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"ANTI MICROBIAL ACTIVITY OF VIDANGA CHURNA (Embelia ribes Burm.) BY CUP DIFFUSION TECHNIQUE"

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Abstract:

The Antimicrobial activity of a drug is generally expressed as its inhibiting effect towards the growth of bacterium in nutrient broth or on nutrient agar. Antimicrobial activity results gave mixed results. In which negative result were found by cup diffusion method. Thus, here Vidanga Churna (Embelia ribes Burm.) drug can be used in bacterial infections but not in fungal infections, which can be confirmed after conducting clinical trials.

Key Words: Anti microbial, Agar, Vidanga.

EVALUATION OF ANTIMICROBIAL ACTIVITY[1,2]

Principle:

The agar dilution technique is used to measure qualitatively the vitro activity of an Antimicrobial agent against the test bacterial.

In this method, the petridishes were filled with inoculated liquefied agar medium to uniform thickness. Then graded amount of test samples (Ex. antibiotics) are incorporated in agar plates and inoculated in spots with the organisms under study. If the organism under study is susceptible to the incorporated test samples, no bacterial growth is expected in agar plates with higher amount of the drugs. Bacterial growth is observed as the test sample concentration in the agar plate diminishes. Inhibition of growth at the minimum or lowest concentration of test sample.

Antimicrobial activity:

The Antimicrobial activity of a drug is generally expressed as its inhibiting effect towards the growth of bacterium in nutrient broth or on nutrient agar.

For this study, following conditions are observed for:

1) The substance or extract must be in contact with the test organism.
2) Conditions must be favorable for the growth of microorganisms in the absence of Antimicrobial substances.
3) There must be means of estimating the amount of growth and thereby percentage of growth of inhibition.
4) The activity of extract should be observed and determined by the growth response of microorganisms.

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Methods:

Tests were done by

1) Cup diffusion method.

In present study, roots powder of "Embelia ribes Burm" and of the same were extracted with water and ethanol as solvents. And all the extracts were subjected for Antimicrobial activity.

Method: Experimental Procedure:

In this present study, the Antimicrobial screening was done by cup plate method / cup diffusion method. This is one of the methods official in I.P. where the Antimicrobial extract is diffused from the cup through an agar layer in a petri dish or plate to an extent such that the growth of added microorganisms is restricted entirely in circular area or zone around the cavity containing the solutions of the test sample. The Antimicrobial activity is expressed as zone diameter in millimeters which is measured with a divider.

All the extracts (ethanol and water) of the roots powders (Embelia ribes Burm.) were screened for Antimicrobial activity against wide spectrum of microorganisms and the activity was compared with appropriate standards. (Chloroform, ethanol and water as control)

Standards used in the study:

For bacteria's (Gram +ve, Gram –ve)

1) OFLOXACIN
2) NORFLOXACIN

For Fungi

3) GRISEOFULVIN

Test organisms used for the study:

1) Bacteria

a) Staphylococcus aureus (Gram +ve)

b) Escherichia coli (Gram –ve)

2) Fungi

a) Candida albicans

Preparation of Antimicrobial agent stock solution:

1) Remove the Antimicrobial agents from the freezer and warm to room temperature before opening to avoid condensation of water.
2) Weigh appropriate amount of the powdered semi liquid Antimicrobial agent.
3) Dissolve the Antimicrobial agent powdered / semi liquid in solvent to make 2 mg/ml and 1 mg/ml concentrations.

Preparation of Standard Solutions:

1) Required amount of standard ofloxacin – 2 micg / disc and norfloxacin – 10 micg/disc solutions are prepared.
2) The concentration of standard equivalent to 100 micg/disc of Griseofulvin was prepared as standard for antifungal activity.

Preparation of Inoculums: Mueller and Hinton agar (Himedia Labs) of the following composition was used for preparation of slants.

Peptone : 5.0 gms
Beef extract : 1.5 gms
Sodium chloride : 5.0 gms
Agar : 15.0 gms
Yeast extract : 15.0 gms
Distilled water to make: 1000 ml.

About 28 gms of prepared medium was taken in 1000 ml of distilled water and boiled to dissolve completely. After being streaked with microorganisms under incubated at 370C + 10C for 24 hours. These 24 hours cultures were used for preparation of inoculums. The suspension of microorganisms was prepared in 10 ml of sterile water and 0.5 ml of this suspense was added to 100 ml of the agar medium.

Culture medium:

Medium types of media have been used according to the types of organism. In the present investigation, test sample medium (Muller – Hinton Agar – Himedia) employed possessing the following composition (Readymade medium).

Agar : 15.0 gms
Beef extract : 1.5 gms
Yeast extract : 3.0 gms
Peptone : 6.0 gms
Distilled water to make: 1000 ml.

About 27 gms of above readymade medium was dissolved in freshly prepared distilled water (1000 ml) by gentle heating.

Sterilization:

Sterilization of the medium, tubes for slants, borer etc was done by autoclaving at 15 lbs/square inches for 20 mints. The glassware like syringes, petridishes, pipettes, and empty test tubes were sterilized by dry heat in an oven at a temperature of 1600C for 1 hour.

Preparation of Agar Plates:

The sterilized medium was cooled at 40OC and 0.5 ml of inoculums per 100 ml of medium was added to the conical flask. This was shaken gently to avoid the formation of air bubbles and then transferred into petridishes so as to obtain 6 mm thickness of medium. The medium in the plate was allowed to solidify at room temperature.

Experimental Procedure:

The sterile borer was used to prepare 10 cups of 8 mm diameter in the medium of each petridish. An accurately measured 0.1 ml solution of each concentration of solutions of extracts and standard samples were added to the cups with the help of micropipette. All the plates were kept at room temperature for effecting diffusion of drug extracts and standards. Later, they were incubated at 37 + 1^0C of 24 hours. The presence of definite zones around the cup of any size indicates Antimicrobial activity. The controls were run simultaneously to assess the activity of ethanol and water, which were used as a vehicle for extracts. The diameter of the zone of inhibition was measured and recorded. The zones inhibitions for the Antimicrobial and Antifungal activities of extracts were calculated by measuring the inhibitory effect towards the growth of bacterial and fungus around nutrient agar cup.

Table No.: 1: Cup and Diffusion method (Mueller and Hinton Agar)

Drug / Compound Tested	Zone of inhibition E-coli	Zone of inhibition Staph. aures	Zone of inhibition Candida-a
Ofloxacin (2 micg/disc)	20 mm	23 mm	-
Norfloxacin(10micg/disc)	22 mm	19 mm	-
Gresiofulvin(100micg/disc)	-	-	17 mm
Water extract 1(2mg/ml)	1 mm	0 mm	0 mm
Water extract 2(2mg/ml)	4 mm	0 mm	0 mm
Water extract 3(2mg/ml)	7 mm	5 mm	1 mm
Water extract 4(2mg/ml)	6 mm	9 mm	2 mm
Alcohol extract 1(2mg/ml)	2 mm	0 mm	0 mm
Alcohol extract 2(2mg/ml)	5 mm	3 mm	0 mm
Alcohol extract 3(2mg/ml)	7 mm	5 mm	0 mm
Alcohol extract 4(2mg/ml)	8 mm	1 mm	1 mm

Note: All samples showed resistant activity / very less zone of inhibition, thus it shows negative results.

Conclusion:

From the results, it is concluded, all extracts are resistant to bacteria as well as fungi, when compared to the standards.

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