

PHYTOCHEMICAL, ANTI-MICROBIAL AND CYTOTOXIC STUDIES ON LEAVES EXTRACT OF *PHYLLANTHUS BEILLEI* H.

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Abstract

Microbial resistant is getting dangerous day by day. To find a new antimicrobial source is very essential. The current research study was designed to assess the antimicrobial activity of methanol extract of *Phyllanthus beillei* H. by disc diffusion method. By measuring zone of inhibition mild antimicrobial activity of *Bacillus subtilis* was found and compared with the standard Ciprofloxacin. From the result it can be concluded that *Phyllanthus beillei* H. plant may be a source of antimicrobial constituents. The plant extract also demonstrated a promising cytotoxic activity against brine shrimps nauplii. The value of LC₅₀ of methanol extract of *Phyllanthus beillei* H. was 2.4119 mg/mL which compared with the LC₅₀ value of standard (0.0171mg/mL). The overall results indicate a strong cytotoxic activity of this plant.

Key words: *Phyllanthus beillei*, antimicrobial activity, cytotoxic activity

Introduction

Plants having medicinal activities plays an important role or the treatment of disease of humankind worldwide. Plants also possess a large number of groups of chemical components such as alkaloids, tannins, glycosides, terpenoids, flavonoids etc exhibited extensive range of antimicrobial activity [1-2].

Throughout the extensive evidence of modern era, it was realized that the efectivity of antibiotic is inadequate as well as increasing of resistance. Thus, novel sources of antibiotics are necessary to investigate [3]. Moreover, it is assumed that the favorable selection of plant for the source of antimicrobial agent may stand for an appropriate source for new antimicrobial agents for therapeutic aspects. Constant investigation of new antimicrobial agent in different plant source would become a great approach to solve the arising threats of existing antibiotic resistances [4].

The term cytotoxicity is used for the quality of any agent for being toxic to the living cells. Several toxic agents are available that ate lethal for the survival of cells. The brine shrimp lethality bioassay was proposed firstly by Michael AS which was later established by Vanhaecke P and Sleet RB. This experiment is assumed as a perfect way for assessing cytotoxicity and can be subjected as an expedient indicator for the finding of novel bioactive compounds from natural sources [5-6].

The plant *Phyllanthus beillei* belong to the largest family of Phyllanthaceae which is commonly found in in Kenya, Tanzania, Malawi, Mozambique, Zambia and Zimbabwe. Also, very disjunct populations in Thailand and Cambodia and also in India and Bangladesh. The *Phyllanthus* genus contain many compounds together with tannins, antioxidants and lignans. Research on the *Phyllanthus* genus exhibited some evidence that some other species of *Phyllanthus* may offer certain benefits like liver, kidney or cancer diseases [7].

On the basis of the extensive literature reviews upon this plant, this study was performed for the investigation of the antimicrobial activity of the plant (*Phyllanthus beillei*) extract against certain pathogenic bacteria as well as also explore the cytotoxic activity tried upon brine shrimp nauplii for evaluating their potential as toxic agents [8-10].

Methods

Plant materials

Fresh leaves of the plant were collected from *Chandrima Uddyan, Dhaka, Bangladesh*. These were then separated from other plant parts and materials, cut into small pieces and dried under the mild sun for ten days. The dried leaves were crushed into coarse powder using suitable grinder. The crushed leaves were kept in an air tight container and stored for the experiment.

Preparation of plant extract: The crushed leaves of the plant were subjected in a fresh, bottle and saturated in methanol for 10 days with frequent shaking and stirring. It was then filtered by cotton and finally through Whatman filter paper. The filtrate was then evaporated to obtain desired crude extract.

Chemicals and Reagents: Standard antibiotic discs (Ciprofloxacin in 5µg/disc), 70 % methanol, Nutrient agar medium, Nutrient broth media.

Microorganisms used for the test: The bacterial strains selected for the antimicrobial assay were *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Escherichia coli*. These bacterial strains were collected from the Microbiology Laboratory of Pharmacy Discipline, Jahangimagar University, Dhaka.

Phytochemical screening methods: In order to phytochemical screening the plant extract was subjected to the available screening methods to investigate the availability of different group of chemical compounds such as glycosides, alkaloids, flavonoids, tannins, saponins and steroids [20].

Cytotoxic activity test (Brine Shrimp Lethality Bioassay):

This test was performed to investigate the cytotoxic activity of the plant extract. The brine shrimps (*Artemia salina* Leach) nauplii Ocean 90, USA were selected as the test organisms. The eggs were subjected in brine with a continuous oxygen supply for 48 hours to confirm hatching. In this assay there are 6 graded doses (eg. 5µg/ml, 10µg/ml, 20µg/ml, 50µg/ml, 100µg/ml, 200µg/ml) were used. DMSO was used as a solvent as well as a negative control. The LC₅₀ values of the test sample was measured by

a plot of % of the dead shrimps vs logarithm of the sample concentration. Vincristine sulfate was used as a reference standard [11-14].

Antimicrobial activity

This experiment for the plant extract was observed through disk diffusion method. The test microorganisms were inoculated on nutrient agar media in a sterile condition and experimental drugs and extracts were applied through a sterile loop under an aseptic condition followed by an overnight incubation at 37°C [15-19]. The test samples/reagent or extracts were used for this experiment as follows (see at the end of text).

Statistical analysis

All values were considered as mean \pm SEM. Statistical analysis was performed by One-way analysis of variance (ANOVA), followed by using student-t test. Results were measured as significant if p values were less than 0.05 ($p < 0.05$).

Results

Phytochemical test results of leaves extract of *Phyllanthus beillei* H.

This test exhibited the presence of different group of constituents such as tannin, alkaloid, glycoside, Saponins and Flavonoid, in methanol extract of *Phyllanthus beillei* H. which exhibit a great deal of pharmacological action. The outcomes of chemical group assay of the plant extract are given in the table 1.

Antimicrobial activity of leaves extracts of *Phyllanthus beillei* H.

In this study, the methanolic extract of *Phyllanthus beillei* H. leaves exhibited antimicrobial activities against *Staphylococcus aureus*, *Bacillus subtilis* and *Enteropathogenic Escherichia coli* (EPEC) in the table-2. This result of plant extract (500 μ g/disc) exhibited near about a similar result of standard drug Ciprofloxacin (5 μ g/disc). From this experiment it was showed that the methanolic extract of *Phyllanthus beillei* H. leaves possesses a promising antimicrobial activity.

Cytotoxic activity of leaves extracts of *Phyllanthus beillei* H.

In this study an increased mortality rate of the brine shrimp was observed alongside the increasing of concentration of the extract. Throughout the plotting of concentration versus response

percentage put on the Ldp line software produced an approximate straight linking between them. The concentrations plant extract at which LC₅₀ of brine shrimp nauplii observed were measured from the graph by extrapolation and was found below in the table 3.

Discussion

Phytochemical test results of leaves extract of *Phyllanthus beillei* H. showed presence of Tannins, Alkaloids, Glycosides, Saponins & Flavonoids but Steroids are absence. The results for the availability of different group of chemical components of *Phyllanthus beillei* H is shown in the Table 1.

Antimicrobial activity of this plant showed promising effect on both gram (+ve) and gram (-ve) microorganism [8]. Zone of inhibitions (mm) showed approximately similar compare to the standard. as shown in Table 2. Thus, it can be hypothesized that the plant extract may have some antimicrobial compounds which can show more promising effect if we increase the amount of extract or use the isolated compound from this extract. But it needs further isolation of compound which can be reduced the doses of the extract.

Through the test of cytotoxic activity, the methanol crude extract of *Phyllanthus beillei*. H. displays a promising activity against the brine shrimp nauplii [14]. The LC₅₀ value of methanol extract was 2.4119 mg/mL compared with the LC₅₀ value of standard (0.0171 mg/mL) exhibited in table 3. Therefore, the response attained in this assay recommends that the extract may contain cytotoxic components. Nevertheless, more investigations are needed to get more information regarding the activities of the plant extracts.

Conclusion

A large number of plants are used for treatment of diseases due to having active chemical components. A variety of studies were accomplished to confirm scientifically uses of these medicinal plants. *Phyllanthus beillei* H. is one of the major medicinal plants among them. In this study, the plant *Phyllanthus beillei* H. exhibited a promising effect regarding antimicrobial and cytotoxic activities. Therefore, a further investigation is required to

discovery of the active chemical ingredients having the targeted activities as well.

References

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Microorganisms Staphylococcus aureus, <i>Bacillus subtilis</i> , <i>Escherichia coli</i>	Standard antibiotic Ciprofloxacin (30 µg/disc)	Plant extract (500 µg/disc)
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Antimicrobial activity

Table1. Results of chemical group tests:

Test groups	Methanol extract of <i>Phyllanthus beillei</i> H. Leaves
Tannins	+
Alkaloids	+
Glycosides	+
Saponins	+
Steroids	-
Flavonoids	+

(+) indicates presence and (-) indicates absence of the components

Table 2. *In vitro* antimicrobial activity of methanol extract of leaves of *Phyllanthus beillei* H.

Bacterial Strains	Type of bacteria	Zone of inhibition (mm)	
		Ciprofloxacin (30 µg/disc)	Extract (500 µg/disc)
<i>Staphylococcus aureus</i>	Gram(+ve)	35±0.22 *	26±0.31
<i>Bacillus subtilis</i>	Gram(+ve)	35±0.34 *	30±0.32 *
<i>Escherichia coli</i>	Gram(-ve)	34±0.15 *	32±0.24 *

The values were expressed in Mean ± SEM. In each experiment (n=5) results were compared to the Control Group. * Designates significant change compared to control group (p<0.05).

Table-3: Result of cytotoxic activity test through brine shrimp lethality bio assay

Group	LC ₅₀ Value (mg/mL)
Control	1655±0.25
Standard	0.0171±0.13 *
Methanol Extract	2.4119±0.35 *

The values were expressed in Mean ± SEM. In each experiment (n=5) results were compared to the Control Group. * Designates significant change compared to control group (p<0.05).