Introduction

In the present day scenario it is absolutely vital to discover novel antibacterial molecules as antibiotic discovery process has not been able to keep the pace with rapidly emerging pathogens and drug resistant isolates [1]. In this process due emphasis is given to understand the mechanisms underlying the antibacterial action of the potential molecules. This helps in identifying the likely targets and predicts possible outcomes of resistant mechanisms that bacteria may develop. Resveratrol is a redox active molecule that readily binds with transition metal ion copper and reduces it. In this process it generates reactive oxygen species (ROS) [2]. ROS can damage vital molecules inside a cell like DNA, proteins and membranes. It also alters the redox status inside a cell leading to pleiotropic effects that could culminate in cell death. Previous studies have shown that resveratrol and its metabolite piceatannol bind copper ions, generate ROS and damage DNA [2,3]. Inhibition of ROS generation by addition of chelators or scavenging ROS by antioxidants prevented this damage [2]. It has been further demonstrated that due to increased availability of copper in certain target cells, polyphenols like resveratrol are able to target them selectively [3].

Resveratrol inhibits a wide array of bacteria including both gram positive and gram negative organisms. It is more effective against gram positive bacteria than gram negative bacteria [4]. in vitro resveratrol inhibits Helio bacter pylori that is responsible for chronic gastritis and peptic ulcer [5]. In rats fed with resveratrol prior to colitis induction, increase in the favourable lactobacilli and bifidobacteria was observed with a concomitant decrease in enterobacteria [6]. In spite of these observations the mechanism(s) behind resveratrol induced inhibition of bacteria remains unknown. The present study aims to understand the antibacterial action of resveratrol. In particular, we sought to probe any role of ROS, as resveratrol is a known ROS inducer. For this we employed a comprehensive array of biochemical and genetic approaches. Our results provided convincing evidence against the direct involvement of diffusible ROS in the resveratrol-mediated inhibition of...
Escherichia coli cells while the inhibition is a consequence of oxidative membrane damage.

Materials and methods

Bacterial strains

The bacterial strains and plasmids used in this study are listed in Table 1. Cultures were grown in Luria-Bertani broth (LB) or plated on LB agar. The sodA sodB double mutant (designated NJ03) of strain MG1655 was constructed by P1-mediated transduction as previously described [7]. In brief sodA sodB double mutant NJ03 was generated by transduction of sodA allele from strain NJ01 mutant to NJ02 background [8].

Reagents and chemicals

The Luria agar components yeast extract, tryptone and agar were from Difco laboratories, USA. Tert-buty1 hydroperoxide was purchased from Lancaster Synthesis, Morecambe, England. Hydrogen peroxide (H₂O₂) was from Merck Specialities Pvt. Ltd., Mumbai, India. L-Buthionine-sulfoximine (BSO), dihydrofluorescein diacetate (DCFDA), dimethyl sulfoxide (DMSO), GSH, Lysozyme, Mitomycin C, N-acetylcycteine (NAC), Propidium iodide (PI) and Paraquat dichloride were purchased from Sigma Aldrich Inc., St. Louis, USA. Live/Dead BacLight bacterial staining kit was from Molecular probes, Eugene, USA.

Enumeration of viable cells

Overnight E. coli cultures were diluted (1:100) in LB and grown afresh at 37 °C. Mid-exponential-phase culture (OD₆₀₀ 0.3–0.5) was exposed to different concentrations of resveratrol for 5 h at 37 °C. All the resveratrol treated samples contained a uniform concentration of vehicle (1% v/v DMSO). At the end of the incubation period the number of cells in each experimental tube was enumerated by plating on Luria agar.

Mid-exponential-phase culture was exposed to different concentrations of resveratrol. At the end of the incubation period the cells were washed with phosphate buffered saline (PBS), incubated in PBS with 10 μM DCFDA in dark for 30 min. The cells were acquired by a flow cytometer (CyFlow Space, Partec, Germany) and fluorescence (excitation 488 nm and emission 530 nm) quantified.

Induction of adaptive response in E. coli

Mid-expontial-phase cells were suspended in saline with 1% glucose and exposed to H₂O₂ or γ-radiation to induce an adaptive response in E. coli. In case of H₂O₂ the cells were exposed to 0.5 mM H₂O₂ or 1 mM H₂O₂ for 30 min. In case of γ-radiation the cells were exposed to 10 Gy at a dose rate of 2.14 Gy/min and remained to stand till 30 min. At the end of 30 min cultures were treated with resveratrol for 5 h in LB and the number of viable cells was enumerated by plating on Luria agar.

Estimation of catalase in E. coli

The E. coli cells were lysed by treatment with lysozyme followed by sonication. The cell lysate was obtained by centrifugation at 4 °C for 30 min at 16,500 g. The amount of catalase per unit protein was estimated spectrophotometrically by a previously established method [9].

Monitoring expression of oxidative stress responsive genes

To monitor the expression of different genes in response to resveratrol, we employed the corresponding reporter strains from Thermo Scientific E. coli promoter collection which employs the E. coli K12 strain MG1655 wherein the promoters of corresponding genes are transcriptionally fused to green fluorescent protein (GFP) on a low copy plasmid pMS201 [10]. The fluorescence of GFP serves as a reporter for transcription initiation from the promoter. The cells were treated with resveratrol or respective positive control in 0.3 mL volume in a 96 well plate in triplicates. The plate was read at a 37 °C in a multiwell plate reader (Tecan Infine M200) up to 22 h. The GFP fluorescence (excitation: 480 nm and emission: 510 nm) and the absorbance (OD₆₀₀) were quantified at 30 min intervals. Specific fluorescence intensities (SFI) were calculated by dividing fluorescence values by OD₆₀₀ for the respective wells. Being a relatively stable protein, the accumulation of GFP fluorescence per cell (SFI) with respect to time gives an indication of the promoter activity. The strains employed from the collection for this study are serial numbers: 1862 oxyR (transcriptional regulator of oxidative stress, regulates intracellular H₂O₂...
Membrane compromise studies

Membrane compromise in *E. coli* was studied by quantifying the leaked potassium ions in the supernate as well as the residual potassium ions in the pellet. The cells were treated with vehicle or resveratrol in a 1 mL volume in PBS. After different time intervals the supernate and pellet were separated by centrifugation. The pellet was digested with 4 N nitric acid overnight. The samples were diluted with Nanopure water to 5 mL and the potassium was quantified by atomic absorption spectrometry. Membrane compromise (up to 3 h) was also studied by staining with Live/Dead BacLight kit followed by fluorescent microscopy according to the manufacturer’s instructions. Pictures were acquired in a Carl Zeiss Axioskop2 Motplus fluorescent microscope. The SYTO–PI ratio was quantified spectrofluorimetrically ([JASCO FP6500](https://www.jasco.com/)). The excitation and emission wavelengths for SYTO were 480 and 530 nm respectively and those of PI were 480 and 630 nm. To further quantify the membrane damage by the uptake of PI the test samples were treated with PI (50 μM) for 5 min in dark and acquired immediately by flow cytometry.

Statistical analysis

Data are expressed as means ± SE. Statistical differences between groups were calculated by the Student’s unpaired *t*-test. *p* ≤ 0.05 was considered to be significant.

Results

The effect of resveratrol on *E. coli* MG1655 was evaluated by exposing the cells in LB to increasing concentrations of resveratrol (57–456 μg/mL; 250–2000 μM). It was found that resveratrol inhibits growth of *E. coli* in a concentration dependent manner up to 342 μg/mL ([Fig. 1a](#fig1a){refig}). The IC50 concentration was found to be 182.5 ± 5.09 μg/mL. This value is in agreement with a previous report of related gram negative organisms albeit using a higher DMSO concentration that influences the solubility of resveratrol and subsequently its efficacy [11]. Further, the minimum inhibitory concentration of resveratrol against many gram negative microorganisms has been reported to be greater than 400 μg/mL [12]. This is due the insolubility of resveratrol beyond 400 μg/mL in aqueous media as observed by us and others [12]. Hence we employed IC50 as an end point in our studies. To evaluate if resveratrol is bactericidal or bacteriostatic we incubated pre-grown *E. coli* cells with resveratrol and monitored the number of viable cells at regular time intervals by plating. The results of both time and dose dependent experiments did not show any reduction in the number of viable cells due to resveratrol treatment, suggesting its bacteriostatic, but not bactericidal ([Fig. 1b](#fig1b){refig}). The dose-dependent effect of resveratrol and the positive control H2O2 on ROS generation was evaluated at 2 h by flow cytometry using DCFDA as the oxidative probe. Compared to the control and vehicle-treated cells, resveratrol dose-dependently increased the DCFDA fluorescence, showing the maximum (16.3 ± 2.8% increase) with resveratrol (228 μg/mL). The positive control, H2O2 (1 mM) induced a robust fluorescence (> 80% at 1 mM) in the cells under similar conditions ([Fig. 2](#fig2){refig}). Separate time-dependent (0, 1, 2 and 3 h) experiments with resveratrol (228 μg/mL) showed a gradual increase in the DCFDA fluorescence up to 2 h when the plateau value as above was attained. External supplementation of the antioxidants, by pre-treating the cells with NAC and GSH (each 5 and 10 mM) did not prevent the cell inhibition by resveratrol (182 μg/mL). The antioxidant supplemented population exhibited the same amount of inhibition (48.83 ± 2.57% to 53.86 ± 2.62%) as that of 182 μg/mL resveratrol (51.75 ± 3.73%) treated cells. The antioxidants NAC and GSH alone did not alter the growth of *E. coli* ([Fig. 3](#fig3){refig}). In a reverse approach we employed BSO to deplete the intracellular antioxidant glutathione. As shown in [Fig. 4](#fig4){refig} *E. coli* cells treated with BSO did not exhibit a hypersensitive phenotype to subsequent resveratrol treatment. The sensitivity of the 0.5, 1 and 2 mM BSO treated cells to resveratrol (182 μg/mL) were 47.35 ± 3.65%, 47.68 ± 2.54% and 42.32 ± 2.64% respectively which was not significantly different than that of BSO untreated cells (47.82 ± 3.26%). Further we employed antioxidant defence deficient *E. coli* strains to investigate the role of ROS upon treatment with resveratrol. Enzymatic antioxidant defences of *E. coli* against superoxide and peroxides comprises of superoxide dismutases (sodA, sodB and sodC), catalase (katG and katE) or alkaline hydroperoxidase (ahpCF) respectively [13,14]. The efficacy of resveratrol on these multiple knock out strains was compared to the wild type strain that had these defences intact. We employed mutants that lack more than one antioxidant defence in this investigation as lack of one gene product is compensated by the other and defeat the purpose of the investigation. All the three catalase and peroxidase double mutants (i.e. JI367, JI372 and JI374) exhibited similar sensitivity to 182 μg/mL resveratrol (49.23 ± 4.26%, 51.60 ± 3.56% and 53.81 ± 4.62% respectively) as that of the wild

![Fig. 1.](#fig1){refig} (a) Effect of resveratrol on the growth of *E. coli*. *E. coli* cells in LB treated with different concentrations of resveratrol for 5 h exhibited reduced growth as enumerated by viable count. (b) Effect of resveratrol on pre-grown *E. coli* cells. A bacteriostatic effect was observed on *E. coli* cells incubated with resveratrol. The number of cells in different samples was enumerated on Luria agar plates in a time dependent manner up to 5 h. The experiments were performed thrice with triplicate samples. The values denoted are mean ± SEM.
type parent strain MG1655 (52.17 ± 3.84%). Moreover the extent of inhibition seen with catalase (katE, katG) and alkylhydroperoxide reductase (ahpCF) mutant JI377 (51.63 ± 3.44%) was not different from the parent strain MG1655. Similarly NJ03 which is the double gene knockout for cytosolic superoxide dismutase activities (SodA and SodB) exhibited similar inhibition (51.60 ± 3.47%) as compared to the wild type parent strain (53.51 ± 4.33%) upon resveratrol treatment (182 μg/mL). However upon paraquat treatment (64 μg/mL), a drug that generates superoxide radicals this double mutant (87.82 ± 2.94%) and the triple mutant JI377 (83.2 ± 3.6%) exhibited significant increase in sensitivity compared to wild type (72.13 ± 3.82%) (Fig. 5).

To gain further evidence, the E. coli cells were exposed to H2O2 or γ-radiation to induce adaptive oxidative stress response in them. Incubation of the cells for 30 min with different concentrations of H2O2 (0.5–10 mM) revealed that 0.5 mM H2O2 did not affect the cell viability, but 1 and 5 mM H2O2 reduced it by 4.36 ± 1.34% and 26.78 ± 2.36% respectively, compared to untreated sample. A similar dose-dependent study revealed that a γ-ray dose up to 10 Gy did not result in reduction in viability (data not shown). Hence H2O2 (0.5, 1 mM) or γ-radiation (dose 10 Gy) was used to investigate the effect resveratrol on adapted cells. The adaptive oxidative stress response in these cells was examined from the levels of catalase as a marker for enhanced antioxidant defence. The first order rate constant which is a measure of the quantity of catalase present in the sample is given in Fig. 6. The
populations, exposed to H₂O₂ or γ-radiation exhibited an enhanced antioxidant defence status compared to the untreated cells (Fig. 6). Untreated and sham irradiated cells exhibited catalase concentrations 0.09 ± 0.001 and 0.05 ± 0.007 k/mg protein while 0.5 and 1 mM H₂O₂ treated cells exhibited 1.1 ± 0.126 k/mg and 1.00 ± 0.068 k/mg protein respectively, a tenfold increase compared to untreated cells. A similar significant increase in catalase levels (0.58 ± 0.782 k/mg protein) was observed upon exposure to 10 Gy γ-radiation. Resveratrol itself did not alter the catalase levels in E. coli. However, none of these adaptively-primed cells showed any significant change in their sensitivity to resveratrol (182 μg/mL). It was anticipated that any molecule that act via a ROS pathway lose its efficacy under oxygen depleted conditions. Under oxygen depleted conditions the inhibitory property of resveratrol towards E. coli remained unaltered compared to the experiment carried out at ambient oxygen level (IC₅₀ = 182 μg/mL in both cases).

Finally to investigate the role of ROS in resveratrol mediated bacterial inhibition we looked into the expression of sensor/regulatory proteins that are sensitive to oxidative stress. In E. coli oxyR regulon is reported to be induced upon exposure to the peroxide stress whereas soxRS is induced by superoxide radicals [15]. Treatment with resveratrol did not induce oxyR, soxS or soxR genes significantly, as observed from the green fluorescent protein (GFP) fluorescence of the respective GFP-tagged genes (Figs. 7 and 8). However a robust induction was seen in case of oxyR when treated with tert-butyl hydroperoxide (Fig. 7). Similarly paraquat treatment induced the soxS significantly (Fig. 8a), although the increase of soxR was not pronounced (Fig. 8b). Resveratrol treated samples exhibited membrane damage as evidenced by the leakage of potassium in the supernate and a corresponding decrease in the pellet (Fig. 9). In resveratrol treated cells the amount of potassium increased in supernate from 2.54 μg/mL at 0 h to 8.88 μg/mL at 4 h. The decrease in potassium quantity in the pellet was found to be 0.76 μg/mL at 4 h from 6.02 μg/mL at 0 h. In case of vehicle treated cells such a change was not observed. This was further confirmed by fluorescent microscopy (Supplementary Fig. 1a). Quantification of SYTO–PI ratio revealed decrease in the ratio in a concentration dependent manner in resveratrol treated samples but not in mitomycin C treated samples (Supplementary Fig. 1b). Further flow cytometry analysis revealed a marked uptake of PI in resveratrol treated cells (Fig. 10) which indicated membrane compromise. Cells treated with 70% isopropyl alcohol (membrane permeabilized cells) were used as positive control.

Discussion

Plant polyphenols are important components of human diet and a number of them are considered to possess chemopreventive
and therapeutic properties. A redox reaction of the polyphenols and Cu(II) in the ternary complex may occur leading to the reduction of Cu(II) to Cu(I), whose reoxidation generates a variety of ROS [2]. This has been attributed as a primary reason behind the cytotoxic potential of several polyphenols including resveratrol [16,17]. Extensive work has been carried out on the health benefits of resveratrol and the associated mechanism of its action [18]. Its antibacterial activity has also previously been reported [11,19]. Kohanski et al. have proposed a unified common mechanism of killing bacteria by antibiotics, involving induction of the most reactive ROS, hydroxyl radicals [20]. Several antibacterial molecules have been suggested to act via this ROS generation pathway [8,21]. In view of these, the role of oxidative stress in the antibacterial activity of resveratrol was examined for the first time in this study. Direct quantification of ROS by different fluorescent dyes has been a useful technique to demonstrate the involvement of ROS in a process. This in conjunction with external supplementation of antioxidants to scavenge the ROS provides valuable insight in to the events leading to macromolecular damage and cellular cytotoxicity. Fluorescence augmentation due to oxidation of DCFDA by ROS has been employed previously in E. coli cells treated with resveratrol (182 μg/mL) analysed for potassium in pellet and supernate by atomic absorption spectrometry. The values denoted are mean ± SEM. *Values are significantly different compared to 0 h values (p ≤ 0.05).

and therapeutic properties. A redox reaction of the polyphenols and Cu(II) in the ternary complex may occur leading to the reduction of Cu(II) to Cu(I), whose reoxidation generates a variety of ROS [2]. This has been attributed as a primary reason behind the cytotoxic potential of several polyphenols including resveratrol [16,17]. Extensive work has been carried out on the health benefits of resveratrol and the associated mechanism of its action [18]. Its antibacterial activity has also previously been reported [11,19]. Kohanski et al. have proposed a unified common mechanism of killing bacteria by antibiotics, involving induction of the most reactive ROS, hydroxyl radicals [20]. Several antibacterial molecules have been suggested to act via this ROS generation pathway [8,21]. In view of these, the role of oxidative stress in the antibacterial activity of resveratrol was examined for the first time in this study. Direct quantification of ROS by different fluorescent dyes has been a useful technique to demonstrate the involvement of ROS in a process. This in conjunction with external supplementation of antioxidants to scavenge the ROS provides valuable insight in to the events leading to macromolecular damage and cellular cytotoxicity. Fluorescence augmentation due to oxidation of DCFDA by ROS has been employed previously in E. coli cells treated with resveratrol (182 μg/mL) analysed for potassium in pellet and supernate by atomic absorption spectrometry. The values denoted are mean ± SEM. *Values are significantly different compared to 0 h values (p ≤ 0.05).

**Fig. 8.** Effect of resveratrol on expression of (a) soxS and (b) soxR in E. coli. E. coli with soxS-GFP or soxR-GFP construct was treated with resveratrol and the expression was followed fluorimetrically. Paraquat was used as a positive control. Filled squares indicate untreated cells, half filled squares indicate vehicle treated cells, stars indicate cells treated with 27 μg/mL paraquat, circles indicate cells treated with 64 μg/mL paraquat, triangles indicate cells treated with 114 μg/mL resveratrol and diamonds indicate cells treated with 182 g/mL resveratrol. The inset shows the magnification of the closely placed data points. The values are mean of triplicates of a single experiment. The experiment was performed twice.

**Fig. 9.** Membrane damage induced by resveratrol quantified by potassium release. E. coli cells treated with resveratrol (182 μg/mL) analysed for potassium in pellet and supernate by atomic absorption spectrometry. The values denoted are mean ± SEM. *Values are significantly different compared to 0 h values (p ≤ 0.05).
Earlier such response negated subsequent toxicity induced by a variety of aldehyde compounds in *E. coli* [25]. Similarly H₂O₂ pre-treatment also protected *E. coli* cells against methylnitronitrosoguanidine induced lethality which is known to progress by ROS generation [26]. But in the current investigation the adaptive oxidative stress response did not aid *E. coli* cells in combating a subsequent resveratrol challenge.

Many of the oxidative stress induced genes are under the control of OxyR in *E. coli*. Mutants that lack oxyR are hypersensitive to H₂O₂ and many oxidants. The spontaneous mutation rate is also elevated in these mutants under aerobic conditions [27]. Similarly soxSR regulon control the expression of at least 10 proteins that are induced upon exposure to superoxide generating agents. Under oxidative stress a conformational change occurs in soxR that acts as a transcriptional activator of soxS. The SoxS protein then activates the transcription of genes that are under the control of soxSR [28]. Although induction of oxyR by tert-butyl hydroperoxide and induction of soxSR by paraquat was observed as a proof of principle in our experiments, resveratrol did not induce the expression of oxyR or soxSR. This provided further evidence of lack of any oxidative stress in the *E. coli* cells due to resveratrol treatment. Previous studies in case of clofazimine, a riminophenazone antibiotic active against *Mycobacterium* it was found that ROS scavengers could not prevent the inhibitory activity of this antibiotic in spite of it being a potential candidate for intracellular redox cycling. The outer membrane appeared to be the primary site of action of this antibiotic [29]. We also found membrane damage after treatment with resveratrol in *E. coli* proved by potassium leakage, SYTO–PI dual staining and PI uptake by flow cytometry. However it is not clear at this point how it is taking place. Although diffusible/scavengable ROS could not be detected, the damage might still be occurring by oxidation where in the transfer of electrons between the target and the oxidizer could be occurring in a very short time frame and in a microenvironment not amenable to the detection techniques employed in this investigation. Membrane damage could also occur due to activation of certain phospholipases in the cell. It has also been observed that resveratrol inhibits purified as well as membrane bound F₁ ATP synthase [30,31]. Since ATP synthase is an important drug target [31], it would be interesting to investigate the link between this and membrane damage. ATP synthase is regulated in response to proton motive force to avoid wasteful ATP hydrolysis. Though it has been showed resveratrol binds to ATP synthase and inhibits it, the inhibition of the same might also be due to loss of proton motive force induced by the membrane damage by resveratrol. Our result also throws up an interesting avenue as it has been reported previously certain organic molecules aid in increasing the efficacy of existing antibiotics that may be ineffective due to the outer membrane architecture of gram negative bacteria [32,33]. Since resveratrol changes the membrane permeability of *E. coli* it would be interesting to see if this property aids in the efficacy of other drugs used against *E. coli* in particular or gram negative bacteria in general. Further detailed experimentation could reveal if co-treatment or pre-treatment with resveratrol would aid some of the standard drugs to be more potent thereby producing a favourable clinical outcome. The bactericidal antibiotics are hypothesized to generate ROS and kill the target bacteria. A few examples were investigated and evidence towards this hypothesis was gathered [20]. Our results also corroborate this, as we found resveratrol to be bacteriostatic and not bactericidal. Taken together,
our data strongly suggests that unlike certain other antibacterials [8] scavengable ROS generation and subsequent oxidative stress do not play an obligatory role in the resveratrol-mediated inhibition of *E. coli* cells. However we find the primary event in resveratrol mediated inhibition of *E. coli* is membrane damage. Further investigation is currently underway towards the mechanism behind membrane damage, direct damage or activation of phospholipases and damage of any secondary targets inside the cells.

**Conflict of interest**

None to declare.

**Funding**

The study is funded by internal department funds of Department of Atomic Energy, Government of India.

**Acknowledgements**

We are grateful to James Imlay (University of Illinois) for providing the strains related to this study. The author M.S. wishes to express his gratitude to Dr. S. Chattopadhyay for his keen interest, support and critical reading of the manuscript.

**Appendix A. Supplementary material**

Supplementary data associated with this article can be found in the online version of http://dx.doi.org/10.1016/j.redox.2014.06.007.

**References**

[1] FischbachMA., WalshCT., Antibiotics for emerging pathogens, Science (New York, NY) 325 (2009) 1089–1093, http://dx.doi.org/10.1126/science.1179315.

[2] SubramanianM., ShadaksharilI., ChattopadhyayS., A mechanistic study on the nucleic acid properties of some hydroxystilbene, Bioorganic & Medicinal Chemistry 12 (2004) 1231–1237, http://dx.doi.org/10.1016/j.bmc.2004.01.035.

[3] LiZ., YangX., DongS., LiX., DNA breakage induced by piceatannol and copper (II): mechanism and anticancer properties, Oncology Letters 3 (2012) 1087–1094 27883397.

[4] PauloL., FerreiraS., GallardoE., QueirozJ.A., DominguesF., Antimicrobial activity of resveratrol on dermatophytes and bacterial pathogens of the skin, Biochemical Pharmacology 63 (2002) 99–104, http://dx.doi.org/10.1016/j.bcp.2006.06.007 11847582.

[5] PauloL., GallardoE., QueirozJ.A., DominguesF., Antimicrobial activity of resveratrol on human pathogenic bacteria, World Journal of Microbiology and Biotechnology 26 (2010) 1533–1538, http://dx.doi.org/10.1007/s11274-010-0872-9.

[6] HadiS.M., UllahMF., AzmaIS., AhmadA., ShimunII., ZubairH., KhanHY., Resveratrol mobilizes endogenous copper in human peripheral lymphocytes leading to oxidative DNA breakage: a putative mechanism for chemoprevention of cancer, Pharmaceutical Research 27 (2010) 979–988, http://dx.doi.org/10.1007/s11120-010-0191-4.

[7] LeeSK., LeeHJ., ParkEJ., LeeKM., AhnYH., ChoYJ., PyeJH., Antibacterial and antifungal action of pinoresinol, a constituent of pine, Fitoerapia 76 (2005) 258–266, http://dx.doi.org/10.1007/s13076-005-0026-6.

[8] GoswamiM., JawaliN., N-acetylcysteine-mediated modulation of bacterial antioxidant susceptibility, Antimicrobial Agents and Chemistry 54 (2010) 3529–3530, http://dx.doi.org/10.1093/acd/baq267.

[9] FitzgeraldMP., MadenJ.M., ColemanMC., TreholM., WestphalS.G., SpitzDR., Rad60, Dominant-2, Transgenic biosynthesis of tryptophanophosphate in *Escherichia coli* from radiation-induced toxicity, Radiation Research 174 (2010) 290–296, http://dx.doi.org/10.1664/10.1664.20726720.

[10] EnangAA., ArianyagamMK., StewartML., BarrettMP., Activity of megazol, a tryptophanoid nitroimidazole, Am. J. Cancer, is associated with DNA damage, Antimicrobial Agents and Chemistry 47 (2003) 3368–3370, http://dx.doi.org/10.1016/j.aca.2003.06.0061.

[11] DempleM., HalfbrookJ., Inducible repair of oxidative DNA damage in *Escherichia coli*, Nature 304 (1983) 466–468, http://dx.doi.org/10.1016/y.nature.1983.06.005.

[12] NunoshibaT., HashimotoM., NishiokaA., Riesoactive adaptive response in *Escherichia coli* caused by pretreatment with *HgCl* against formaldehyde and other aldehyde compounds, Mutation Research 235 (1991) 265–271, http://dx.doi.org/10.1016/0027-5107(91)90543-P.

[13] HassettD.J., SchweizerH.P., OhmanD.E., Pseudomonas aeruginosa sodA and hmp (flavo-hemoglobin) gene expression in *Escherichia coli* ATCC 25922, http://dx.doi.org/10.1016/0029-5034(79)90072-9.

[14] SeaverL.C., ImlayJ.A., Alkyl hydroperoxide reductase is the primary scavenger of reactive oxygen species in *Helicobacter pylori* infection: mechanisms for their direct and indirect activities, International Journal of Antimicrobial Agents 31 (2008) 199–208, http://dx.doi.org/10.1016/j.ijantimicag.2004.06.007 18301047.