of flavonoid, terpenoid, tannin, phenol, alkaloid, and glycoside in the sample. Among them, phenolic and flavonoid components are considered as more significant classes of phytochemicals [4]. The antioxidant activity test was conducted by DPPH assay. DPPH is a stable free radical that contains an odd electron that is utilized for detection of radical scavenging potential in chemical assessment. Antioxidants counteract free radicals through multiple mechanistic pathways, principally Hydrogen Atom Transfer (HAT), Single Electron Transfer (SET), and Sequential Proton Loss-Electron Transfer (SPLET). In the HAT mechanism, antioxidants donate a hydrogen atom to radical species, effectively neutralizing them and terminating chain-propagating reactions. The SET pathway involves electron donation from the antioxidant to the radical, often coupled with proton transfer (SET-PT), resulting in the formation of a relatively stable antioxidant-derived radical [18]. In the DPPH assay, the extract revealed potential DPPH free radical scavenging activity with an IC_{50} value of 65.75 µg/mL. Because of the presence of free hydrogen, phenolic compounds are responsible for significant antioxidant activity [19]. Furthermore, flavonoid, tannins, and reducing sugar were present in the extract, which can be responsible biochemicals for the antioxidant potential [20].

The ferric reducing power assay is one of the methods to estimate the antioxidant efficiency of substances. The basis of this assay is the redox reaction of antioxidant which reduces ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) in an acidic medium [13]. Increase in absorbance with concentration is considered as the parameter to

ascertain reducing power. In this assay, the extract showed an increasing trend of absorbance with the increasing concentration. This study also exposed the high phenolic (61.76 ± 0.15 mg GAE/g) and flavonoid content (77.28 ± 0.16 mg QE/g) in the methanolic extract of *Excoecaria agallocha.* The presence of prominent content of flavonoid and phenolic compounds in the extract showed various pharmacological activities.

The cytotoxic activity test was conducted by brine shrimp lethality bioassay, which is a simple and convenient assay for screening bioactive compounds having cytotoxic activity in crude extract. There is a positive co-relationship between brine shrimp bioassay and cytotoxicity [21]. In this assay, the extract showed significant cytotoxic activity with the LC_{50} value of 26.05 µg/mL.

5. Conclusion

This study explored the phytochemical properties and pharmacological activities of methanolic extract of leaves of *E. agallocha*. The findings of this study revealed that different bioactive phytochemicals existed in the extract. Because of the resulting free radical scavenging efficiency and the presence of a high content of phenolic and flavonoid content, *E. agallocha* can be a great source for developing novel natural antioxidant and anticancer agents. Thus, as the findings are based on in vitro screening, in vivo studies are required to validate the observed pharmacological effects and would provide a more balanced scientific perspective. This research has served as a background for further studies to isolate, purify, and identify the responsible bioactive compounds for clinical use against various disease conditions.

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Authors' Contribution

Kazi Shubarna Rahman: Conceptualization, conducting experiments, data analysis, and manuscript writing. Satyajit Roy Rony: Reagents and chemicals arrangement, and conceptualization. Md. Hossain Sohrab: Supervision, conceptualization, review, and editing. Firoj Ahmed: Supervision, conceptualization, review, and editing.

Data Availability

Raw data associated with this study are available upon request from the corresponding authors.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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