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Evaluation of the *in vitro* antimicrobial effect of resveratrol on human pathogens

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Abstract
Resveratrol, the naturally occurring polyphenol compound present in plant kingdom was identified to have significant antimicrobial inhibitory activity against human clinical pathogens which causes harmful diseases to human beings by this antimicrobial study. In this *in vitro* study, eight strains of bacteria and five strains of fungi were used. Among bacteria, the growth of *Pseudomonas aerogenosa* and *Staphylococcus aureus* were highly controlled by resveratrol followed by other strains. In antifungal activity, *Aspergillus fumicatus* and *Aspergillus niger* shows high zone of inhibition against resveratrol and no activity was recorded with other tested strains of fungi.

Keywords: resveratrol (3, 4, 5-trihydroxystilbene), antibacterial, antifungal, clinical pathogens, agar disc diffusion method, standard antimicrobial drugs, zone of inhibition

Introduction
Resveratrol (3, 4, 5-trihydroxystilbene) is a polyphenol naturally found in different group of plants including grapes, peanuts, cocoa, chocolate, tomato fruit skin, hops, jackfruit, blueberries, cranberries, mulberries, and bilberries [1, 3]. It was first detected in the roots of white hellebore (*Veratrum grandiflorum*) [4] in 1940, and later in 1963, separated from the roots of *Polygonum cupsidatum*, a plant used in traditional Chinese medicine [5]. A wide range of scientific researches has shown that resveratrol possesses various therapeutic effects, including antioxidant, antimicrobial, cardioprotective, anti-tumor, anti-diabetes, anti-obesity and anti-aging effects [6, 14]. Resveratrol inhibit the growth of some pathogenic microorganisms such as Gram-positive and Gram-negative bacteria and fungi [15, 23].
Resveratrol has antimicrobial, antiparasitic and anti-inflammatory effects showing the potential to be used for microbial food safety against the infections endangering people [24].The resveratrol inhibits the growth of human pathogenic fungi by preventing its germination [25, 26].
In view of this, the antimicrobial activity of resveratrol against various strains of human clinical isolated pathogens *i.e.* bacteria and fungi were assessed by this study to evaluate the potential of resveratrol as an antimicrobial agent.

Materials and Methods
Resveratrol
Resveratrol (3,4',5-trihydroxystilbene) compound was purchased from Sigma - Aldrich, USA.

Bacterial strains
Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli, Proteus mirabilis, Micrococcus species, Bacillus cereus, Pseudomonas aerogenosa and *Proteus vulgaris*.
Fungal strains: *Aspergillus niger, Aspergillus fumicatus, Aspergillus flaves, Aspergillus terreus* and *Rhizopus oryzae.*

Bacterial media: Muller Hinton agar.
Fungal media: Potato dextrose agar.
Positive control for bacteria and fungi: Norfloxacin and Fluconozale.
P reparation of sterile disc: Each sterile disc (6mm, Himedia) was incorporated individually with 5, 10, 15, 20, 25 and 30µg/µl of compound (resveratrol) using micropipette.
Agar Disc Diffusion Method: Agar disc-diffusion method was developed in 1940 by N.G.Heatley [27] and this method is used in many clinical microbiology laboratories for routine antimicrobial susceptibility testing. The antibacterial and antifungal activity of human clinical isolated bacteria and fungi against resveratrol was done according to the standard procedures of Parekh and Sumitra (2007), Selvamohan *et al.* (2012) and Parasharami *et al.* (2014) [28, 30].
Agar plates are inoculated with a standardized inoculum of the test microorganism *i.e*., bacteria and fungi. Then, filter paper discs (about 6 mm in diameter), containing the resveratrol at different concentrations 10, 15, 20, 25 and 30 µg/µl, were placed on the agar surface and the Petri plates were incubated under suitable conditions. The incubation period for bacteria was 37◦C for 24 hrs and for fungi 37◦C for 48 h. Norflaxin (20 µg/µl) served as positive control for the bacteria and fluconozole (20 µg/µl) for fungi. Antimicrobial activities were evaluated by measuring the inhibition zone against the test organisms. Resveratrol diffuses into the agar and inhibits germination and growth of the test microorganism and then the diameters of inhibition growth zones are measured in mm. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms by the method of Collins and Lyne (1987) [31].

Results and Discussion
The antimicrobial activity of resveratrol was assessed and the results are tabulated. The bacterial strains used in this present study were chosen on the basis of their clinical importance.

Eight bacterial strains *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Proteus mirabilis, Proteus vulgaris, Micrococcus species, Pseudomonas aerogenosa* and *Bacillus cereus* were tested with 10µg, 15µg, 20µg, 25µg and 30µg concentrations of resveratrol along with norflaxin 20µg/µl was used as positive control. In this study, significant antibacterial activity was recorded with *Pseudomonas aerogenosa* followed by *Staphylococcus aureus, Micrococcus species* and *Proteus vulgaris* and no activity was recorded with *Bacillus cereus.* (Table.1). Similar results were recorded with *Bacillus cereus* by Vaz *et al.* 2012 [32] where wine proved a strong inactivation effect against vegetative cells of two strains of *B. cereus.*

Resveratrol displayed potent antifungal activity against *Aspergillus fumicatus* followed by *Aspergillus niger* (Table.2). In other tested species such as *Rizhopus oryzae, Aspergillus terreus and Aspergillus flaves,* resveratrol failed to inhibit the growth *ie*. no zone of inhibition. Fluconazole was used as positive control for this study. From the data it can be concluded that antimicrobial activity increased comparatively by increasing the concentration of resveratrol *in vitro*. Figure 1 to 13 clearly indicates the antimicrobial activity of resveratrol.

As far as we know, this study is the first demonstration of the remarkable antimicrobial abilities of resveratrol, a polyphenolic compound against human clinical isolated pathogens. Most importantly, the antimicrobial mechanism of resveratrol is due to its action on the cell wall and cytoplasmic membrane. These results are in agreement with the findings of the authors of some plant studies [33]. Based on this study it can be concluded that resveratrol can be used as human antimicrobial agent.

Table 1: Antibacterial activities of resveratrol

Bacteria Strains	Zone of inhibition in mm					
	10µg/µl	15µg/µl	20µg/µl	25µg/µl	30µg/µl	Norflaxin 20µg/µl
Staphylococcus aureus	0	1.5	1.7	1.9	2.5	4.8
Escherichia coli	0	0	0	0	1.5	4.1
Klebsiella pneumonia	0	0	0	0	0.5	4
Proteus mirabilis	0	0	0	0.5	0.7	4
Proteus vulgaris	0	0.2	0.4	0.5	0.7	4.3
Micrococcus species	1	1.1	1.3	1.4	1.6	4.7
Pseudomonas aerogenosa	1.1	1.2	1.6	2.3	2.5	4.8
Bacillus cereus	0	0	0	0	0	4

Table 2: Antifungal activities of resveratrol

Fungi Strains	Zone of inhibition in mm					
	10µg/µl	15µg/µl	20µg/µl	25µg/µl	30µg/µl	Fluconazole 20µg/µl
Aspergillus niger	0.3	0.5	0.7	1.2	1.6	3.4
Rhizopus oryzae	0	0	0	0	0	0
Aspergillus terreus	0	0	0	0	0	2.5
Aspergillus flaves	0	0	0	0	0	4
Aspergillus fumicatus	1.2	1.4	1.6	1.9	2	1.5

Antibacterial activities of resveratrol at different concentrations

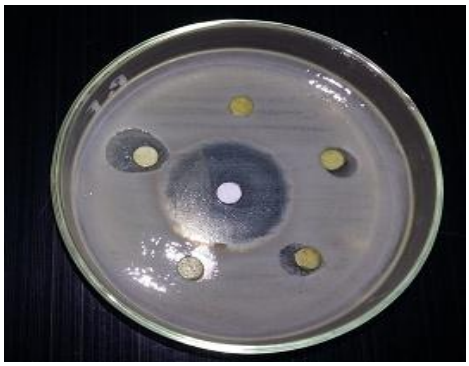

Fig 1: *Staphylococcus aureus*

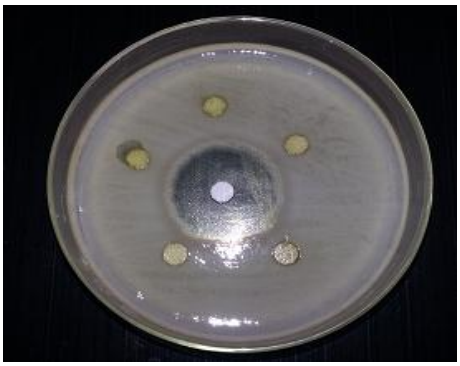

Fig 2: *E. coli*

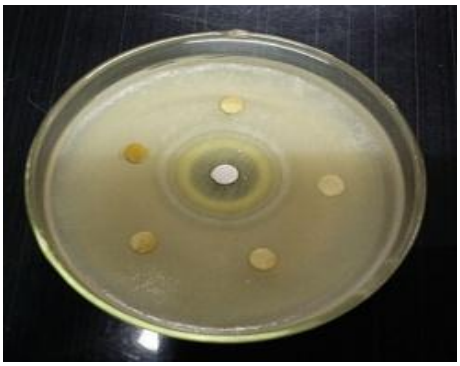

Fig 3: *Klebsiella pneumonia*

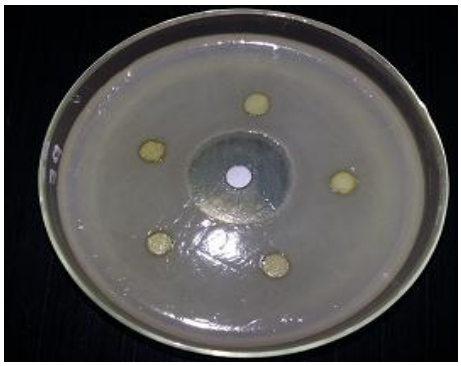

Fig 4: *Proteus mirabilis*

Fig 5: *Proteus vulgaris*

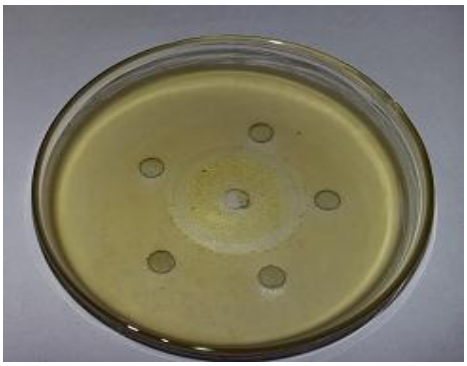

Fig 6: *Micrococcus species*

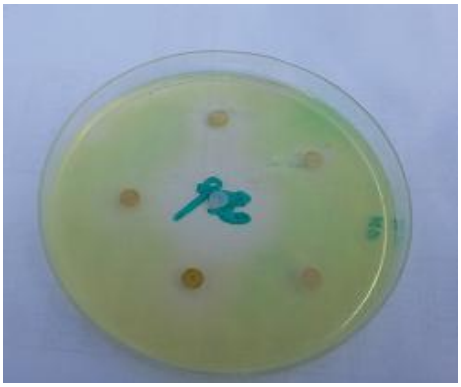

Fig 7: *Pseudomonas aerogenosa*

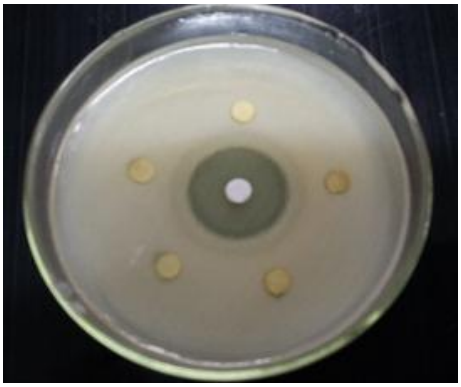

Fig 8: *Bacillus cereus*

Fig 1 to 8: markings A to E starting from 12'O clock position and F at the centre of the plate. A- 10µg/µl, B- 15µg/µl, C- 20µg/µl, D- 25µg/µl, E- 30µg/µl, F- NORFLAXIN 20µg/µl

Antifungal activities of resveratrol at different concentrations

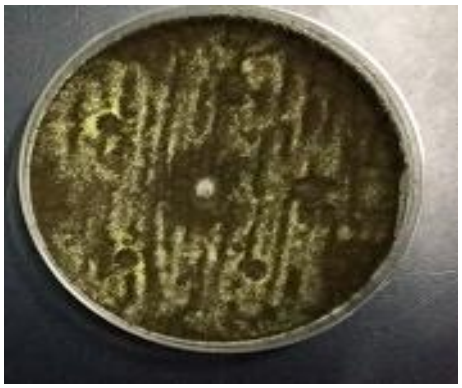

Fig 9: *Aspergillus niger*

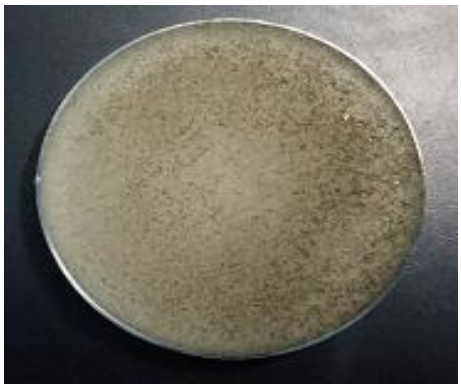

Fig 10: *Rhizopus oryzae*

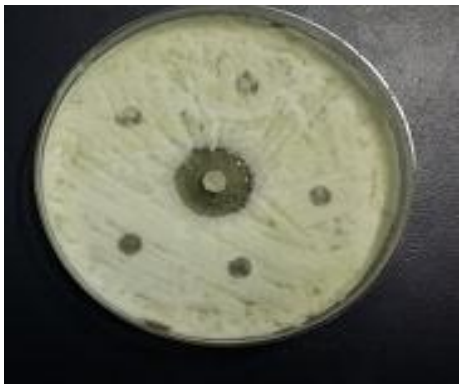

Fig 11: *Aspergillus terreus*

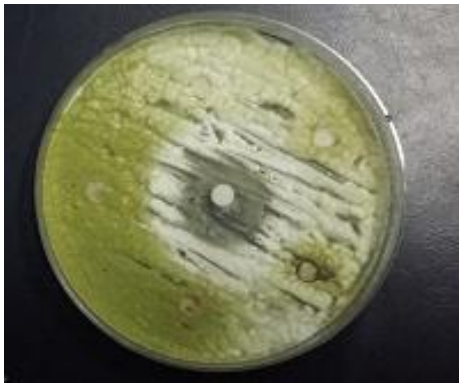

Fig 12: *Aspergillus Flavus*

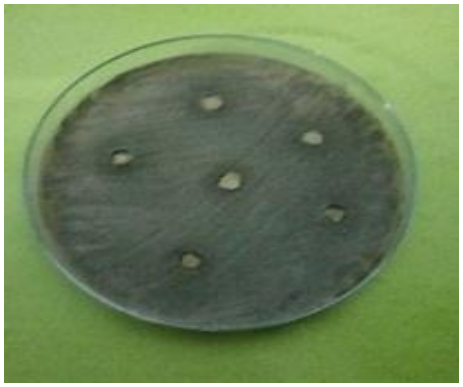

Fig 13: *Aspergillus fumigatus*

Fig 9 to 13: markings A to E starting from 12'O clock position and F at the centre of the plate. A- 10µg/µl, B- 15µg/µl, C- 20µg/µl, D- 25µg/µl, E- 30µg/µl, F- FLUCONOZOLE 20µg/µl

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