

## Comparative Evaluation of *In-vitro* Antimicrobial Activity of Alcoholic Polyherbal Extract IMMU 4 Plus.

K.Radha<sup>1\*</sup>, Padmaja V<sup>2</sup>, Ajithkumar P<sup>3</sup>, Helen William<sup>1</sup>

<sup>1</sup>College of Pharmaceutical Sciences, Medical College, Kottayam, Kerala, India.

<sup>2</sup>College of Pharmaceutical Sciences, Medical College, Thiruvananthapuram, Kerala, India.

<sup>3</sup>Narayana Institute of Medical Science, Ernakulam, Kerala, India.

Available Online: 1<sup>st</sup> February, 2015

### ABSTRACT

Development of multi-drug resistance in pathogenic microbes necessitates a search for new antimicrobial agents from other sources, including plants. Four indigenous plants viz *Azadirachta indica* (Meliaceae), *Centella asiatica* (Apiaceae), *Tinospora cordifolia* (Menispermaceae) & *Withania somnifera* (Somnifera) traditionally known for immunomodulatory activity were evaluated for antimicrobial activity against bacterial strains *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus mutans*, and *Klebsiella pneumonia* and antifungal activity against *Candida albicans* and *Aspergillus niger*. IMMU 4plus formulation which is on the way of development, may be used as an adjuvant along with other immunomodulatory agents to produce broad spectrum antimicrobial activity along with other therapeutic properties.

**Keywords:** Broad spectrum, anti-microbial activity, immunomodulatory activity, indigenous plants

### INTRODUCTION

Plant extract shows more than one therapeutic property. Four indigenous plants viz *Azadirachta indica* (Meliaceae), *Centella asiatica* (Apiaceae), *Tinospora cordifolia* (Menispermaceae), & *Withania somnifera* (Somnifera) traditionally known for immunomodulatory activity were evaluated for antimicrobial activity against bacterial strains *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus mutans*, and *Klebsiella pneumonia* and antifungal activity against *Candida albicans* and *Aspergillus niger*.

*Azadirachta indica* commonly known as Margosa tree, Neem, Ariyaveppu, Nimbah is a large white flowering tree, common in dry deciduous forest. The bark, leaves, flowers, fruits and seeds are used in Ayurveda, folk, Homoeopathy, Tibetan and Unani. Bark is used in skin diseases. Leaves are antiseptic, applied to boils in the form of poultice. Decoction is given for ulcers and eczema. Flowers are stomachic and tonic. Berries are purgative and emollient. Seed oil is used in skin troubles. Fresh tender twigs are used to clean teeth particularly in pyorrhoea. Bark, leaves and seeds are used in rheumatism, intestinal worms, impurity of blood, eye diseases, diabetes, small pox, chicken pox and other cutaneous affection, ulcers, ringworm, scabies, etc. Bark, leaf, flower, seed and seed oil are used for leprosy, liver disorders, cough, dyspnoea, polyuria, wounds, fevers, poisoning and eye diseases.<sup>1</sup>

*Centella asiatica* commonly known as Indian Pennywort, Spade leaf, Brahmanduki, Kudangal, Mandukaparni is

trailing herbs with flowers brownish, distributed in Tropical Asia, Africa and generally found near reservoirs and streams of water. Whole plant is used in Ayurveda, folk, Homoeopathy, Tibetan and Unani. Whole plant is used for epilepsy, polyuria, distaste, psychosis, fever, bronchial asthma and stammer. It is a brain tonic, rejuvenator, nervine and cardiac tonic, improves memory power, physical strength, voice and digestive power.<sup>1</sup>

*Tinospora cordifolia* commonly known as Bile liller, Moon creeper, Amrita, Guduchi etc. is climber flowers greenish yellow distributed from India to Indo-China, evergreen, moist deciduous forest, sacred groves and plains. The stems are used in Ayurveda, folk, Homoeopathy, Tibetan and Unani systems of medicine. Stems are used in fever, jaundice, thirst, burning sensations, diabetes, piles, skin ailments, respiratory disorders, neurological disorders and rheumatism. It also improves intellect and imparts youthfulness, vitality and longevity.<sup>1</sup>

*Withania somnifera* is commonly known as Winter cherry, Amukkuram, Aswagandha, etc. is shrub flowers greenish-yellow and is distributed in Paleotropics, Cultivated. Roots and leaves are used in Ayurveda, folk, Homoeopathy, Siddha, Tibetan and Unani. Roots are used in goiter, fainting, insomnia, worm infection, blood disorders, dyspnoea, leprosy, tuberculosis, emaciation, tonic, aphrodisiac, important rejuvenative which improves physical vigour and strength, cures cough, impotence, rheumatism, toxicosis and leucoderma. Leaf paste is used for tumors and scrofula.<sup>1</sup>

Table 1: Zone of Inhibition

Antimicrobial Activity (Antibacterial & Antifungal Activity)		Zone of inhibition (mm)						
Micro organisms	Conc.	Control		Alcoholic extract				
		Cotrimazole 0.1g in 1ml DMSO	Gentamycin 40mg/ml	<i>Centella asiatica</i>	<i>Withania somnifera</i>	<i>Tinospora cordifolia</i>	<i>Azadiracta indica</i>	Polyherbal extract
<i>Candida albicans</i>	50µl	20.7+/- 0.12	NA	00	00	00	00	00
	100 µl		NA	00	00	00	00	00
<i>Asperigillus niger</i>	50 µl	24.0+/- 0.15	NA	00	00	00	00	00
	100 µl		NA	50.0+/- 01.5	06.0+/- 1.20	00	00	00
<i>Pseudomonas auroginosa</i>	25 µl	NA	31.0+/- 05.2	00	00	00	00	00
	50 µl	NA		00	00	00	00	09.0+/- 01.1
<i>Staphylococcus aureus</i>	100 µl	NA		00	12.0+/- 02.1	00	00	11.0+/- 02.0
	25 µl	NA	24.0+/-02.5	00	00	00	00	00
	50 µl	NA		00	00	00	00	00
	100 µl	NA		10.0+/- 02.1	00	00	00	13.0+/- 01.5
<i>Streptococcus mutants</i>	25 µl	NA	36.0+/-05.8	00	10.0+/- 01.1	00	00	00
	50 µl	NA		00	11.0+/- 01.2	00	12.0+/- 01.4	11.0+/- 01.1
<i>Klebsiella pneumonia</i>	100 µl	NA		00	15.0+/- 01.7	00	14.0+/- 01.6	15.0+/- 01.8
	25 µl	NA	29.0+/- 03.7	00	00	00	00	00
	50 µl	NA		00	00	10.0+/- 01.4	00	00
	100 µl	NA		11.0+/- 01.2	10.0+/- 01.3	10.0 +/- 01.4	00	00

Development of multi-drug resistance in pathogenic microbes necessitates a search for new antimicrobial agents from other sources, including plants. Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties.<sup>2,3</sup> It is expected that screening of plant extracts against wide variety of test micro-organisms will be helpful in obtaining broad-spectrum herbal formulation as well as new antimicrobial substances.

## MATERIAL AND METHODS

### Crude drug

Stems of *Tinospora cordifolia*,  
Roots of *Withania somnifera*,  
Whole plants of *Centella asiatica*,  
Leaves of *Azadiracta indica*

### Collection of crude drug

The authenticated crude drugs were collected from Kerala Ayurveda Limited, Angamali, Ernakulam, Kerala and dried in the shade and then exposed to sunlight for 3 days, and subjected to powdering.

**Polyherb:** Equal proportion of coarsely powdered crude drug

### Preparation of Plant extracts<sup>4</sup>

The sun dried crude drugs were subjected to physical evaluation. The standardised coarse powdered crude drugs ( sieve size 60 ) were subjected to alcoholic extraction by Soxhlet Extractor.

### Plant Extracts

Aqueous extract of Polyherbs  
Alcoholic extract of Polyherbs  
Alcoholic extract of *Tinospora cordifolia*  
Alcoholic extract of *Withania somnifera*  
Alcoholic extract of *Centella asiatica*  
Alcoholic extract of *Azadiracta indica*

### Preparation of Polyherbal extracts(IMMU 4plus)

Crude drugs were powdered to coarse size ( sieve size 60 ) separately and mixed in equal ratio by weight. Alcoholic extract of mixed crude drugs were prepared by Soxhlet Extraction Method.

### Microorganism

Antibacterial and antifungal activity were carried out at Microbiology lab, College of Pharmaceutical Sciences, Govt. Medical College, Thiruvananthapuram.

### Microorganisms used

**Antibacterial activity:** The organisms used were *Klebsiella pneumonia*, *Pseudomonas aeruginosa*(Gram-ve) and *Staphylococcus aureus*, *Streptococcus mutants*(Gram+ve).

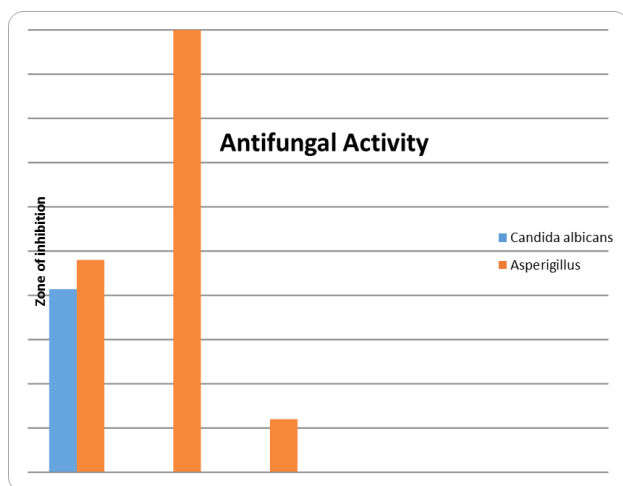


Fig 1: Antifungal Activity

**Antifungal activity:** The organisms used were *Candida albicans* and *Asperigillus niger*.

#### Antimicrobial Activity

**Agar- Well Diffusion Method:** The antimicrobials present in the plant extract are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters.

#### Reagents

**Muller Hinton Agar Medium (1 L):** The medium was prepared by dissolving 33.9 g of the commercially available Muller Hinton Agar Medium (HiMedia) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten.

**Nutrient broth (1L):** One litre of nutrient broth was prepared by dissolving 13 g of commercially available nutrient medium (HiMedia) in 1000ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

**Gentamycin** (standard antibacterial agent, concentration: 40mg / ml)

#### Procedure

Petriplates triplicates containing 20ml Muller Hinton medium were seeded with 24hr culture of bacterial strains such as *Pseudomonas auroginosa*, *Staphylococcus aureus*, *Streptococcus mutans*, and *Klebsiella pneumoniae*. Wells of approximately 10mm was bored using a well cutter and sample of 25, 50, and 100 µl conc: were added,. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well.<sup>5</sup> Gentamycin was used as a positive control.

Sample concentration: 0.1 gm (sample) in 1 ml DMSO

## RESULTS AND DISCUSSION

### Antifungal activity

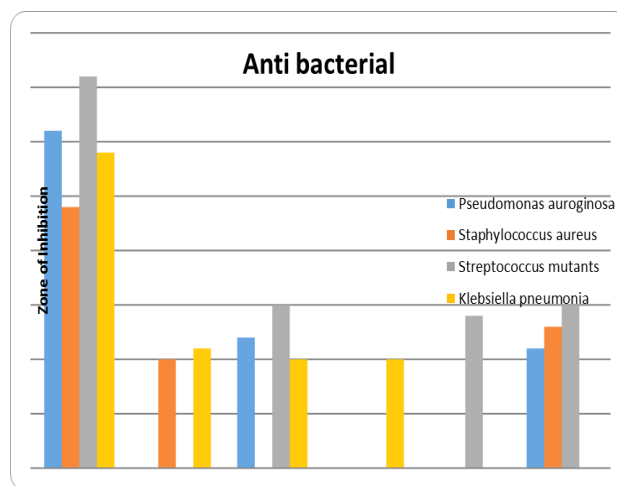


Fig 2: Antibacterial Activity

Antifungal activity study shows that polyherbal extract wherein individual plant are in subtherapeutic level has no antifungal activity. But *Centella asiatica* extract showed very significant antifungal activity against *Asperigillus niger* and no activity against *Candida albicans*. *Withania somnifera* shows comparatively less antifungal activity against *Asperigillus niger*.

#### Antibacterial activity

Figure1 bar diagram shows the comparative antibacterial activity of each individual plant alcoholic extract against microorganisms namely *Pseudomonas aurognosa*, *Staphylacoccus aureus*, *Streptococcus mutants* and *Klebsiella pneumonie*. Alcoholic extract of *Centella asiatica* has maximum activity against *Klebsiella pneumononiae* than *Staphylacoccus aureus* and minimum activity against *Pseudomonas aeruginosa* and *Streptococcus mutants*. The antibacterial activity is significant when compared to Gentamycin. *Azadiracta indica* showed significant activity only against *Streptococcus mutants* while *Tinospora cordifolia* showed significant activity only against *Klebsiella pneumononiae*. Polyherbal extract showed maximum activity against *Streptococcus mutants* followed by *Staphylacoccus aureus* and then *Pseudomonas aeruginosa*, at sub.therapeutic level. Polyherbal Extract shows antibacterial activity against both gram +ve and gram -ve bacteria wherein each plant extract is at sub-therapeutic dose. Hence polyherbal extract can be formulated with broad spectrum activity.

## CONCLUSION

IMMU 4plus formulation which is on the way of development may be used as an adjuvant along with other immunomodulatory agents to produce broad spectrum antimicrobial activity along with other therapeutic properties.

Thus screening of plant extracts against wide variety of test micro-organisms will be helpful in obtaining broad spectrum herbal formulation as well as new antimicrobial substances.

## REFERENCES

1. Udayan P.S & Indira Balachandran, Medicinal Plants of Arya Vaidya Sala Herb Garden, 1<sup>st</sup> edition, Arya Vaidya Sala, Kottakkal, May 2009, 64-65, 103
2. Bruneton. J (1995). Pharmacognosy, Phytochemistry, Medicinal plants. France: Lavoisier Publishing Co., pp.265-380
3. Chopra. R.N., S.L Nayer, I.C Chopra (1992). Glossary of Indian Medicinal Plants, 3rd Edn. New Delhi: Council of Scientific and Industrial Research pp.7-246
4. S.S Agarwal & M.Paridhavi, Herbal Drug Technology, 2<sup>nd</sup> edition, Universities Press, 2012, pp 666-669
5. National Committee for Clinical Laboratory Standards. (1993a). Performance Standards for Antimicrobial Disk Susceptibility Tests—Fifth Edition: Approved Standard M2-A5. NCCLS, Villanova, PA