

RESEARCH ARTICLE

EVALUATION OF ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF ETHANOLIC POLYHERBAL EXTRACT

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ABSTRACT

Antimicrobial activity of an ethanolic polyherbal extract was examined against the bacterial strains, *Escherichia coli*, *Proteus mirabilis*, *Gardnerella vaginalis* and *Pseudomonas aeruginosa* and the fungal strains, *Candida albicans* and *Aspergillus niger* using disc diffusion method. The polyherbal extract was prepared from the combined ethanolic extracts of *Saraca indica*, *Symplocos racemosa*, *Hemidesmus indicus*, *Aloe vera*, *Asteracantha longifolia*, *Erythrina indica* and *Tribulus terrestris*. Antibacterial activity was determined using Mueller Hinton Agar (MHA) medium and antifungal activity was studied using Potato dextrose agar (PDA) medium. The polyherbal extract showed significant antimicrobial activity against the tested microorganisms. The minimum inhibitory concentration (MIC) value was also determined which was found to be 1 mg/ml against the selected microorganisms.

Key Words: Antibacterial activity, antifungal activity, polyherbal extract, minimum inhibitory concentration

INTRODUCTION

Herbal medicine, sometimes referred to as Herbalism or Botanical Medicine, is the oldest form of healthcare known to mankind. Herbs had been used by all cultures throughout history for their therapeutic or medicinal value. A herb as a whole or plant part is valued for its medicinal, aromatic or savoury qualities. Herbal plants contain therapeutically active chemical substances that act upon the body¹. Many drugs commonly used today are of herbal origin. Herbal medicine is a major component in all indigenous traditional medicine and a common element in Ayurvedic, Homeopathic, Naturopathic, Traditional oriental and Native Indian medicine.

Historically, natural products, derived mainly from plant sources, have proved to be a fruitful source of new therapeutic agents. Herbal medicines are frequently considered to be less toxic and free from side effects than synthetic ones²⁻⁴. With the advancement of phytochemical knowledge, many active ingredients responsible for therapeutic action have been identified and isolated from plant sources. Better therapeutic effect, good patient compliance and cost effectiveness are the reasons for choosing drugs from natural origin.

An herbal formula consists of a selective combination of individual herbal ingredients that are formulated for a specific ailment or group of disease conditions. When herbs are combined together, they become more potent and effective within the body than single herb due to their activating or catalyzing influence upon one another. These combinations act as powerful catalysts with synergistic effects in order to activate individual healing energies which permeate the entire organism and reside in each and every cell in our body.

In the present work a polyherbal extract has been prepared from the combined ethanolic extracts of various plants viz., *Saraca indica*, *Symplocos racemosa*, *Hemidesmus indicus*, *Aloe vera*, *Asteracantha longifolia*, *Erythrina indica* and *Tribulus terrestris*. The polyherbal extract was screened for

antibacterial and antifungal activities against selected microorganisms.

MATERIALS AND METHODS

Collection of plant material

The plants were collected from Palode, Kerala and Papanasam, Tamil Nadu. The plants were authenticated by Botanical Survey of India, Coimbatore. The extraction process was carried out in Rumi Herbals R & D, Chennai.

Preparation of extract

The collected plant parts were cleaned and shade dried. The dried plant parts were coarsely powdered. Equal portions by weight of all the ingredients were homogeneously mixed and subjected to soxhlet extraction using ethanol. The extract obtained was evaporated in rotary evaporator to get a powdery mass. The powder extract obtained was then subjected to phytochemical analysis to detect the chemical constituents present.

The phytoconstituents present in the extract was detected by performing preliminary phytochemical tests. The detection of various phytoconstituents was carried out by performing qualitative chemical tests. The results of phytochemical analysis showed the presence of alkaloids, phenolic compounds, tannins, saponins, phytosterols, gums, mucilage, carbohydrates, proteins, amino acids, triterpenoids and flavones.

Preparation of Inoculum

Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Mueller-Hinton Broth (MHB) for bacteria and Sabouraud Dextrose Broth (SDB) for fungi that were incubated without agitation for 24 h at 37°C and 25°C respectively. The cultures were diluted with fresh Mueller-Hinton and Sabouraud dextrose broth to achieve optical

densities corresponding to 10^6 colony forming units (CFU/ml) for bacteria & 10^5 spore/ml for fungal strains.

Antibacterial activity

The effect of the polyherbal extract was studied on several bacterial strains by Disc diffusion method.⁵⁻⁷

Culture Medium

Mueller Hinton Agar (MHA) (B.No LOT 0000054948, Exp. date Mar. 2013)

Nutrient Broth(B.No. LOT0000010128, Exp.Dat 01/2013)

Mueller Hinton Agar Medium

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

Nutrient broth

One liter 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 min.

Bacterial Strains

S.No	Bacterial Pathogen	Code Number
1	<i>Proteus mirabilis</i>	RHPM001
2	<i>Gardnerella vaginalis</i>	RHGV001
3	<i>Escherichia coli</i>	RHEC001
4	<i>Pseudomonas aeruginosa</i>	RHPA001

Method

Growth inhibition activities for polyherbal extract against *Escherichia coli* (RHEC001), *Pseudomonas aeruginosa* (RHPA001), *Proteus mirabilis* (RHPM001) and *Gardnerella vaginalis* (RHGV001) were tested using disc diffusion method. One milliliter of inoculum suspension was used to inoculate by flooding the surface of Mueller-Hinton Agar petridishes. Excess liquid was airdried under a sterile hood. Three different dilutions of extracts (1000, 2000 and 3000 µg/disc) and Ciprofloxacin standard (100 µg/disc) were loaded on 6 mm sterile disc. Dimethylsulphoxide (DMSO) was used as negative control. The loaded disc was placed on the surface of medium and the compound was allowed to diffuse for 5 min and the plates were kept for incubation at 37°C for 24 h. At the end of incubation, minimum inhibitory concentration and inhibition zones formed around the disc were measured with transparent ruler in mm. The experiments were conducted in triplicate.

Determination of MIC

1 ml of standard test inoculum of bacterial suspension was added to the tubes containing 1 ml of broth with different concentration of PHE. The tubes were incubated at 37°C for 24 h. Subcultures were made from these tubes on Mueller Hinton Agar Medium and the presence, reduction or absence of growth was observed. If growth was observed, the particular compound was considered as inactive against the particular organism. If the growth was absent, the particular concentration of antibacterial agent was considered as effective in killing the microorganism. MIC is read as lowest concentration of antibacterial agent that inhibits the visible

growth of the test bacterium or that reduces the number of colonies of the test bacterium. The results were compared with positive control containing media with specific organisms and negative controls containing plain media with no organism.

Antifungal activity

Determination of antifungal activity of polyherbal extract was accomplished by agar disc diffusion method on Potato Dextrose Agar^{8,9}.

Culture Medium

Potato Dextrose Agar

Sabouraud Dextrose Agar Medium

Potato Dextrose Agar

Potato infusion was made by boiling 300 g of sliced potatoes in approximately 1 liter water for 30 min and then decanting or straining the broth through cheesecloth. Distilled water is added such that the total volume of the suspension is 1 liter. 20 g dextrose and 20 g agar powder are then added and the medium is sterilized by autoclaving at 15lbs for 15 min.

Sabouraud dextrose agar medium

One liter of Sabouraud Dextrose Agar Medium was prepared by dissolving 65 g of Sabouraud dextrose agar in 1000ml of distilled water. The medium was sterilized by autoclaving at 121°C for 15 min at 15 lbs.

Fungal Strains

S.No	Fungal Pathogen	Code Number
1	<i>Aspergillus niger</i>	RHAN001
2	<i>Candida albicans</i>	RHCA001

Method

Three different dilutions of extracts (1000, 2000 and 3000 µg/disc) and Ketoconazole standard (20 µg/disc) were loaded on 6 mm sterile disc. The loaded disc was placed on the surface of medium and the compound was allowed to diffuse for 5 min and the plates were kept for incubation at 30°C for 72 h. DMSO was used as the negative control. At the end of incubation, MIC and inhibition zones formed around the disc were measured with transparent ruler in mm. All tests were assayed in triplicate.

Statistical Analysis

Results are expressed as the mean value \pm standard error of mean (S.E.M.). Significant difference between control and experimental groups was assessed by student's t test.

Determination of MIC

After preparation of a suspension of the fungus, the stock solution of these essences was prepared with DMSO. Then 1 ml of this stock solution was combined with Sabouraud dextrose agar medium to prepare diluted compound. 1 ml Sabouraud broth was added to 11 tubes and 1 ml of diluted compound added to the first tube and after shaking, 1 ml of this solution added to the second tube. This work was continued to 9th tube. Finally, 1 ml of mixture of essence diluted compound and culture medium was removed from number 9 tube. After this, 50 µL of fungal suspension was added to first 10 tubes and then these tubes were incubated at 37°C for 24 h. After incubation the tubes were examined for microbial growth by observing turbidity after 24, 48 and 72 h. The MIC

values were interpreted as the highest dilution of the sample which showed no growth. After 24 h, concentration of that tube in which there is growth of one or two colonies was considered as the MIC. In this test, the 11th tube is negative blank and 10th tube is positive blank.

RESULTS AND DISCUSSION

The antibacterial activity was studied in Muller Hinton Agar medium by Disc diffusion method using Ciprofloxacin as standard. The bacterial strains used were *Proteus mirabilis*, *Gardnerella vaginalis*, *Escherichia coli* and *Pseudomonas aeruginosa*. The extract showed good antibacterial activity. The MIC was found to be 1mg/ml for all the bacterial strains used.

Antifungal activity was studied using Potato Dextrose Agar medium by Disc diffusion method using Ketoconazole as standard. The fungal strains used were *Aspergillus niger* and *Candida albicans*. The extract exhibited significant antifungal activity. The MIC value was found to be 1mg/ml for both *Aspergillus niger* and *Candida albicans*. The zone of inhibition produced by the polyherbal extract with the selected organisms and the MIC values are shown in table 1.

The antimicrobial properties of the polyherbal extract may be attributed to the presence of alkaloids, flavonoids, saponins and tannins and is confirmed by the results of phytochemical analysis. The antimicrobial action of these compounds is well-known and is related to their ability to denature proteins,

which in general renders them to be classified as surface-active agents.

Saponins have been reported to possess a wide range of biological activities. The mode of action of antibacterial effects of saponins seem to involve membranolytic properties, rather than simply altering the surface tension of the extracellular medium, thus being influenced by microbial population density.

Flavonoids are naturally occurring phenols which possess numerous biological activities including anti-inflammatory, antiallergic, antithrombotic and vasoprotective effects. Since they are known to be synthesized by plants in response to microbial infection, it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. More lipophilic flavonoids may also disrupt microbial membranes.

Alkaloids isolated from plant are commonly found to have antimicrobial properties. The mechanism of antimicrobial action is attributed to their ability to intercalate with DNA.

The antifungal activity may be attributed to various chemicals detectable in the extract such as saponins. The action mechanisms of saponins may lie in damage to the membrane and leakage of cellular materials, ultimately leading to cell death.

Table 1: Zone of inhibition against selected microorganisms and MIC values

Sample	Concentration (µg/disc)	Zone of inhibition (in mm)					
		<i>P.mirabilis</i>	<i>G.vaginalis</i>	<i>E.coli</i>	<i>P.aeruginosa</i>	<i>C.albicans</i>	<i>A.niger</i>
DMSO	300	-	-	-	-	-	-
Ciprofloxacin	100	12 ± 0.57	10 ± 0.33	16 ± 0.57	14 ± 0.00	NA	NA
Ketoconazole	20	NA	NA	NA	NA	12 ± 0.33	10 ± 0.00
PHE	1000	6 ± 0.57 ^a	2 ± 0.33 ^a	10 ± 0.57 ^a	8 ± 0.34 ^a	5 ± 0.57 ^a	4 ± 0.34 ^a
	2000	8 ± 0.33 ^a	4 ± 0.33 ^a	12 ± 0.33 ^a	10 ± 0.33 ^a	8 ± 0.34 ^a	6 ± 0.57 ^a
	3000	10 ± 0.57 ^a	8 ± 0.34 ^a	14 ± 0.11 ^a	12 ± 0.57 ^a	10 ± 0.33 ^a	8 ± 0.33 ^a
MIC		1 mg/ml	1 mg/ml	1mg/ml	1 mg/ml	1 mg/ml	1 mg/ml

Values are mean ± SEM, n = 6. t-value represents significance at ^ap < 0.05 when compared with solvent control group

CONCLUSION

The polyherbal extract was found to possess significant antibacterial and antifungal activities. Thus it may be concluded that the antimicrobial properties of the polyherbal extract is due to the presence of the phytoconstituents present in the extract.

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