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"EVALUATION OF ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF RUTIN AND BERBERINE"

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ABSTRACT

The aim of study is to investigate the antibacterial and antifungal activity of natural compounds like Rutin (bioflavonoid) and Berberine (isoquinoline alkaloid) by using disc diffusion method. Antibacterial activity was determined using two gram positive cultures (Bacillus subtilis, Staphylococcus aureus) and two gram negative cultures (Pseudomonas aeruginosa, Escherichia coli). Antifungal activity was evaluated against Aspergillus flavus and Fusarium verticillioides fungal cultures. Both Rutin and Berberine were tested at concentration of 50 µg/ml and 100 µg/ml. The isoquinoline alkaloid Berberine at 50µg/ml has significant antibacterial activity against Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis and Staphylococcus aureus. At 100 µg/ml it has highly significant antibacterial activity against all cultures compared to control group. Rutin has no significant antibacterial activity at 50 µg/ml however at 100 µg/ml it has shown significant activity against Bacillus subtilis. Rutin has no significant antifungal activity at both the doses. Berberine at 50 µg/ml did not show any significant activity against Aspergillus flavus but it was shown lower significant activity against Fusarium verticillioides however at higher concentration (100 µg/ml) it has shown highly significant activity against both the cultures. Berberine has shown higher anti-bacterial and antifungal against all cultures and the activity was better when compared to Rutin. It was concluded that the antibacterial and antifungal potential of these natural products might be due to interference of microbial protein synthesis, so these natural compounds might be better therapeutic targets for bacteria and fungi clinically for development of safe and efficacious antimicrobials.

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INTRODUCTION

Antibiotics are the substances produced by various species of microorganisms (bacteria, fungi, actinomycetes) that suppress the growth of microorganisms. Hundreds of antibiotics have been identified and developed to the stage where they are of value in the therapy of infectious diseases [1].

Antibiotics includes antibacterial, antifungal, antiviral, antiparasites etc. these agents may act as either bacteriostatic (reduce the growth) or bactericidal (kills the bacteria) in case of antifungal drugs the term used is fungistatic or fungicidal. Majority of these antimicrobial drugs act through following mechanisms. Inhibition of cellwall synthesis, inhibition of protein synthesis, and inhibition of nucleic acid synthesis [2].

Commonly used antibacterial are Penicillins, Cephalosporins, Quinolones and antifungals are Azoles, Echinocandins, Polyenes etc. They act by complex pathways that control metabolism and growth of the bacteria and fungi resulting in static or cidal effect.

Above said natural, synthetic antibacterial and antifungal are associated with severe toxicity and the major drawback is associated with its use is quick development of resistance on long-term usage [3].

Hence the present research is targeted to identify safe and efficacious natural products like bioflavonoid and alkaloids [4].

Bioflavonoids are the natural compounds belongs to phenolic groups of products which are classified as flavonols, flavones, isoflavonones etc. flavonoids of different compounds have various pharmacological activities. So in the present study natural flavonols like Rutin is evaluated forantimicrobial activity [4].

Berberine is an isoquinoline alkaloid present in a number of clinically-important medicinal plants, including Hydrastiscanadensis (goldenseal), Coptischinensis (coptis or goldenthread), Berberisaquifolium (Oregon grape), Berberis vulgaris (barberry), and Berberisaristata (tree turmeric). Berberine was proven to exhibit significant antimicrobial activity against a variety of bacteria, fungi, protozoanshelminths, and viruses. In the following study bereberine was evaluated for antimicrobial activity against bacteria and fungi [5].

MATERIALS AND METHODS

Table 1: Drugs and chemicals.

S. no.	Drugs and chemicals	Source
1	0.25% Ethanol	
2	Rutin	Sigma
3	Ciprofloxacin	Sigma
4	Berberine	Sigma
5	Ketoconazole	Sigma

Selection of micro organisms:

Microorganisms were collected from department of microbiology, govt medical college, MBNR, Telangana, India. Bacteria were maintained in nutrient broth at 37˚C. Fungus was maintained in potato dextrose agar at 28˚C [6].

2: Microorganisms.

S. no.	Microorganisms used	
	Bacteria	Fungi
2	Pseudomonas aeruginosa	Aspergillus flavus
3	Escherichia coli	Fusarium verticillioides
4	Bacillus subtilis	
5	Staphylococcus aureus	

Preparation of Inoculums:

The gram positive(Bacillus subtilis, Staphylococcus aureus) and gram negative(Escherichia coli, Pseudomonas aeruginosa) were pre-cultured in nutrient broth overnight in a rotary shaker at 37˚c, centrifuged at 10,000 rpm for 5 minutes. The fungal inoculums (Aspergillus flavus, Fusarium verticillioides) were prepared from 7 days old culture grown on potato dextrose agar medium. 10 ml of distilled water is added to Petri dishesand the density of the spores adjusted spectrophotometrically to obtain 10^5 spores/mlfinal concentration [7].

Procedure for evaluation of Antimicrobial activity:

Antibacterial activity:

Disc diffusion method [8]:

Different concentrations of the extracts were prepared by reconstituting with ethanol. (50 and 100 µg/ml).The test organisms were transferred into respective medium by spread plate technique [9].10µl of 24 hours bacterial cultures were grown in broth. After solidification, filter paper discs (4mm diameter) coated with extracts are placed in respective microorganism seeded plates. Rutin and Berberine was termed as test drugs and ethanol used as a vehicle control. Ciprofloxacin is used as positive control [10].The zones of growth inhibition were measured after 24 hours of incubation at 37˚C. Zone of inhibition of bacterial growth is expressed in mm.

Table 3: Experimental design in Antibacterial activity.

Groups	Treatment
Group-I	Vehicle control (ethanol 0.25%)
Group-II	Rutin (50µg/ml)
Group-III	Rutin (100µg/ml)
Group-IV	Berberine (50µg/ml)
Group-V	Berberine(100µg/ml)
Group-VI	Standard drug (Ciprofloxacin 10 µg/ml)

Antifungal activity:

The antifungal activity was evaluated by using disc diffusion method [11].Sterile filter paper discs (4mm diameter) impregnated with test compounds were placed in respective microorganism seeded Petri plates[12].Rutin and Berberine were termed as test drugs and ethanol used as a vehicle control. Azole antifungal Ketoconazole is used as positive control.

Table 4: Experimental design in Antifungal activity.

Groups	Treatment
Group-I	Vehicle control (ethanol 0.25%)
Group-II	Rutin (50µg/ml)
Group-III	Rutin (100µg/ml)
Group-IV	Berberine (50µg/ml)
Group-V	Berberine(100µg/ml)
Group-VI	Standard drug (Ketoconazole 10 µg/ml)

Statistical Analysis:

Data is expressed as mean±SEM (triplicates). All groups were compared by control group using Two-way ANOVA followed by dunnets test. Probability (P) value <0.05 taken as the level of significance.

RESULTS AND DISCUSSION

Table 5: Antibacterial activity of Rutin and Berberine against bacterial species tested by disc diffusion assay.

Groups	Zone of inhibition(mm)			
	Pseudomonas Aeruginosa	Escherichia Coli	Bacillus Subtilis	Staphylococcus Aureus
G-I Ethanol (0.25%)	2±0.12	3±0.15	3±0.11	2±0.18
G-IIRutin (50µg/ml)	3±0.55	2±1.08	6±0.76	4±0.67
G-IIIRutin (100µg/ml)	5±0.22	4±0.43	9±0.26***	5±0.55
G-IVBerberine (50µg/ml)	6±0.95**	8±0.42**	10±0.55***	12±0.92***
G-V Berberine(100µg/ml)	14±0.96***	17±0.95***	13±0.55***	15±0.45***
G-VI Ciprofloxacin(10 µg/ml)	25±0.66***	28±0.79***	24±0.27***	23±0.67***

Values are expressed as the mean ± SEM for triplicates. * P < 0.05; ** P < 0.01; ***P < 0.001 compared with control. Data is analyzed by Two-way ANOVA followed by dunnets test.

Table 6: Antifungal activity of Rutin and Berberine against bacterial species tested by disc diffusion assay.

Groups	Zone of inhibition(mm)	
	Aspergillus flavus	Fusarium verticillioides
G-I Ethanol (0.25%)	1±0.60	1.1±0.60
G-II Rutin (50µg/ml)	1±0.90	1±0.70
G-III Rutin (100µg/ml)	1.8±0.95	2.1±1.22
G-IVBerberine (50µg/ml)	7.0±1.00	9.0±1.90*
G-V Berberine(100µg/ml)	13±1.15***	14±1.92***
G-VIKetoconazole(10 µg/ml)	22±1.47***	27±1.96***

Values are expressed as the mean ± SEM for triplicates. * P < 0.05; ***P < 0.001 compared with control. Data was analyzed by Two-way ANOVA followed by dunnets test.

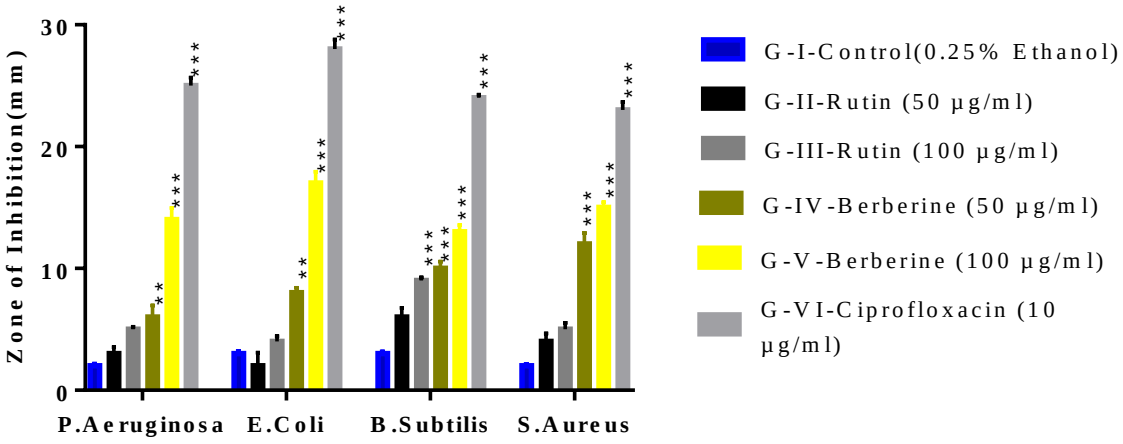

Figure 1: Antibacterial activity.

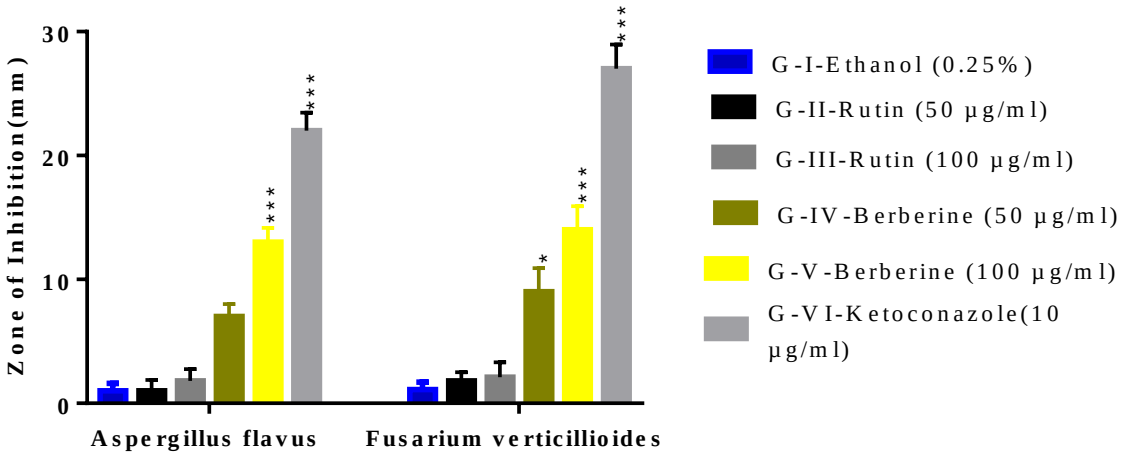

Figure 2: Antifungal activity.

Antibacterial activity:

Antibacterial activity of natural compounds like Rutin (bioflavonoid) and Berberine (isoquinoline alkaloid) was determined by using disc diffusion method. The alkaloid Berberine at 50 µg/ml did not show any antibacterial activity againstEscherichia Coli, Bacillus Subtilis, Staphylococcus Aureus. It has shown significant antibacterial activity againstPseudomonas aeruginosa(**p<0.01),Escherichia coli(**p<0.01), Bacillus subtilis(***p<0.001), Staphylococcus aureus(***p<0.001) and at 100 µg/ml it has shown highly significant antibacterial activity (***p<0.001) against all cultures compared to control group. Rutin didn't show significant antibacterial activity against all bacterial cultures at 50 µg/ml however at 100 µg/ml it was shown significant activity against (***p<0.001) Bacillus subtilis. Ciprofloxacin is an extended spectrum antibiotic which was shown highly significant antibacterial activity (***p<0.001) against all these pathogens. The antibacterial activity was higher when compared to Rutin and Berberine treated groups.

Antifungal activity:

Rutin has no significant antifungal activity at both the doses. Berberine at 50 µg/ml did not show any significant activity against Aspergillus flavus but it was shown slightly significant activity against Fusarium verticillioides (*p<0.05). Berberine at 100 µg/ml has shown highly significant activity (***p<0.001) against both the cultures. Ketoconazole is a fungicidal agent which was showed more significant (***p<0.001) antifungal activity against Aspergillus and Fusarium compared to vehicle, Rutin and Berberine treated groups.

CONCLUSION

Present study was investigated for the antibacterial and antifungal activity of natural compounds like Rutin (bioflavonoid) and Berberine (isoquinoline alkaloid) by disc diffusion method. The results of this study clearly indicates that the Rutin at both doses doesn't have significant antibacterial and antifungal activity. Berberine has shown higher anti-bacterial and antifungal against all cultures and the activity was better when compared to Rutin. It was concluded that the antibacterial and antifungal potentialof these natural products might be due to interference of microbial protein synthesis, so these natural compounds might be better therapeutic targets for bacteria and fungi clinically for development of safe and efficacious antimicrobials. Further studies are being carried out to isolate the pure active constituents responsible for the activity and to identify the exact mechanism of action for antibacterial and antifungal activity.

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