



QUANTITATIVE ANALYSIS AND ANTIMICROBIAL ACTIVITIES OF *PHYLLANTHUS EMBLICA* (L) LEAF EXTRACTS AGAINST FISH PATHOGEN *AEROMONAS HYDROPHILA*

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ABSTRACT

The present study has been to assess the qualitative screening of phytochemical constituents and quantitative study of total flavonoid content, total phenol content, total tannin and *in-vitro* antioxidant activity; these were determined by aluminium chloride colorimetric method, Folin-ciocalteu method, and ferric reducing antioxidant power method respectively. The anti-bacterial activity of aqueous leaf extracts of different concentration of (25mg/ml, 50mg/ml, 75mg/ml and 100mg/ml) *Phyllanthus emblica* against *Aeromonas hydrophila* was done by agar well diffusion methods.

Qualitative study reported the presence of tannins, saponins, flavanoids and phenols. Quantitative investigation showed variable amounts of phytochemical constituents such as tannins, flavonoid, and phenol and antioxidant activity in the leaf extract. The most efficient inhibitory activity was examined in higher concentration (100mg/ml) of aqueous extract leaf extracts of *Phyllanthus emblica*.

From the results, the present study indicated that *Phyllanthus emblica* not only contains high amount of phytochemical compounds but showed higher medicinal value. *Phyllanthus emblica* possess powerful antimicrobial activity against *Aeromonas hydrophila* and can be used in various ayurvedic treatments.

Keywords: *Phyllanthus emblica*, phytochemical constituents, Quantitative, Qualitative analysis, *Aeromonas hydrophila*.

1. INTRODUCTION

Medicinal plants and herbs are always being proved to be very significant to the health of the individuals' and societies. In current years, several scientific investigations of traditional herbal remedies for many diseases have been tried out and this has lead in the expansion of alternative drug and therapeutic approaches [1]. Gram negative rod shaped, and facultative intracellular aquatic bacteria *Aeromonas hydrophila* commonly occurs in foods such as fish, poultry, milk and vegetables besides aquatic habitats and is the major causative agent of ulcerative disease syndrome (UDS) in fishes [2].

Medicinal plants serve as source for preparation of various effective therapeutic agents because they are used for protective, promotional and therapeutic applications by over 1.5 million practitioners of traditional medicinal system since past [3]. From time immemorial as important part of human diet; besides boosting the flavor of foods herbs and spices are also known for their preservative and medicinal value [4].

1.1. *Phyllanthus emblica*

Phyllanthus emblica (L) is commonly known as Indian gooseberry *P. emblica* (syn. *Embllica officinalis*) (amla) belongs to family Euphorbiaceae as a potent regenerating herb and has been widely used in therapeutic potentials and as a palatable plant. In Ayurveda all parts of the plant like fruits, leaves, seed, bark, roots and flowers are used for therapeutic purposes, it is reported as a vital dietary source of vitamin C, minerals and amino acids.

In tropical and subtropical parts of the China, India, and Indonesia as well as in Malay Peninsula *Phyllanthus emblica* L grows as a small or moderate size tree with greenish-grey bark and greenish-yellow flowers formed in auxiliary clusters. The leaves are feathery linear oblong with an acute apex and rounded base. The amla fruits are green fleshy, lobes and shining green changes from light yellow to brick red when mature [5].

The major metabolites in *Embllica officinalis* active against bacteria include flavanoids, ascorbic acid, Gallic acid, alkaloids and hydrolysable tannins. In the

treatments of anti-inflammatory and antipyretic disease usually leaves have been used. Today to treat the infectious diseases, indiscriminate antimicrobial drugs are used due to which the pathogenic bacteria have developed resistance against existing antibiotics [6].

For a long period of time there is a rapid increase of antibiotic resistance in our country, this difficulty has led to the continuous search and study of medicinal plants for new drugs with the objective of getting cheaper and better management options. So researchers are taking interest in exploring traditional medicines, looking for new way to develop better drugs against microbial infections.

The present study aims is to explore the phytochemical constituents and antibacterial property of the aqueous leaf extracts of amla *Phyllanthus emblica* (L) against gram negative *Aeromonas hydrophila* bacteria.

2. MATERIAL AND METHODS

2.1. Collection of Plant Material and Authentication of the plant.

The leaves of the *Phyllanthus emblica* (L) was collected from St. Aloysius' College (Autonomous), Jabalpur garden then *Annona* species was identified and authenticated by a Professor of Botany, Dr. Shikha Bansal St. Aloysius' College (Autonomous), Jabalpur. The leaves were washed thoroughly first in tap water and then rinsed with distilled water. Then it was dried completely in shade at room temperature for 30 days. The plant material were crushed and blended to fine powder in an electronic grinder (mixer) and stored in air tight plastic container till further use.

2.2. Preparation and screening of the plant extracts

For aqueous extraction, the fine powder of leaves of *Phyllanthus emblica* (L) was subjected for decoction process. It is done by boiling 10g of the leaf powder in 100ml distilled water in water bath at 70-80°C for 15 min. After boiling, it was then filtered through Whatman filter paper no.1, autoclaved at 121°C for 15 min and stored at 4°C for further use [6].

2.3. Test microorganism-*Aeromonas hydrophila*

Lyophilized cells of *Aeromonas hydrophila*-MTCC1739 was supplied by the Institute of Microbial Technology, Chandigarh, and was sub-cultured and maintained in nutrient broth at 4°C in the refrigerator The experiment was performed under strict aseptic conditions.

2.4. Antibacterial activity Studies

The antibacterial activity of aqueous leaf of *Phyllanthus emblica* (L) extracts was determined by Agar well diffusion assay [7]. The different concentrations (25mg/ml, 50mg/ml, 75mg/ml and 100mg/ml) of aqueous leaf extract were prepared and their antimicrobial activity against *Aeromonas hydrophila* bacterial culture was determined. 16g of Nutrient agar medium was dissolved in 500 ml of distilled water and autoclaved at 121°C for 15 min. Before swabbing the bacterial culture, the media was poured in sterilized Petri plates then it was allowed to cool, after the solidification of media the culture suspensions was then spread (flood) inoculated onto the surface of sterile nutrient agar media (NAM) plates. The wells, 10mm in diameter, were created using sterile agar borer and the wells were filled adding 25µl of different aqueous concentration of leaf of *Phyllanthus emblica* (L) extracts and was incubated at 37°C for 12-24hrs. One set was kept as control. Three replicates were prepared from each concentration. The aqueous concentration of leaf extract of *Phyllanthus emblica* (L) having antimicrobial activity inhibit the microbial growth and the clear zones were formed. The zone of inhibition was measured in millimeters. Observations were analyzed statistically applying standard deviation and data presented in observation table with ±SD.

3. RESULTS

3.1. Qualitative analysis

Preliminary phytochemical screening of aqueous leaf extract of *Phyllanthus emblica* (L) showing the presence of various bioactive constituents like alkaloid, flavonoids, phenol, saponin, quinones, glycosides, carbohydrate, coumarine and tannins were the most prominent and the result of phytochemical test has been summarized in the Fig.1 & 2.

3.2. Quantitative analysis

3.2.1. Estimation of total phenol and tannin content by UV spectroscopic method

The following regression equation given below was used in calculation of amounts of total phenol content present in test sample: $Y = 0.0529x + 0.0121$ ($R^2 = 0.989$) Where, X= concentration of the sample Y= absorbance (Fig.3). Total phenolic content of test sample- (3.66µg/ml). Total tannin content of test sample- (14.34µg/ml).

3.2.2. Estimation of total flavonoid content by UV spectroscopic method

The following regression equation given below was used in calculation of amounts of total flavonoid content present in test sample $Y = 0.0191x + 0.0979$ ($R^2 = 0.992$) Where, X= concentration of the sample Y= absorbance (Fig. 4). Total flavonoid content of test sample - (41.05 $\mu\text{g/ml}$).



Fig. 1: Preliminary phytochemical screening of leaves of *Phyllanthus emblica*



Fig. 2: Leaf powder of *Phyllanthus emblica*

3.3. Ferric Reducing Power Ability (FRPA) assay

FRPA assay is one of the methods used to reveal the potential of the *Phyllanthus emblica* extract to reduce the ferric-ferricyanide complex to the ferrous-ferric cyanide complex using ascorbic acid as standard. The aqueous extract of *Phyllanthus emblica* (L) demonstrated a significant reducing effect. The following regression equation given below was used in calculation of amounts of antioxidant activity present in test sample: $Y =$

$0.0641x + 0.5059$ ($R^2 = 0.968$) Where, X= concentration of the sample Y= absorbance (Fig. 5). In-vitro antioxidant activity of test sample - (4.2 $\mu\text{g/ml}$).

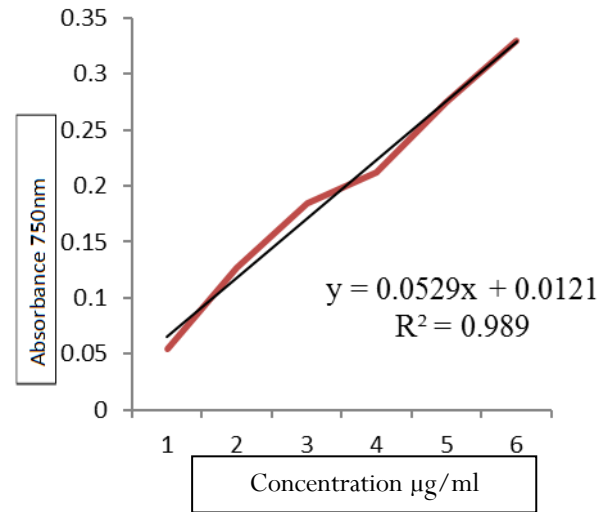


Fig. 3: UV spectroscopy method-linear curve for standard gallic acid

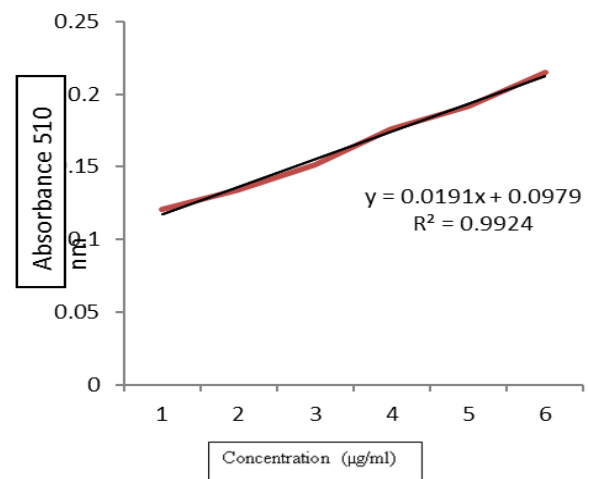


Fig. 4: UV spectroscopy method-linear curve for standard quercetin

3.4. Antibacterial activity

The outcome obtained from the analysis showed that almost all different concentration of leaf extracts of *Phyllanthus emblica* (L) showed significant antibacterial activity against gram negative bacteria *Aeromonas hydrophila*. The 100mg/ml concentration of leaf extract of *Phyllanthus emblica* (L) was found to be the most effective with highest inhibition zone of 3.5cm, followed by 75mg/ml concentration of leaf extract, inhibition zone was found to be 3cm. In 50mg/ml

concentration the inhibition zone was 2.5cm and least zone of inhibition 2cm was seen with 25mg/ml aqueous extracts respectively. The positive control streptomycin disc was used, distilled water was used as negative control, no inhibition zone was observed in control. The antibacterial activity of leaf extract is presented in Fig. 6.

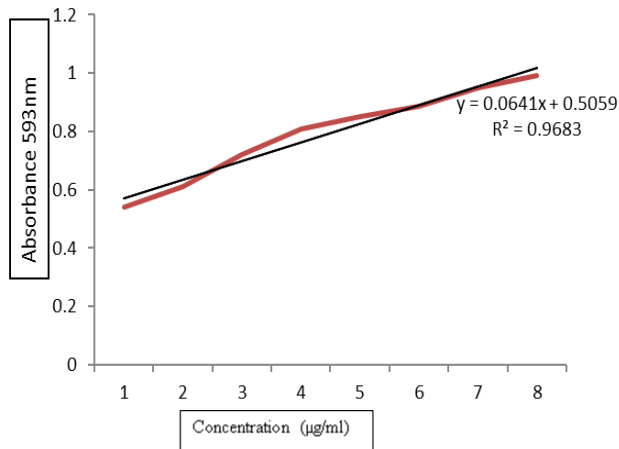


Fig.5: FRPA Method -Standard curve for Ascorbic acid

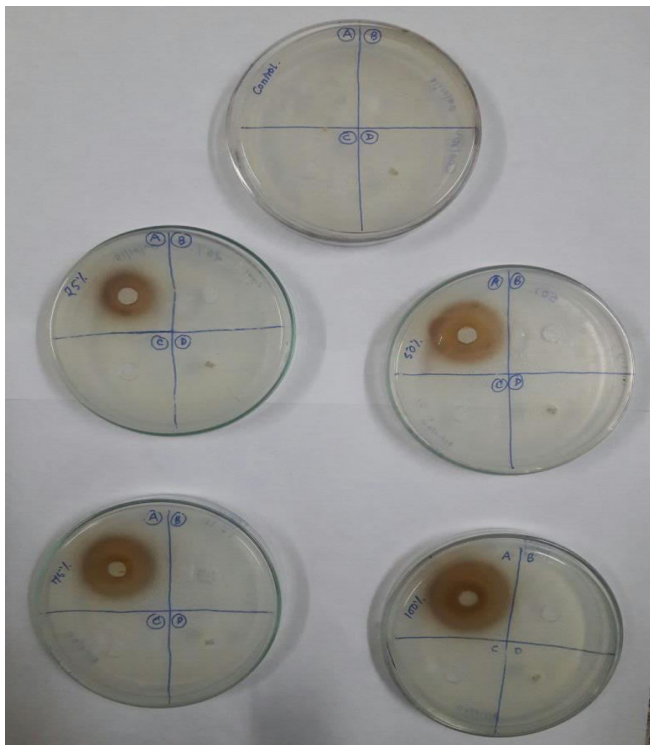


Fig. 6: Antibacterial activity of various conc. 25mg/ml, 50mg/ml, 75mg/ml & 100mg/ml of Phyllanthus emblica leaf extract

4. DISCUSSION

Medicinal plants (plant remedies) are deeply rooted component of the cultural heritage of many people from diverse cultures and countries are as such closely linked to the maintenance of good health [8]. Research currently is being carried out on the herbs and herbal extracts that have been shown to modulate the immune system of fish. These products are generally regarded as harmless and can be used as novel methods of minimizing disease risk and as a good substitution for antibiotics in aquaculture [9].

The current study agrees with the results and they found that the aqueous crude extract of *Emblica officinalis* and *Phyllanthus niruri* crude extracts showed antibacterial activity against bacterial pathogens [10]. Similar study was carried out and it was found that the *Phyllanthus emblica* L. extracts in different solvents shows inhibitory action towards seven different bacterial strains (four gram and three gram-negative bacteria) [11]. Same work was done by researcher and assessed the aqueous and methanolic extract of Amla leaf (*Emblica officinalis*) combined with some antibiotics have antibacterial activity against *E. coli* and *B.subtilis* [6].

Our investigation exhibited that the antibacterial activity shown by the leaf extracts of the *Phyllanthus emblica* is found to be active against *Aeromonas hydrophila*, possibly due to the presence of phytochemicals i.e. *tannins*, *phenols* and *flavonoids*. The outcome of the present research supports the practice of traditional therapeutic plants in curing of disease in human and animal to a certain extent. These results recommend the presence of considerable amount of bioactive compounds and signifying the occurrence of a wide spectrum of antibacterial compounds present in the plant which shows good antibacterial potency.

5. CONCLUSION

The aqueous leaf extracts used in current study revealed the presence of various bioactive compounds such as phenols, tannin, flavonoids. It also possesses antioxidant and antimicrobial activities which may be beneficial in controlling various infectious disease and its complications caused by *Aeromonas hydrophila*. The highest concentration of aqueous leaf extracts possesses effective inhibitory activity against the gram negative bacteria. The study revealed that the leaves of *Phyllanthus emblica* (L) contain a substantial amount of phenolic - flavonoid component that were considered to be the chief contributor for their antioxidant and

antibacterial activities. Hence it can be concluded that the leaf extract of *Phyllanthus emblica* can be used for separation of bioactive particles which further can be utilized in treatment of various diseases. From this study results showed that aqueous leaf extract of *Phyllanthus emblica* may be used as potent drug of natural origin.

6. REFERENCES

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