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Isolation and study of antimicrobial activities of polar and non polar flavanoids from the leaves of *Phyllanthus emblica*

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ABSTRACT

Polar flavanoids and non polar flavanoids have been isolated from the leaves of Phyllanthus emblica (Amla) by chemical method. The isolated extract was screened for antimicrobial activities against Proteus vulgaris, Staphylococcus aureus, Escherichia coli and Salmonella typhi. The extracts showed good to moderate activity against the pathogens.

Keywords : Phyllanthus emblica, Polar flavanoids, non polar flavanoids antimicrobial

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INTRODUCTION

Medicinal plants are widely used in Aurvedic medicines. Variety of plants have been extensively documented in the literature. The present study is aimed to explore some new out comings by experimenting the known plants in our surrounding nature, one such as Phyllanthus emblica.

Phyllanthus emblica has been reported to possess antiviral and antimicrobial properties [1]. It has also been reported to have potential efficacy against laboratory models of disease, such as for inflammation, age-related renal disease, and diabetes [2,3,4]. Due to diverse biological activities of Amla, it is worth experimenting to isolate particular class of chemical constituents from Amla and test their antimicrobial activity against pathogenic microbes. In the present work, Polar flavanoids and non polar flavanoids have been isolated from the leaves of Phyllanthus emblica by chemical method [5]. The isolated extract was screened for antimicrobial activities against *Proteus vulgaris*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*. The extracts showed good to moderate activity against the pathogens.

MATERIALS AND METHODS

1. Phyllanthus emblica leaves were collected , dried in shade and preserved in sealed container.
2.
A) Extraction of Polar flavanoids: - 5 g of dried leaves were boiled in 100% methanol for 5 min. The solvent was evaporated in water bath at 40^0C. Then residue was collected and small amount of petroleum ether was added. Green layer of ether was discarded. The process was repeated until the extract with no green layer was obtained. The residue was dried and used for antimicrobial activities test.

B) Extraction of Non Polar flavanoids: - 5 g of dried leaves were taken in soxhlet apparatus and extraction was done by n-hexane. The extract so obtained was collected and evaporated up to dryness and used for antimicrobial activities test.

Antimicrobial screening

1) Nutrient agar: Nutrient Agar was used as the medium for the growth of culture.

Composition of Nutrient Agar.

Sodium chloride (5 g/lit)
Beef extract (1.5 g/lit)
Yeast extract (1.5 g/lit)
Agar powder (1.5 g/lit)
$_{p}$H. 7.4 $\pm$ 0.2

Medium was prepared by dissolving the ingredients in distilled water followed by sterilization at 121^{0}C temperature & 15 lbs/inch^2 pressures for 25 min in an autoclave. After autoclaving, medium was allowed to cool to about 50^{0}C (as solidification starts at 45$\pm$ 2^{0}C) poured carefully into sterile petridishes and allowed to solidify.

2)Nutrient Broth :

Nutrient Broth was used for determination of minimum inhibitory concentration (MIC) values of test compounds against various microbes by broth micro dilution method.

Composition of Nutrient Broth

Sodium chloride - 5.0 g/lit
Peptone - 5.0 g/lit
Beef extract - 1.5 g/lit
Yeast extract - 1.5 g/lit
$_{p}$H - 7.4 $\pm$ 0.2

The ingredients were dissolved in appropriate quantity of distilled water, mixed thoroughly and distributed 0.5 ml volumes each in glass test tubes (12 x 75 mm). All the tubes were plugged with cotton and sterilized in autoclave for 15-20 min at 125^{0}C (15 lbs/inch^2 pressure). After sterilization tubes were cooled to room temperature.

Preparation of the inoculum:

Stock inoculum of the microbes was prepared by the inoculation the 50 ml nutrient both with test organisms and incubating it at 37 $\pm$ 2^{0}C for 24 hrs.

Preparation of the stock solution extracted polar flavanoids and non polar flavanoids

The dried extract of polar flavanoids was dissolved in distilled water and dilutions were made as 100mg/ml and 500mg/ml and 1000mg/ml. Same procedure was adopted for non polar flavanoids

Antimicrobial study by cup plate method

Cup plate method :- The sterilized nutrient agar medium was poured into the petridishes and allowed to solidify. The lawn of the culture was prepared by spreading the microbial suspension on the surface of the medium with the help of sterilized triangular loop. Petridishes were allowed to remain for 10 min, after which excess of nutrient broth cultures were taken out aseptically using pasture pipettes. Standard 8 mm size cups were then prepared in the solidified medium with the help of pre-sterilized steel cylinder of 8 mm diameter. The wells were then filled with the 0.5 ml stock solution of the test samples.

RESULTS AND DISCUSSION

The zone of inhibitions is recorded in the following table. The zones are recorded including the well diameter of 8 mm.

1. Antimicrobial activity of polar flavanoides

Conc.	*Proteus vulgaris*	*S. Aurius*	*E.coli*	*Salmonella typhi*
100mg/ml	16mm	13mm	15mm	17mm
500mg/ml	17mm	18mm	18mm	18mm
1000mg/ml	16mm	17mm	19mm	19mm

2. Antimicrobial activity of non-polar flavanoides

Conc.	*Proteus vulgaris*	*S. Aurius*	*E.coli*	*Salmonella typhi*
100mg/ml	15mm	16mm	16mm	15mm
500mg/ml	17mm	18mm	19mm	17mm
1000mg/ml	19mm	18mm	17mm	17mm

The extracts showed good to moderate activity against the pathogens.

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