



IN-VITRO ANTI BACTERIAL AND ANTHELMINTIC ACTIVITY OF *TERMINALIA CHEBULA*, *MORINGA OLEIFERA* AND *ALLIUM SATIVUM*

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The objective of the current research work is to investigate the in-vitro anti-bacterial and anthelmintic activity. The combined tri-herbal methanolic extract made up of equal quantities of leaves of *Moringa oleifera*, seeds of *Terminalia chebula*, and fresh bulbs of *Allium sativum* was evaluated for its in-vitro anti-bacterial and anthelmintic activity and was compared with its individual methanolic extracts of *Terminalia chebula*. The anti-bacterial activity was evaluated against gram negative and gram positive bacteria. Streptomycin was used as a standard drug. The Anthelmintic activity was evaluated against *Pheretima posthuma*. Albendazole was used as a standard drug. The experimental results showed that tri-herbal methanolic plant extracts possess better activity on both microorganisms and earthworms. The combined activity of *Moringa oleifera*, *Terminalia chebula* and *Allium sativum* has been reported for the first time.

KEYWORDS: IN-vitro, Anthelmintic, Microorganisms, Streptococcus, Bacillus, Pseudomonas

1. INTRODUCTION

The use of natural medicines for treatment or prevention of bacterial infections or disorders is safe for maintenance of good health. There are so many inventions and research works done on natural drugs due to their high activity and less side effects¹. *Allium sativum* commonly known as garlic which belongs to the family Liliaceae. Its biological activities are mainly related to heart and blood systems like blood pressure, heart attack and atherosclerosis. Louis paster was the first scientist who discovered the anti-bacterial activity of garlic on both gram positive and gram negative bacteria. Garlic

has strong anti-bacterial activity². *Terminalia chebula* commonly known as myrobalan which belongs to the family Combretaceae. It shows its inhibitory activity against *Helicobacter pylori*, *Xanthomonas campestris* sp. Citri and *salmonella typhi*. It is used in ayurvedic formulation triphala as main ingredient which is used for kidney and liver dysfunctions³.

Moringa oleifera commonly known as moringa belongs to the family Moringaceae. It has anti-bacterial activity against *E.coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and *Streptococcus*. Other biological activities are anti-oxidant,

anti- fungal, anti- inflammatory, anti- tumor and anti- ulcer. Now a days helminth infections are most common and spread widely throughout the world. Anthelmintic drugs are used for treatment of parasitic diseases. Over usage of these drugs may induce toxicity in humans. In order to overcome of these side effects natural medicines have been discovered.

2. MATERIALS AND METHODS

Collection of plant:

The fresh leaves of *Moringa oleifera* were collected near our house located in Visakhapatnam district and the seeds of *Terminalia chebula* and fresh bulbs of *Allium sativum* were purchased from the local market in Visakhapatnam.

Preparation of plant materials:

Fresh leaves of *Moringa oleifera* were collected, washed thoroughly and dried under shade and then made into fine powder using dry grinder. The seeds of *Terminalia chebula* were collected and dried under shade and then made into moderate powder. Fresh bulbs of *Allium sativum* were collected and the cloves are separated, peeled and washed and then dried under shade and were crushed using mortar and pestle. They were kept aside for two to three days crumbled with a blender to a fine powder and kept for further analysis.

Extraction process:

The extracts were taken in three separate round bottomed flask in equal ratio to this methanol is added and they were subjected to maceration process for two to three days

followed by soxhlation. Then hydro distillation was carried out which is responsible for recollection of methanol. The extracts were stored in sample bottles in a refrigerator for further studies.

IN- VITRO ANTI-BACTERIAL ACTIVITY

Agar diffusion method is applied for screening of anti-bacterial activity.

Strains for anti- bacterial activity:

Pseudomonas aeruginosa, *Bacillus subtilis*, *Streptococcus aureus*

Culture media:

Culture media was procured by our college. Media was prepared using specified quantities of antibiotic assay medium and were thereafter sterilized by autoclaving at 15lb pressure at 121°C for 20min.

Media for bacterial growth:

Nutrient agar medium 4g of agar powder, 1.5g of pectin, 1.5g of bees wax, 0.5g of sodium chloride in 250ml distilled water. This solution is autoclaved at 121°C 15lb pressure for 20min.

Agar disk diffusion method:

Agar disk diffusion method is widely used to evaluate anti- bacterial activity of plants or microbial activity. Agar plates were prepared and test microorganisms are inoculated. The tri-herbal plant extracts were prepared in various concentrations (50,100,150µg/ml). The individual plant extract concentrations were prepared in various concentrations (100,200µg/ml). Streptomycin was taken as standard drug. The standard drug was prepared in concentrations of 10µg/ml.

Filtered paper discs of 6mm in diameter were soaked in extracts of different concentration and placed on the surface of agar plates. The agar plates are incubated at 34°C for 24 hours. After 24 hours agar plates are examined and diameter of zone of inhibition is measured.

IN- VITRO ANTHELMINTIC ACTIVITY

Earthworm collection:

Indian adult earth worms (*Pheretima posthuma*) were collected from moist soil and washed thoroughly with water in order to remove the unwanted matter.

In-vitro anthelmintic activity:

The in-vitro anthelmintic activity of plant extracts were evaluated against *Pheretima posthuma*. All the drug extracts were prepared and kept aside before starting the experiment. Various concentrations of tri-herbal and individual (100, 80, 60, 40, 20 mg/ml) of extracts are prepared and tested against earthworms to determine the time of paralysis and death. Albendazole was taken as reference drug. The various concentrations of reference drug are taken as 20, 40 mg/ml. The earthworms are placed in Petri dishes containing different concentrations of methanolic extracts (combined form and individual form) and reference drug preparation and observed for time taken to cause paralysis and death. The time taken for paralysis is noted down if there is no movement for a period of time then it is regarded as death followed by fading of body colors.

3. RESULTS AND DISCUSSION:

In-vitro anti- bacterial activity:

In this study the tri herbal methanolic extract own anti- bacterial effect against *Streptococcus aureus* and *Pseudomonas* and the sensitivity of the bacterial gradually increased with increasing of concentration. Methanolic extract of *Terminalia chebula* own potent anti- bacterial activity against *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Streptococcus aureus* (Table 1). All the results were compared to that of the reference drug (Table 1).

In-vitro anthelmintic activity:

The data revealed that tri- herbal methanolic extract showed better anthelmintic activity against earthworms. The results were comparable with that of the reference drug. By increasing the concentration of the extracts it procured better activity against earthworms. Methanolic extract of *Terminalia chebula* procured affective anthelmintic activity against earthworms (Table 2).

4. CONCLUSION:

On the basis of the above results it can be concluded that tri herbal methanolic plant extract a combination of three plants exerts eloquent anti- bacterial and anthelmintic activity because of the presence of the active biological principles.

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Table 1: Anti-bacterial activity of the methanolic extract in-vitro

Drug	Concentrations (µg/ml)	Streptococcus (mm)	Pseudomonas (mm)	Bascillus (mm)
<i>Terminalia chebula</i>	100	30	26	28
	200	40	36	32
<i>Tri- herbal extract</i>	50	12	12	16
	100	20	20	28
	150	24	22	20
Standard	10	17	12	11

Table 2: Anthelmintic activity of methanolic extracts:

Drug	Concentrations ($\mu\text{g/ml}$)	Paralysis (min)	Death (min)
<i>Terminalia chebula</i>	100	40.54	50.08
	80	30.44	60.34
	60	40.47	65.23
	40	50.23	74.45
	20	65.33	85.10
<i>Tri-herbal extract</i>	100	10.23	15.56
	80	20.36	25.35
	60	25.47	35.06
	40	40.38	50.22
	20	45.12	63.15
Standard	20	25.15	48.08
	40	23.50	50.26

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