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THERAPEUTIC POTENTIALS OF *PREMNA LATIFOLIA*

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Abstract

In the present study antibacterial, antiviral, anthelmintic and phytochemical screening of *Premna latifolia* extract was evaluated. Turbidimetric method and disc method were used for antibacterial analysis. The strains of bacteria *Klebsiella pneumoniae and Salmonella typhi* were used for this study. In turbidimetric method, there was a concentration dependent inhibition of growth of bacteria observed in *Premna latifolia* treated cultures. In disc method, *Premna latifolia* extract showed potential inhibitory action against *Klebsiella pneumoniae* and *Salmonella typhi.* The diameter zone of inhibition was found to be 14.7 mm (100 µg/ ml) against *Klebsiella pneumonia* and 23.7mm (100 µg/ ml) against *Salmonella typhi*. The minimum inhibitory concentration (MIC) of the ethanolic extract of *Premna latifolia* against *Klebsiella pneumoniae* and *Salmonella typhi* was found to be 6.25 µg/ml and 3.125 µg/ml respectively. In Newcastle Disease (NDV) virus alone treated embryonated chik eggs, the embryos were poorly developed and were shown haemorrhage and developmental abnormalities. Treatment with the plant extract at 500 µg/egg reversed the NDV induced cytopathic changes. The heamagglutination titre was found to be 512 in NDV virus alone treated group, while in Premna *latifolia* extract (500 µg/egg) treated group it was reduced to 4. Anthelmintic activity was also performed with the ethanolic extract of *Premna latifolia* against Indian adult earthworm, *Pheretima posthuma*. When compared to standard drug Albendazole, in 50 mg/ml of *Premna latifolia* treated groups the earthworm get paralyzed after 2 min and died after 5 min. It was found to be closer to control treated groups. Preliminary phytochemical analysis showed the presence of alkaloid and phenols in this extract.

Keywords: *Premna latifolia*, Antibacterial activity, Newcastle Disease virus, Anthelmintic activity, *Pheretima posthuma*, Alkaloid, tannin

Introduction

Plants have been used for medicinal purposes long before prehistoric period. They are the potent source of many pharmacological activities. They act as sources of drugs and therapeutic agents. Herbal medicine, based on their traditional uses in the form of powders, liquids or mixtures, has been the basis of treatment for various ailments in India since ancient times. Numerous studies have shown that aromatic and medicinal plants are sources of antioxidant and antimicrobial properties which can protect the human body against both diseases and pathogens (Banothu *et al*., 2007). Despite of tremendous progress in human medicines, infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health. Their impact is particularly large in developing countries due to relative unavailability of medicines and the emergence of widespread drug resistance.

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Current research on natural molecule and products primarily focuses on plants since they can be sourced more easily and be selected based on their ethno-medicinal uses. This interest primarily stems from the belief that green medicine is safe and dependable, compared to costly synthetic drugs which are invariably associated with adverse effects. Now-a-days there is widespread interest in evaluating drugs derived from plant sources. Because a large number of plant species still need to be analyzed for their therapeutically effects on various pathogens (Joshi *et al*., 2011).

Considering the vast potentiality of plants as sources for antimicrobial drugs with reference to antibacterial agents, a systematic investigation was undertaken to screen the antimicrobial activity of *Premna latifolia*.

Materials and Methods

Preparation of plant extract

*Premna latifolia (*family-Verbenaceae) plant was

collected locally. It was washed, dried and powdered. 10 gm powdered *Premna latifolia* was taken in a conical flask. 75 % ethanolic extract was made. Filtrate was evaporated to dryness. The yield of the preparation was found to be 0.6g.

Determination of antibacterial activity of *Premna latifolia* extract by turbidimetric and disc method

Antimicrobial activity of plant extract was investigated against two bacterial strains, *Klebsiella pneumoniae* and *Salmonella typhi*. They were collected from Veterinary College, Mannuthy. The reference strains of bacteria were maintained on Nutrient agar (Hi Media, Mumbai). The cultures were subcultured regularly (every 30 days) and stored at 4^0C. From stock culture by aliquot quantity of culture inoculated into 25 ml of nutrient broth and incubated overnight at 37^0C.

In turbidimetric method, varying concentrations of *Premna latifolia* extract were added into the identical tubes in triplicate. 2.5ml nutrient broth and 0.1 ml culture were also added to the same tubes. Tubes with 0.1 ml culture suspended in 2.5 ml nutrient broth are taken as control. All tubes were incubated at 37^0C and optical density of the control and experimental tubes were measured at different time intervals ranging from 1hr, 5hr and 24hr at 530nm (Brown, 1966).

In disc method, 15 ml of nutrient agar medium was dispensed into pre-sterilized petridishes to yield a uniform depth for bacterial inoculation. The discs were impregnated with varying concentrations of the *Premna latifolia* extract and dried for 10-15 minutes. The dried discs were placed on the agar surface with flamed forceps and gently pressed down to ensure contact with the agar surface. Tetracyclin (10µg) was used as positive control. The discs were spaced far enough to avoid overlapping rings of inhibition. Finally, the petridishes were incubated for 24 hours at 37^0C for bacteria. The diameter of zone of inhibition (ZI) (mean of triplicates ± SD) as indicated by clear area which was devoid of growth of microbes was measured (Davis and Stout, 1971). The activity index of the crude plant extract was calculated as

$$Activity\ index\ (A.I.) = \frac{Mean\ of\ zone\ of\ inhibition\ of\ the\ extract}{Zone\ of\ inhibition\ obtained\ for\ standard\ antibiotic\ drug}$$

Determination of the minimal inhibitory concentration (MIC)

The antibacterial activity of the plant extracts was determined using 96-well plates. The minimal inhibitory concentration (MIC) values, which represent the lowest concentration of extract that completely inhibits the growth of microorganisms, were determined by a micro-well dilution method (Zarai *et al*., 2011). The inoculum of each bacterium was prepared and the suspensions were added to each well. Extract was dissolved in 100% ethanol and then dilutions series were prepared in a 96-well plate. Each well of the micro plate included 40 µl of the growth medium, 10 µl of inoculums and 50 µl of the diluted sample extract. Ethanol is used as negative control. The plates were then covered with the sterile plate and incubated at 37°C for 24 h. After that, 40 µl of 3- (4, 5-dimethyl-thiazol-2-yl)- 2,5-diphenyl-tetrazolium chloride (MTT) at a final concentration 0.5 mg/ml freshly prepared in water was added to each well and incubated for 30 min. The change to red colour indicated that the bacteria were biologically active. The MIC was taken to the well, where no change of colour of MTT was observed. The MIC values were done in triplicate.

Determination of antiviral activity of *Premna latifolia* extract against Newcastle Disease (NDV) virus in chick embryonated eggs

Seven-day-old chick embryonated eggs were wiped with alcohol and kept at 39^0C with 60% humidity before the experiment. On day seven, eggs were candled and live eggs were taken. The eggs were divided into control, normal and NDV+different concentration of *Premna latifolia* extrat treated along with NDV groups (5 eggs in each group). After inoculation eggs were sealed and were kept back in the incubator at 37^0C. The eggs were candled every day for mortality. On 13^{th} day, eggs were chilled at 4^0C and harvesting was done on the next day.

For harvesting eggs were open on the top and using a sterile pipette allantoic fluid was collected into a sterile container. After collecting allantoic fluid the embryo was taken into a sterile petridish, growth and morphology of embryos and Chorioallantoic memebrane were checked. It was compared with normal untreated, virus alone treated and virus+drug treated embryos. For heamagglutination assay, 0.1ml chicken RBC was added to each well, mixed gently and incubated for 20 min. 0.1 ml allantoic fluid was serially diluted in these wells. After incubation clumping was observed and the viral strength of fluid was expressed as the reciprocal of the highest dilution that causes agglutination (Vijayan *et al.,* 2004).

Determination of anthelmintic activity of *Premna latifolia* extract against earthworm, *Pheretima posthuma*

Identified adult earthworms (*Pheretima posthuma)* were collected from Kerala Agricultural University, Mannuthy. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used for all the experimental

protocol. The anthelmintic assay was carried out as per the method of Ajaiyeoba *et al.*, (2001). The assay was performed *in vitro* using adult earthworm *Pheretima posthuma* as it is having anatomical and physiological resemblance with the intestinal round worm parasites of human beings for preliminary evaluation of anthelmintic activity. *Premna latifolia* extract was prepared at the concentrations 50 and 25 mg/ml in distilled water and three worms *i.e. Pheretima posthuma* of approximately equal size (same type) were placed in each nine cm petri dish containing 15 ml of above test solution of extracts. Albendazole (20 mg/ml and 40 mg/ml) was used as reference standard as advocated earlier. All the test solution and standard drug solution were prepared freshly before starting the experiments. Observations were made for the time taken for paralysis when no movement of any sort could be observed and time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously.

Separation of active compound from *Premna latifolia* extract by thin layer chromatography (TLC)

Identification Test for Phenols

TLC plate was loaded with *Premna latifolia* extract. After drying it was run with the solvent methanol: chloroform in the ratio 1:5. After drying, it was sprayed with Folin– Ciocalteu reagent and kept for drying. Band was visualized under UV inspection box (Chhetri *et al*., 2008).

Identification Test for Alkaloid

TLC plate was loaded with *Premna latifolia* extract. After drying it was run with suitable the solvent methanol: chloroform in the ratio 1:5. After drying, it was sprayed with Dragendroff's reagent and kept for drying. Band was visualized under UV inspection box. It imparts orange precipitate when alkaloid is present in the sample (Chhetri *et al*., 2008).

Statistical analysis

The experimental results were repeated in triplicates. All the results were statistically expressed as the mean ± standard error of mean (SEM).

Results and Discussion

In the present study, the optical density of the control and experimental tubes were measured at different intervals of time and values were listed in table 1. The turbidity readings for *Klebsiella pneumoniae* was found to be 1.214, while the reading for *Premna latifolia* extract (50 and 100 µg/ml) against *Klebsiella pneumoniae* were 0.896, 0.774 respectively. The turbidity readings for *Salmonella typhi* was found to be 1.264, while the reading for *Premna latifolia* extract (50 and 100 µg/ ml) against *Salmonella typhi* were 0.905, 0.701 respectively. There was a concentration dependent inhibition of growth of bacteria observed in *Premna latifolia* treated cultures.

In disc method, *Premna latifolia* extract showed potential inhibitory action against *Klebsiella pneumoniae* 14.7 mm (100 ìg/ ml) and 10.6 mm (50 µg/ml). Activity index of *Premna latifolia* extracts against this bacterium varies from maximum 0.80 for (100 µg/ml) to the minimum 0.24 for (10 µg/ml). Maximum zone of inhibition for tetracycline was found to be 18.2 mm. Zone of inhibition of *Premna latifolia* extract against *Salmonella typhi* was found to be 23.7mm (100 µg/ml) and 17.3 mm (50 ìg/ ml) while inhibition zone for tetracycline was found to be 24.3mm. Activity index of *Premna latifolia* extracts against this bacterium varies from maximum 1 for (100 µg/ml) to the minimum 0.45 for (10 µg/ml). The results were summarized in table 2.

The minimum inhibitory concentration (MIC) of the ethanolic extract of *Premna latifolia against Klebsiella pneumoniae* and *Salmonella typhi* was found to be 6.25 µg/ml and 3.125 ìg/ml respectively.

Premna latifolia extract did not produce any kind of toxicity to chick embryos all the concentrations studied. In NDV alone treated embryos were poorly developed and were associated with haemorrhage and developmental abnormalities. Treatment of embryos with the plant extract reversed the NDV induced cytopathic changes. Higher concentration treated (500 µg/egg) embryos almost look alike the normal. The heamagglutination assay was done against chicken RBCs. The virus alone treated group had a titre of 512, while the administration of *Premna latifolia* extract (500 µg/egg) reduced the titre value to 4. In 200 µg/egg *Premna latifolia* extract treated groups titre value was 32 and in 100 ìg/egg it was 64. The results of heamagglutination titre were given in table 3. *Premna latifolia* extract showed significant antiviral activity against Newcastle Disease virus.

The anthelmintic activity of *Premna latifolia* extract and standard drug Albendazole were studied by observing the time taken for paralysis and death of earthworm, *Pheretima prosthuma*. In 40mg/ml of standard drug Albendazole treated groups, the earthworm get paralysed and died after 2min and 6min respectively. In 20mg/ml of Albendazole the time for paralysis and death of *Pheretima posthuma* was found to be 4min and 18min respectively. When compared to control, 50mg/ml of *Premna latifolia* extract the earthworm get paralysed after 2 min and died after 5 min. But in 25 mg/ml, paralysis was observed after 5 min and died after 24 min respectively (table 4).

Table 1: Antibacterial activity of *Premna latifolia* extract by turbidimetric method

Strain of Bacteria	Concentration	Optical density at 530nm		
		1h	5h	24h
Klebsiella pneumoniae	Control	0.356	0.684	1.214
	Premna latifoilia (50 µg/ml)	0.338	0.547	0.896
	Premna latifoilia (100 µg/ml)	0.301	0.435	0.774
Salmonella typhi	Control	0.365	0.696	1.264
	Premna latifoilia (50 µg/ml)	0.363	0.555	0.905
	Premna latifoilia (100 µg/ml)	0.328	0.415	0.701

Table 2: Antibacterial activity of *Premna latifolia* extract by disc method

Strain of Bacteria	Concentration	Zone of inhibition (mm)	Activity Index
Klebsiella pneumoniae	Control (10 µg/ml)	18.2 ± 2.0	
	Premna latifoilia (10 µg/ml)	4.3 ± 2.5	0.24
	Premna latifoilia (50 µg/ml)	10.6 ± 1.5	0.58
	Premna latifoilia (100 µg/ml)	14.7 ± 1.5	0.80
Salmonella typhi	Control (10 µg /ml)	24.3 ± 1.5	
	Premna latifoilia (10 µg/ml)	11.0 ± 2.6	0.45
	Premna latifoilia (50 µg/ml)	17.3 ± 2.1	0.71
	Premna latifoilia (100 µg/ml)	23.7 ± 3.1	1.0

Table 3. Effect of Premna latifolia on HA titre in NDV inoculated embryonated eggs

Group	Haemagglutination titre
NDV alone	512
0.1 ml NDV+100 mg/egg *Premna latifolia*	64
0.1 ml NDV+200 mg/egg *Premna latifolia*	32
0.1 ml NDV+500 mg/egg *Premna latifolia*	4

Table 4. Anthelminthic activity of *Premna latifolia extract* against earthworm *Pheretima posthuma*

S No	Treatment Groups	Concentration (mg/ml)	Time Taken in minutes	
			Paralysis	Death
1	Albendezole	20	4± 0.4	18±1.6
		40	2±0.47	6±0.81
2	*Premna latifolia*	25	5±0.81	24±1.69
		50	2±0.47	5±0.81

Plants are important source of potentially useful structures for the development of new therapeutic agents. The best alternative over modern synthetic drugs is plant derived medicine. The results obtained from this work revealed that the plants contained bioactive agents such as alkaloid and tannins. The presence of phytochemical namely alkaloids, flavonoids, steroids, gallic tannins, catecholic tannin plays the vital role in the plant defense mechanisms (Tariq *et al*., 2010).

Preliminary phytochemical analysis of the extract showed that this plant extract contains alkaloid and phenols. Secondary metabolite is crucial for plant defenses which has enabled plants to survive; e.g.- phenolics, alkaloids, steroids, terpenes, saponins, *etc*. Phenolic is one of the major groups of phytochemical that can be found ubiquitously in certain plants. Phenolic compounds are potent antioxidants and free radical scavenger which can act as hydrogen donors, reducing agents, metal chelators and singlet oxygen quenchers. Purified alkaloids as well as their synthetic derivatives are used as medicinal agents for their various biological effects such as analgesic, antispasmodic and bactericidal (Kaur and Arora, 2009). These secondary metabolites exert antimicrobial activity through different mechanisms. Several proposed mechanisms include membrane damage, changes in intracellular pH, membrane potential, and ATP synthesis (Sanchez *et al*., 2010).

Albendazole binds to free ß-tubulin, inhibiting polymerisation and thus interfering with microtubule dependent glucose uptake by the worms. Alkaloids may act on central nervous system and caused paralysis of the earthworm. It may suppress the transfer of sucrose from the stomach to the small intestine. Together with its antioxidant effect which can reduce the nitrate generation and interfere in local homeostasis of helminths. The possible mechanism of action of tannins may be interfere with energy generation by uncoupling oxidative phosphorylation, or may interfere with glycoprotein of cell surface, or can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and cause death (Basha *et al.*; 2011; Bauri *et al.;* 2015).

The crude extract of the medicinal plants are a complex mixture of many compounds which makes it difficult to unravel the property of a specific compound. So further investigations are needed to explore the mechanisms of action of these plant extract as well as their potential role in the biological activity.

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