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Comparative Antibacterial activities of the Combined crude Leaf extract of Eucalyptus Globulus, Azadirachta Indica and Ocimum Scantum

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ABSTRACT

The aim of the present study, whether combination therapy shows greater antibacterial effect than their individual effect. In the present study relived that the antibacterial activity of combinational plants Azadiracta indica, Ocimumsanctam and Eucalyptus globulus ethanol extract shown good inhibitory activity on the test organisms. The MIC was observed in 100µg ml in ethanol extract. Ethanol extracts of individual plants crude drugs inhibits the growth of micro organism in a small area on agar media, When using combinational plant extracts inhibits micro organism in a higher area on agar media. So the combinational crude extracts shows greater inhibition than the individual plant extracts.

Key words: Azadirachta indica, Ocimum scantum, Minimum Inhibitory Concentration and Combretaceae.

INTRODUCTION

Herbs were historically used for specific disease indications. However, the modern practice of use of herbs in crude form was targeted as prophylactic in an effort to prevent health problems ^[1]. There have been conflicting views with respect to the mechanisms of reported therapeutic efficacy of 10 herbal products.

A total of 122 biologically active compounds have been identified, derived only from 94 species of plants. A conservative estimate of the number of flowering plants occurring on the planet is 2,50,000. Of these, only about 6% have been screened for biological activity and a reported 15% have been evaluated phytochemically. Consistent findings should be carried out to discover a probable abundance of medicinal extracts in these plants ^[2].

While the exact mechanism of most of them is still unclear, herbal products have been widely explored as supplements, in folk and traditional medicines and as alternatives to conventional medicine. However, for an efficient exploration of this resource new methods are required that enable the rapid identification or falsepositives and known active compounds. This can help in developing several new chemical entities from ethno medicine ^[3].

Still constitutes a scientific backbone in the development of active therapeutics based upon traditional medicine of various ethnic groups with the ultimate aim of validating these traditional preparations, either through the isolation of active substances or throug various pharmacological findings ^[4].

Combination therapy:

Combined treatment is more than the sum of each component's individual effects4. In the herbal medicine context, however, it refers to the idea that certain components in a plant extract can improve the therapeutic effect of active agents in the patient5. One herb can enhance the effect of another given at the same time. It can also mean that the combined effect of a number of herbal components is actually greater than the sum of each of the individual components ^[5].

"Triphala", contains several bioactive markers which are known to have therapeutic activity and its finger print profile has been narrated. *'Triphala'* is a well known polyherbal formulation (churna) in Indian system of medicine (ISM) particularly in Ayurveda since ancient time; used for several therapeutic purposes.

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SVS group of institution, SVS institute of pharmacy, Bheemaram, Hasanparthy, Warangal -506015, Telangana, INDIA. *E-Mail: rajendarcology@gmail.com This formulation was prepared as powdered preparation, in combination of dried fruits of *Emblica officinalis* Gaertn (Family: Euphorbiaceae), *Terminalia belerica* Linn (Family: Combretaceae) and *Terminalia chebula* Retz (Family: Combretaceae), in equal proportions as described in Ayurvedic Formulary of India (AFI). Traditionally this formulation has been prescribed as a first line treatment for many ailments such as laxative in chronic constipation, detoxifying agent of the colon, food digestive problems and rejuvenator of the body etc ^[6].

In the present study, combination therapy shows synergistic antibacterial effect than their individual effect, in which 3 medicinal plants leaves ethanol extracts combinely shows greater effect than their individuals against three different standard strains of microorganisms

MATERIALS AND METHODS

Collection of Plant Material: Fresh leaves three different plants viz., Azadiracta Indica, Ocimum sanctum & Eucalyptus free from disease were collected from Warangal district. The leaves were washed thoroughly 2-3 times with running water and once with sterile distilled water ^[7].

Solvent Extraction: Thoroughly washed dried leaves of three plats azadiracta indica, ocimum sanctum and eucalyptus plant material were dried in shade for two weeks and then powdered with the help of shade for three days and then powdered with the help of warring blender. 25g of shade – dried powder was filled in the thimble and extracted successively with ethanol solvent in Soxhlet extractor for 48th. The solvent extracts were concentrated under reduced pressure and preserved were concentrated under reduced pressure ad preserved at 35° C in airtight bottle until further use.

Medium preparation: Accurately weigh 55 gm of to agar and make up to the volume (1000ml) using distilled water (used for Escherichia coli).Nutrient agar medium prepared for (pseudomonas, staphylococcus aureus) and the components are Agar (15gr), Beef extract (3gr), sodium chloride (5gr), peptone (5gr) make up to 11t using distilled water .Above prepared mediums are autoclaved at 121 temperature, 15lb pressure for 15 minutes.

Stock solutions of various plants crude extracts were prepared by mixing well appropriate amount of dried extracts and suitable solvents (distilled water) to give raise the final concentrations.

Petri dishes (size 100 mm diameter) containing 18 ml of cool and molten agar (at 40 c) was seeded with 100µl inoculums of bacterial strain. Media was allowed to solidify and then individual Petri dishes were marked for the bacteria inoculated. Wells of 6mm diameter were cut into solidified agar media with the help of sterilized core borer. Aliquot 100μ of each extract was poured in the respective well and plates were incubated at 37 c overnight. Solvents (distilled water), in which extracts concentration were prepared, were used as negative control while Streptomycin antibiotic of one unit strength was used as positive control. The experiment was performed in triplicate under strict aseptic conditions. The antibacterial activity for each of the extract evaluated was expressed in terms of the average of the diameter of zone of inhibition (in mm) produced by the respective extract at the end of incubation period. Standard deviations were also calculated and represented in the respective table against each extract.

Determination of minimum inhibitory concentration (MIC):^[8]

The minimum inhibitory concentration (MIC) was determined by comparing the various concentrations of plant extracts which have different inhibitory effect and the lowest concentration of extract showing inhibition. The MIC had done by 96 well U bottom plates. The MIC plates were filled with agar, agar and various concentrations of plant extracts, antibiotic-Streptomycin or solvent control. Finally the MHB medium with overnight test organism was equally distributed. All the samples were prepared in triplicates and incubated at 37 c for 24 hrs. The least concentration (highest dilution) of the extract that inhibits colony formation on solid agar medium after incubation at 37 c for 24 hr considered as MIC.

RESULTS

The use of antibacterial agents is critical to the successful treatment of infectious disease. Although there are numerous classes of drugs ^[9] that are routinely used to treat infections in humans, pathogenic micro organisms are constantly developing resistance to these drugs because of indiscrimate use of antibiotics ^[10, 11].

Results obtained in the present study relived that the antibacterial activity of combinational plants Azadiracta indica, Ocimumsanctam and Eucalyptus ethanol extract shown good inhibitory activity on the test organisms taking reference crude drug is the standard drug. The MIC was observed in 100 μ g ml in ethanol extract.

Combination of ethanol Azadiracta indica, Ocimum sanctum and Eucalyptus extracts shown better antibacterial activity compared to ethanol extracts of individual plants extracts, Azadiracta indica, Ocimum sanctum and Eucalyptus.

Table No. 1: Minimum Inhibitory Concentraion (MIC) of Azadirecta Indica, Ocimum

Compounds	Eschirichia coli	Psudomonas	Staphylococus	
Azadirecta Indica	75	100	100	
Ocimum Sanctam	100	100	100	
Eukalyptus	100	75	100	

Bacteria		Neem leaves E		Streptomycin	Saline	
	100	250	500	1000	10µg/ml	
E.coli	6±0.65	9 ± 0.71	9±0.72	9±0.78	19±0.71	0.28 ±0.75
pseudomonas	6 ±0.72	7 ±0.71	6±0.65	<i>10</i> ±0.65	19 ±0.78	0.28±0.65
Staphylo coccus	6±0.71	6±0.68	7±0.71	<i>10</i> ±0.78	18±0.65	0.28±0.78
Bacteria	Т	hulasi leaves	Streptomycin	Saline		
	100	250	500	1000	10µg/ml	
E.coli	6±0.65	6± 0.71	6±0.72	9±0.78	<i>18</i> ± <i>0</i> .71	0.28 ±0.75
pseudomonas	6±0.72	7 ±0.71	7±0.65	8±0.65	<i>19</i> ±0.78	0.28±0.65
Staphylo coccus	6±0.71	5±0.68	6±0.71	9±0.78	<i>18</i> ±0.65	0.28±0.78
Bacteria	Eucalyptus leaves Ethanol extract				Streptomycin	Saline
	100	250	500	1000	10µg/ml	
E.coli	6±0.65	6± 0.71	7±0.72	10 ±0.78	19±0.71	0.28 ±0.75
pseudomonas	7 ±0.72	7 ±0.71	8±0.65	9±0.65	19 ±0.78	0.28±0.65
Staphylo coccus	10±0.71	12 ±0.68	14±0.71	11±0.78	18±0.65	0.28±0.78
Bacteria	Neem+Thu	lasi+Eucalypt	Streptomycin	Saline		
	100	250	500	1000	10µg/ml	
E.coli	10±0.65	11± 0.71	12±0.72	16±0.78	19±0.71	0.28 ±0.75
pseudomonas	9±0.72	9±0.71	10±0.65	17±0.65	19 ±0.78	0.28±0.65
Staphylo coccus	10±0.71	9 ±0.68	11±0.71	16±0.78	18±0.65	0.28±0.78

Table No. 2: Sanctum and Eukalyptus

CONCLUSION

Based on discussion, nowadays multi drug therapy shows synergistic effect, acts on multiple target sites in pathogens in certain disorders. Further tests, toxicities studies are needed to these traditional combinational plants extracts.

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