academicJournals

Vol. 12(21), pp. 3171-3180, 22 May, 2013
DOI: 10.5897/AJB12.2467
ISSN 1684-5315 ©2013 Academic Journals
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Evaluation of the genetic effects of the *in vitro* antimicrobial activities of *Rhazya stricta* leaf extract using molecular techniques and scanning electron microscope

Adel A. E. El-Tarras[1,2], Mohamed M. Hassan[1,3] and Mohamed A. M. El-Awady[1,2]

[1]Biotechnology and Genetic Engineering Research Unit, Scientific Research Center, Taif University, Kingdom of Saudi Arabia.
[2]Genetics Department, Faculty of Agriculture, Cairo University, Cairo, Egypt.
[3]Genetics Department, Faculty of Agriculture, Minufiya University, Sheben El-Kom, Egypt.

Accepted 8 March, 2013

Rhazya stricta **plants have always played a major role in the treatment of human and animal diseases and it has main role in the folk medicine. The aim of this study was to explore the potential antimicrobial activities of the aqueous leaves extract of *R. stricta* on Gram-negative and Gram-positive food-borne bacteria and evaluate the antimicrobial effect at the molecular level. The results indicate that the aqueous leaves extract of *R. stricta* exhibited the antimicrobial activity against tested microorganisms. A clear, but significantly smaller, inhibition zones were formed after the treatment of two Gram-negative bacteria (*Escherichia coli and Aeromonas hydrophila)* and one Gram-positive bacteria (*Staphylococcus aureus*) with the aqueous leaves extract of *R. stricta* (50 mg) comparing with those formed after the treatment with streptomycin (15 mg). Moreover, the results obtained after the treatments of bacterial strains with elevated concentrations of aqueous extracts of the wild plant of *R. stricta* leaves reveled that the extract has potent lethal activities as the growth turbidity decreased as the concentration or time of exposure increased. In addition, the observation by the scanning electron microscope showed that cells of the bacterial strains were damaged after the treatment with plant extracts. The noticed antimicrobial effect was explored at the molecular level, using restriction fragment length polymorphism (RFLP) analysis of the plasmid DNA and random amplification of polymorphic DNA (RAPD) analysis of the genomic DNA extracted from the control (untreated) and *R. stricta* leaf extract-treated bacterial strains. The results demonstrate polymorphic band pattern for most treated microbes compared with the wild type (untreated) strain. Concerning gene expression under the same conditions, total protein contents of the three treated bacteria showed significantly gradual increase in all of the treatment doses compared to control. In addition, the SDS-PAGE of the bacterial cellular proteins resulted in the induction of some protein bands under the treatment conditions. All these results strongly point out the mutagenicity, lethal and antimicrobial effect of the leaves extract of *R. stricta*. The results indicate the possibility of using the leaves extract of *R. stricta* as a source of antibacterial compounds for treatment of infections caused by multi-drug resistant (MDR) bacterial pathogens.**

Key words: Medicinal plants, *Rhazya stricta,* antimicrobial, mutagenicity, RAPD, RFLP, SEM, *E. coli*, *S. aureus* ,*A. hydrophila.*

INTRODUCTION

Medicinal plants are the most exclusive source of life saving drugs for the majority of the world's population. Bioactive compounds currently extracted from plants are used as food additives, dyes, insecticides, cosmetics, perfumes and fine chemicals. These compounds belong to a group collectively known as secondary metabolites. About 80% of world population is reliant on medicinal plants to maintain their health and to cure their ailment. The main advantage of natural agents is that they do not enhance the "antibiotic resistance", a phenomenon commonly encountered with the long-term use of synthetic antibiotics (Vukovic et al., 2007). Medicinal plants are the most exclusive source of life saving drugs for the majority of the world's population. Bioactive compounds currently extracted from plants are used as food additives, dyes, insecticides, cosmetics, perfumes and fine chemicals. These compounds belong to a group collectively known as secondary metabolites. About 80% of world population is reliant on medicinal plants to maintain their health and to cure their ailment. The main advantage of natural agents is that they do not enhance the "antibiotic resistance", a phenomenon commonly encountered with the long-term use of synthetic antibiotics (Vukovic et al., 2007). With growing interest world-wide in medicinal plant as a source of medicine, there is need to introduce new important plants of established therapeutic values used either in modern or traditional system of medicine which are sold widely in world market, for herbal, homeopathic, allopathic formulations (Ahmad et al., 2004).

The flora of Saudi Arabia is one of the richest biodiversity areas in the Arabian Peninsula and comprises very important genetic resources of crop and medicinal plants. In addition to its large number of endemic species, the components of the flora are the admixture of the elements of Asia, Africa and Mediterranean region (Rahman et al., 2004). Thus Saudi flora offers great possibilities for the discovery of new compounds with antimicrobial activities.

Rhazya stricta Decne belongs to Apocynaceae family and is widely distributed throughout the world. It is abundantly found in Western Asia from Yemen to Saudi Arabia, to the North West Province of India and various regions of Pakistan. Its leaf extracts were prescribed in folkloric medicine for the treatment of various disorders such as diabetes, sore throat, helminthiasis, inflammatory conditions and rheumatism (Ali et al., 2000; Ahmed et al., 2004). The plant extract contains mainly alkaloids, glycoside, flavonoides, tannins and triterpenes (Al-yahya et al., 1990; Badreldin et al., 2000; Szabó., 2008). Phytochemical analysis has identified more than 100 alkaloids that have several pharmacological properties (Atalay, 2001; Gilani et al., 2006). It is consider as important resources of natural biological compounds useful in human medicine, plant protection (Tanira et al., 2000; Baeshin et al., 2008). It is also a source for phenolic compounds (Sharaf et al., 1997; Lambert et al., 2005), triterpenoids (Xü et al., 1998) and steroidal glucosides compound (Baeshin et al., 2008). In addition, in South Asia and Middle East Countries, *R. strictais* is used traditionally as an indigenous medicinal herbal drug in treatment of different types of diseases such as skin diseases, stomach diseases and antihypertensive (Mukhopadhyay et al., 1981). The leaves, flowers and fruit are also used in joint infections and for cancer (Rahman and Qureshi, 1990; Khan and Khan, 2007). It is also used for throat sour, in fever, general debility and as curative for chronic rheumatism and tumor.

Several studies on rats and mice reported the biological effect of the leaf extract from *R. stricta*. The leaf extract causes sedation, analgesia and decrease in motor activity; furthermore, it has anti-depressant and anti-oxidant activities, as well as complex effects on brain endogenous monoamine oxidase activity and centrally-mediated hypotension (Tanira et al., 2000; Ali et al., 2000). Moreover, the genotoxic effect of the *R. stricta* leaf aqueous extract as antifungal agent was demonstrated, for the first time against *Saccharomyces cerevicae* auxotrophic mutant by the genuine study of Baeshin et al. (2005). The biochemical and molecular evaluation of genetic effects of *R. stricta* (Decne) leaves extract on *Aspergillus terreu* were reported by Baeshin et al. (2008). However, the antimicrobial effect of the leaf extract is poorly studied.

Thus, the aim of this study was to explore the potential antimicrobial activities of the aqueous leaves extract of *R. stricta* on three food-borne bacterial species (*Escherichia coli*, *Staphylococcus aureus, Aeromonas hydrophila*) and evaluate its genetic effects at the molecular level using molecular genetics techniques and SEM.

MATERIALS AND METHODS

Bacterial strains

Three food-borne bacterial species were used in this study (*E. coli*, *S. aureus and A. hydrophila)* to evaluate the antibacterial activity. The bacteria were obtained as kindly gift of Dr. Ahmed Fadel, Professor of microbiology, Biotechnology and Genetic Engineering Unit, Scientific Research Center, Taif University, KSA.

Plant materials

The green *R. stricta* plants were collected from Al-Yamaniya village, Taif governorate, KSA. Fresh leaves were washed well with running water to get rid of dust and sands, hand-minced into small pieces,

*Corresponding author. E-mail: Mohamed_elawady@yahoo.com. Tel:00966-503459387. Fax: 00966-02-7274299.

grinded in liquid nitrogen and kept in freezer until used. The freeze extract then was dissolved in phosphate buffered saline (PBS, pH 7.0 to 7.2) to the final concentration of 500 mg/ml. Then the mixture was left for 24 h at room temperature with mild hand-shaking at regular intervals and sterilized by filtration through 0.22 µm sterilizing Millipore express filter (Millex-GP, Bedford, OH).

Determination of antibacterial activity

Diameter of inhibition zone (DIZ) measurement

The agar-well diffusion method was employed for determination of antibacterial activities **(**NCCLS, 1999). All bacteria were suspended in sterile water and diluted to ~10^6 CFU/ml. The suspension (100 µl) was spread onto the surface of the Plate count agar (PCA) medium. Wells (7 mm in diameter) were cut from the agar with a sterile borer and 50 and 100 µl extract solutions were delivered into them. Thus two concentrations (25 and 50 mg/well) of the leave extracts were applied. Negative controls were prepared using PBS solution. The artificial Streptomycin with a concentration of (15 mg/well) was used as positive reference standards to determine the sensitivity of each microbial species tested and to compare the relative percent of antibacterial activity. The inoculated plates were incubated at 35°C for 24 h. Antibacterial activity was evaluated by measuring the diameter of inhibition zone (DIZ) of the tested bacteria. DIZ was expressed in millimeters. All tests were performed in triplicate.

Monitoring bacterial growth

To study the antibacterial effect of *R. stricta,* different concentrations of its leaves extract were diluted with nutrient broth media at final concentrations of 0.1, 0.3, 0.7, 1.0, 1.5 and 2.0% extract. Single colonies of the tested bacteria were used for inoculation of Luria-Bertani (LB) media containing the six leaves extract concentrations and were incubated at 30°C for 48 h. The antibacterial activities of the *R. stricta* extract was evaluated by measuring the optical density of bacterial growth turbidity 6, 12, 24 and 48 h after the treatment at 590 nm according to Baeshin et al. (2005).

Scanning electron microscopy (SEM)

The bacteria that were susceptible to the plant extracts were prepared for scanning electron microscope (SEM). Small agar pieces were cut out from the inhibition zone and they were fixed in 3% (v/v) glutaraldehyde buffered with 0.1 M sodium phosphate buffer (pH 7.2) for 1 h at room temperature and then washed four times in sodium phosphate buffer. The pieces were then post-fixed in 1% (w/v) osmium tetroxide (OsO4) for 1 h and then washed four times in the buffer. They were dehydrated in a graded alcohol series. The last stages of dehydration were performed with propylene oxide ($CH_3CH.CH_2.O$). The samples finally were examined in a JEOL JSM 6390 LA scanning electron microscope.

Restriction fragment length polymorphism (RFLP) of plasmids DNA

Plasmids were extracted from the bacterial strains after the treatment with the different concentrations of leaves using the Gene JET ™ plasmid mini-prep kit (Jena Bioscince) according to the manufacturers provided with the kit. The isolated plasmids were then digested with the restriction enzymes *Eco RI, Bam HI* and *Hind III* using the standard protocol and digested plasmid DNA was separating in 1% agarose gel, stained with ethidium bromide and photographed using the gel Bio-Rad Gel documentation system (Germany).

Random amplification of polymorphic DNA (RAPD) analysis of the genomic DNA

The bacterial genomic DNA was extracted from bacterial strain using the procedure explained by the Jena Bioscience bacterial DNA preparation kit (Germany). Five random primers (OPG-10, OPG-07, OPG-05, OPG-02 and OPC-05) were used for RAPD fingerprinting of the three treated and untreated bacterial isolates. The PCR amplification for RAPD reactions was performed in a 20 µl reaction mixture containing 2 µl 10 X amplification buffer, 200 µM dNTPs mix, 10 pmole of primers, 40 ng templates DNA, 1 unit Taq polymerase (Go taq polymerase, Promega, USA) and the volume was completed to 20 µl using sterilized distilled water. The reaction was carried out using preheated thermal cycles of Eppendorf (Germany) according to the following program: 35 cycles of denaturation at 94°C for 1.5 min, annealing at 35°C for 1.5 min and extension at 72°C for 2 min and finally incubated at 72°C for 5 min. PCR products were separated in 1% agarose, stained with ethidium bromide and photographed using Bio-Rad Gel documentation system (Germany).

Protein concentration

The protein content in the culture filtrates was estimated by the dye-binding method of (Bradford, 1976). The amount of protein was calculated using bovine serum albumin as standard.

Protein profile

Total extracted protein was separated by denatured polyacrylamide gel electrophoresis (SDS-PAGE) with 8% stacking gel and 12% separation gel according to Baeshin et al. (2008). Protein samples were diluted in loading buffer (1% SDS, 1% mercaptoethanol, 20% glycerol, 0.02% bromophenol blue, 0.01 M Tris-HCl and pH 8.0) at 100°C for 5 min before electrophoresis. Electrophoresis was performed at room temperature for approximately 2 h and the system was programmed to a two-step mode with applying constant current 10 mA in stacking gel and 20 mA in the separation gel. Gels were stained with Commase brilliant blue R 250 stain.

Statistical analysis

All experiments were repeated three times. The growth turbidity of the three tested strains treated with different concentrations of *R. stricta* leaves extract was calculated by one way analysis of variance (ANOVA) for the relationship between the growth turbidity and the concentrations of crude extract Both Microsoft Excel 2007 and SPSS (version 16) were used in such analysis.

RESULTS

Antimicrobial activity of *R. stricta* leaves extract

Disc diffusion assay

Antimicrobial activity of *R. stricta* leaves extract was firstly tested using the inhibition zone width method. A clear inhibition zone of the three studied bacteria was caused

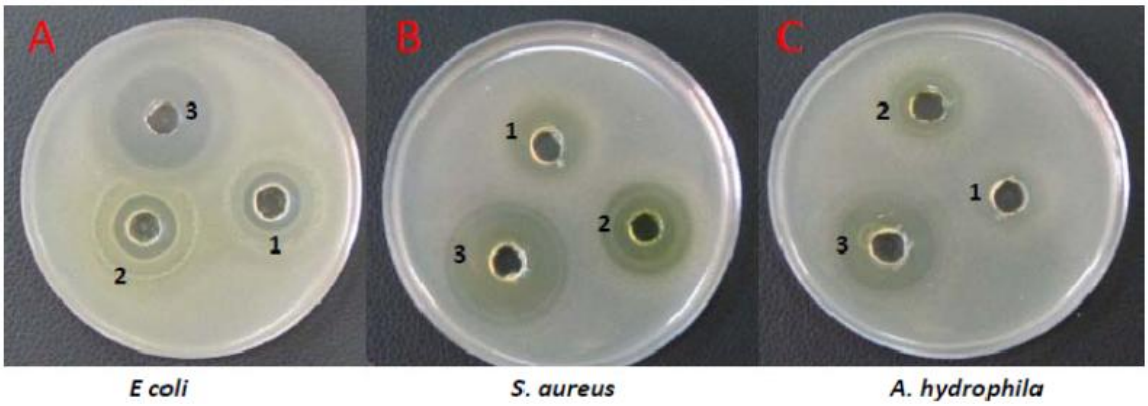

Figure 1. Pictures of inhibition zone test of three bacterial species which caused by leaves aqueous extract of *Rhazya stricta.* 1, 25 mg leave extract; 2, 50 mg leave extract; 3, 15 mg of Streptomycin.

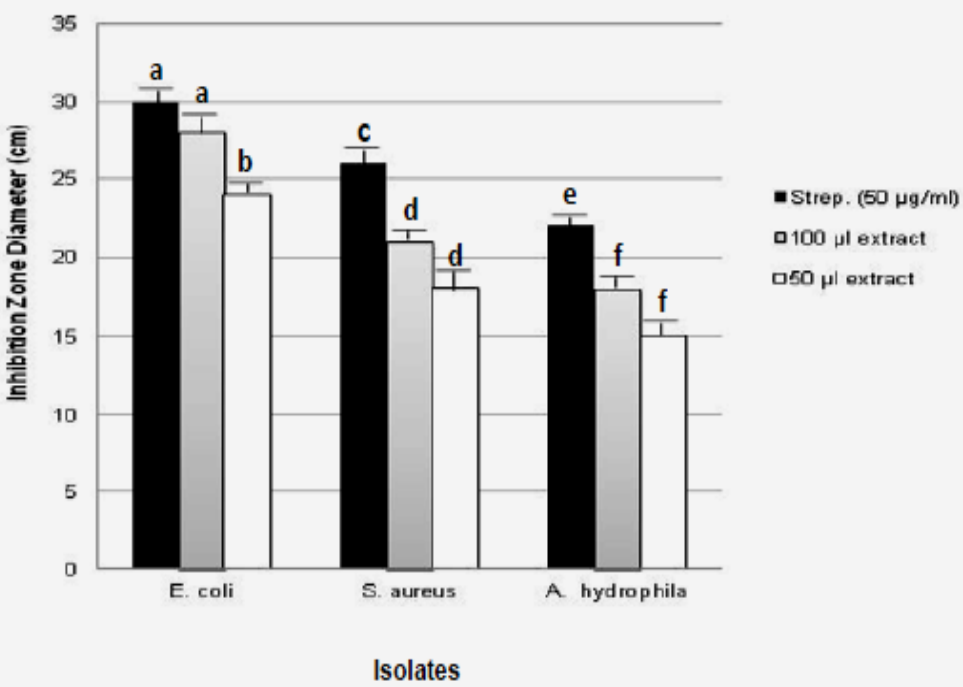

Figure 2. Inhibition zone diameter in three bacterial strains which caused by leaves aqueous extract of *Rhazya stricta.* Values are mean of three replicates and error bars represent standard error. Different letters mean significant difference.

by the treatment with (25 and 50 mg/well) of *R. stricta* leave extract as well as by the treatment with (15 mg/well) streptomycin (Figure 1). The diameter of all inhibition zones (DIZ) were measured and compared (Figure 2). While, increase of the treatment from 25 to 50 mg of the leaves extract resulted in significant increase in the DIZ formed with *E. coli*. However, there was no significant difference in the DIZ caused by same treatments with both of *S. aureus and A. hydrophila*. Remarkably, the DIZ caused by the treatment of *E. coli* with 15 mg/well of the antibiotic streptomycin was not changed significantly comparing with that caused by the treatment with 25 mg/well of the leaves extract (Figure 2).

Monitoring the bacterial growth

Antimicrobial activity of *R. stricta* leave extracts against the three tested bacteria was further explored by incubiting them in medium containing different concentrations of

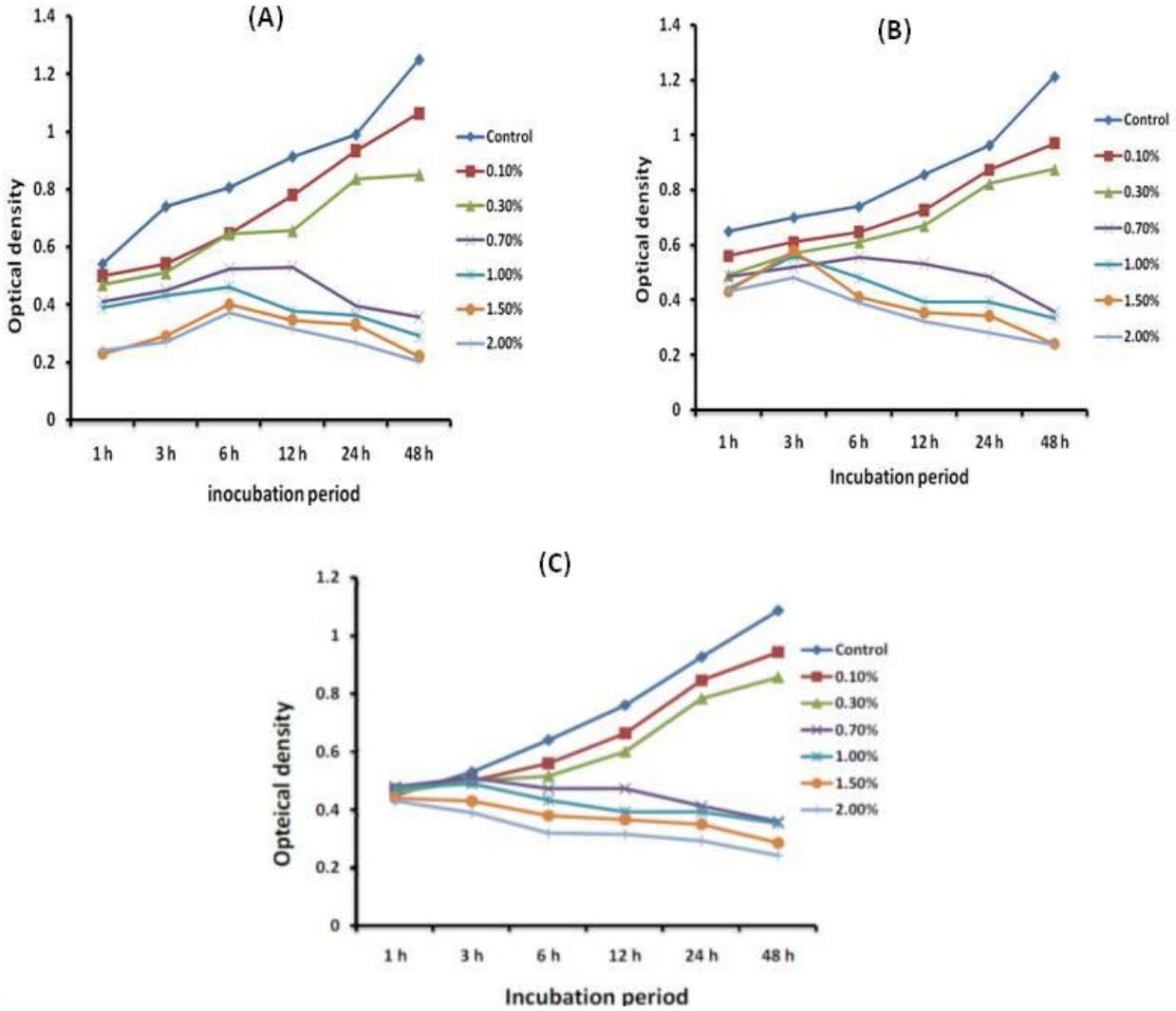

Figure 3. Growth turbidity of **(A)** *Escherichia coli*, **(B)** *Staphylococcus aureus* and **(C)** *Aeromonas hydrophila* strains treated with different concentrations of Rhazya stricta leaves extract.

the leaves extract and monitoring the bacterial growth. It generally noticed that the increase in extract concentration and exposure time lead to a decrease in survival and growth turbidity of the three tested strains (Figure 3). Under the control and low concentration treatments (0, 0.1 and 0.3%) of leave extract, the bacterial growth turbidity increased continuously during the whole period of treatment (48 h). However, under the higher concentrations treatment (0.7 to 2%) of the leave extract, the growth turbidity increased until 6 to 12 h after the treatment and then gradually decreased to the least at 48 h after the treatment (Figure 3).

Scanning electron microscope observations

To observe morphological alteration after the cell was treated with plant extracts, we used scanning electron microscope. When the bacterial cells treated with plant extracts were compared with untreated cells, a degradation of the cell walls and leaking of the cell contents were shown (Figure 4).

The genetic effects of the aqueous leaves extract of *R. stricta*

In order to explore the genetic effects in the treated bacteria as a results of the antimicrobial activities of the aqueous leaves extract of *R. stricta* at the molecular level, the changes in the bacterial genetic material due to the treatment was evaluated using RAPD analysis for genomic DNA and RFLP analysis for plasmid DNA.

Results of RFLP analysis of the plasmid DNA extracted from *A. hydrophila* and *E. coli* both untreated and those treated with different concentration of leave extract after digestion with *Hind III* are shown in Figure 5. As a consequence of molecular changes in the genetic mate-

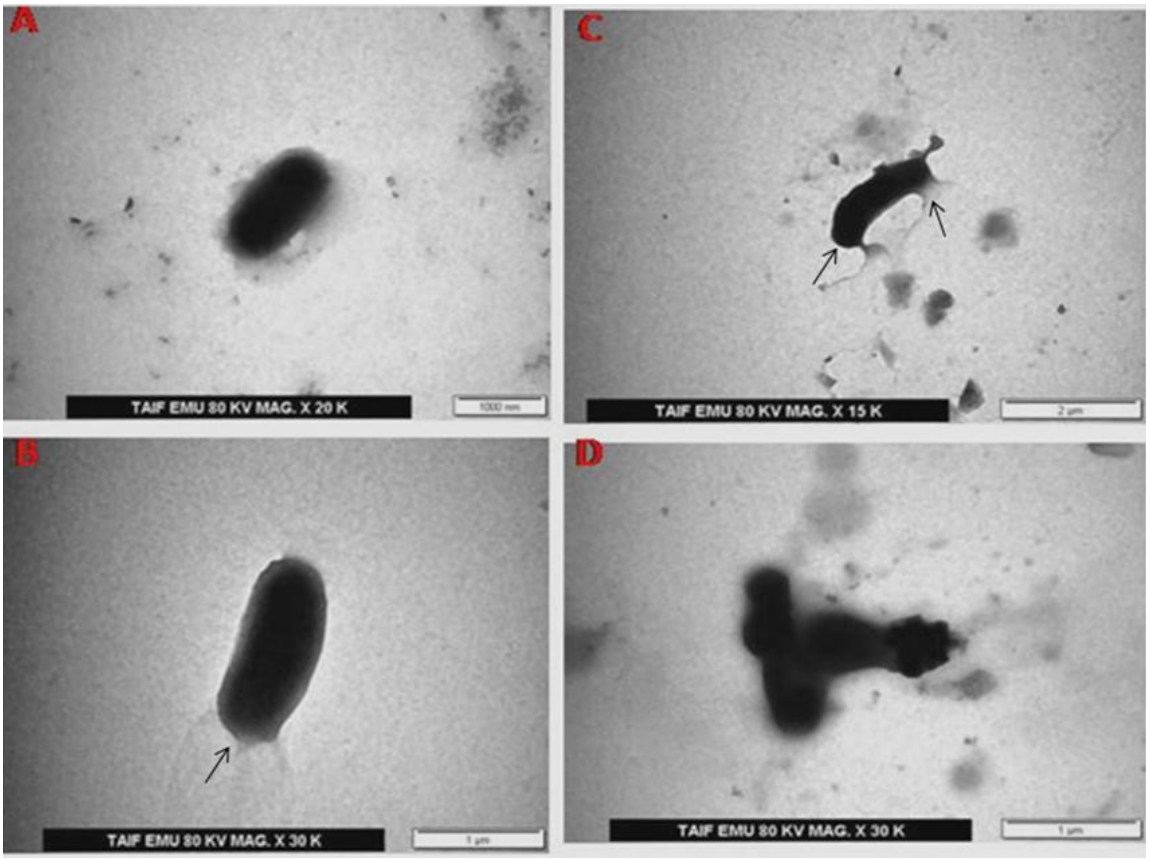

Figure 4. Scanning electron microscope image of untreated and damaged *S. aureus* cells after treatment with the aqueous leaves extract of *R. stricta*. **A)** The control untreated cell. **B)** Moderate damaged cell (arrows indicate the beginning of leaking). **C and D)** Cells with damaged cell membrane and leaking of the cell contents.

Table 1. Polymorphic bands of each genetic primers and percentage of polymorphism in *E. coli* Treated with different concentration of LE.

Primer	Total band	Number of monomorphic band	Number polymorphic band	Percentage monomorphic band	Percentage Polymorphic band
OPG- 10	11	7	4	64.0	36.0
OPG-05	17	12	5	70.5	29.5
OPG-02	13	8	5	61.5	38.5
OPH-04	11	8	3	73.0	27.0
OPC-05	13	8	5	61.5	38.5
Total	65	43	22		

rials of the treated bacteria, the electrophoretic products of digested plasmids showed a polymorphic banding pattern in both cases (Figures 5A and B).

The stability of the bacterial genomic DNA after the treatment with the *R. stricta* leaves extract was evaluated using RAPD analysis. The RAPD results illustrated in Tables 1, 2, 3 and Figure 6 show polymorphic numbers of the genetic bands, which were the electrophoretic products of PCR for treated bacteria compared with those of untreated bacteria. Table 1 illustrates that the highest number of polymorphic bands among treated *E. coli* was generated in reactions with the primers OPG-02 and OPC-05 which was five genetic bands and represented 38.5% of the total bands. While, among treated *S. aureus* and *A. hydrophila*, the reaction with the primer OPG-10 and OPG-07 resulted in the highest number of polymorphic bands (seven and six) that represent 47 and 43% of the total obtained bands (15 and 14), respectively (Tables 2 and 3).

A significant increase in the total cellular protein

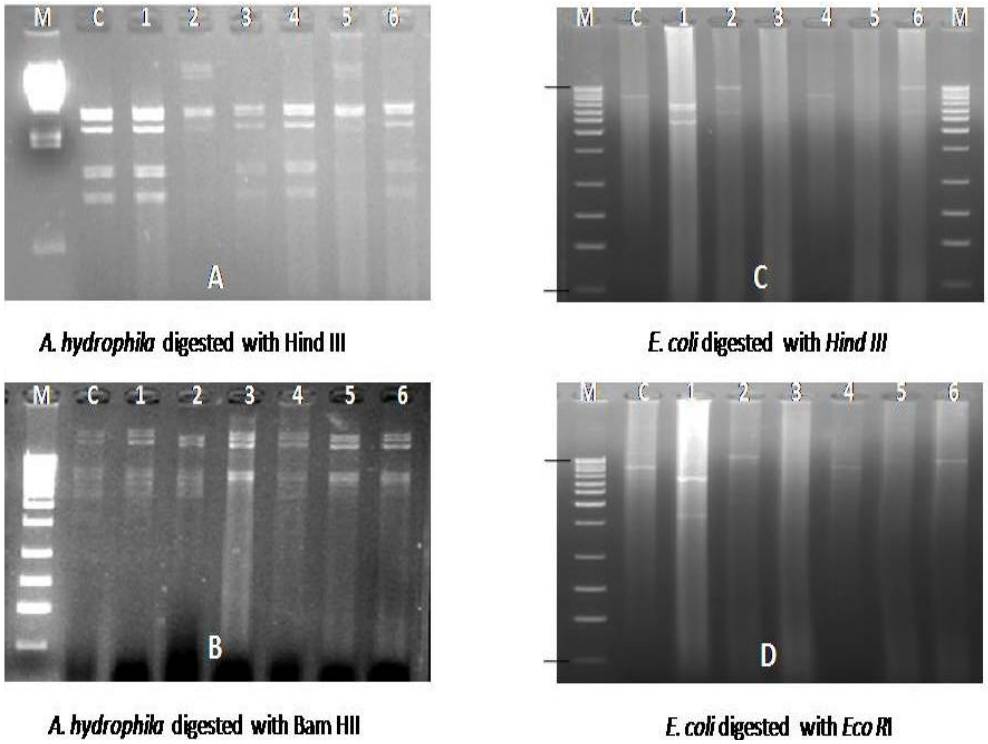

Figure 5. RFLP profile of Plasmid DNA extracted from *A. hydrophila* and *E. coli* after treatment with different concentrations of the leaves aqueous extract (LE) of *Rhazya stricta*, whereas; C, untreated; 1, treated with 0.10% LE; 2, treated with 0.30% LE; 3, treated with 0.70% LE; 4, treated 1.0% LE; 5, treated with 1.5% LE; 6, treated with 2.0 % LE.

Table 2. Polymorphic bands of each genetic primers and percentage of polymorphism in *Staphylococcus* treated with different concentration of LE.

Primer	Total bands	Number of monomorphic band	Number polymorphic band	Percentage Monomorphic band	Percentage Polymorphic band
OPG- 10	15	8	7	53	47
OPG-05	11	5	6	45	55
OPG-02	8	3	5	37.5	62.5
OPH-04	14	10	4	71	29
OPC-05	7	5	2	72	28
Total	55	31	24		

Table 3. Polymorphic bands of each genetic primers and percentage of polymorphism in *Aeromonus* treated with different concentration of LE.

Primer	Total band	Number of monomorphic band	Number polymorphic band	Percentage monomorphic band	Percentage Polymorphic band
OPG-10	15	10	5	67	33
OPG-07	14	8	6	57	43
OPG-05	10	9	1	90	10
OPG-02	11	7	4	64	36
OPC-05	11	9	2	82	18
Total	61	43	18		

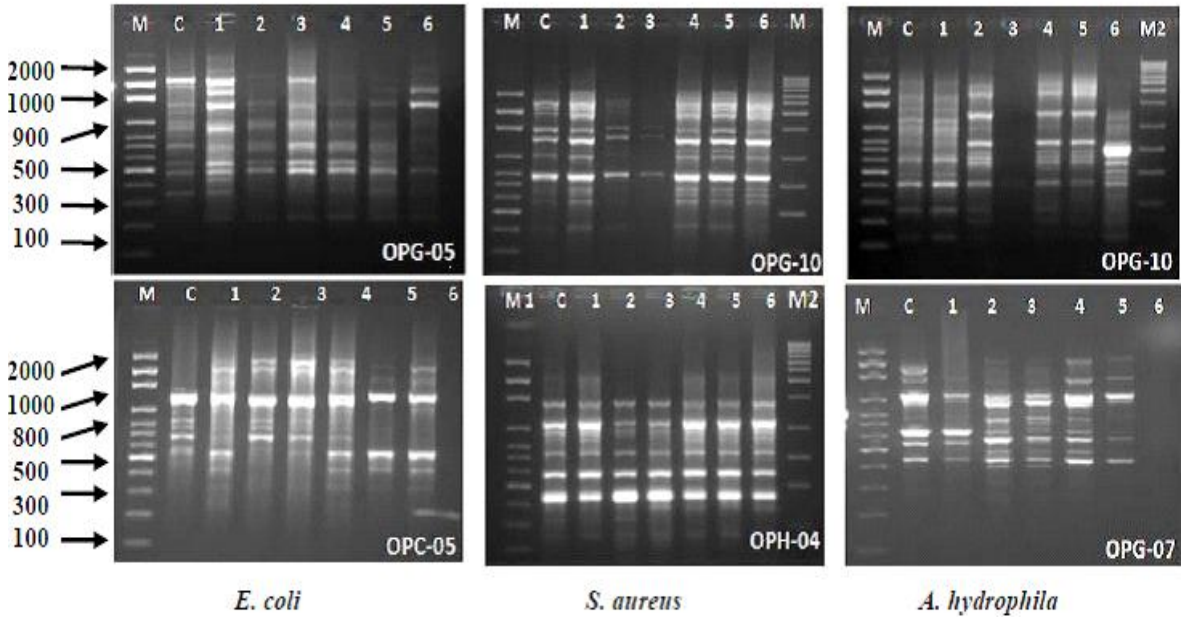

Figure 6. RAPD profile of three bacterial strains after the treatment with different concentrations of leaves aqueous extract (LE) of *Rhazya stricta;* whereas; C, untreated; 1, treated with 0.10% LE; 2, treated with 0.30% LE; 3, treated with 0.70% LE; 4, treated 1.0% LE; 5, treated with 1.5% LE and 6, treated with 2.0 % LE.

Table 4. Total protein contents of the bacterial strains treated with different concentrations of *Rhazya stricta* leaves extract (LE).

Treatment	Protein content (mg ml^{-1})		
	E. coli	***S. aureus***	***A. hydrophila***
0.0% LE	0.252 ±0.032	0.166±0.020	0.136±0.026
0.1% LE	0.260±0.025	0.214±0.028	0.197±0.025
0.3% LE	0.317±0.017	0.254±0.019	0.243±0.021
0.7% LE	0.374±0.019	0.298±0.025	0.289±0.019
1.0% LE	0.434±0.023	0.354±0.017	0.316±0.028
1.5% LE	0.480±0.017	0.366±0.023	0.350±0.032
2.0% LE	0.502±0.020	0.412±0.032	0.394±0.019

Values are mean of three replicates ± the standard error. Control = culture without *Rhazya stricta* leaves extract.

concentration due to the increase of leaves extract concentration with all tested bacteria was shown in Table 4. The protein concentration increased in treated bacteria with 2.0% leaves aqueous extract (LE) compared with those of untreated from 0.252 to 0.502 in *E. coli*, from 0.166 to 0.412 in *S. aureus* and from 0.136 to 0.394 in *A. hydrophila,* respectively. Moreover, SDS-PAGE of total cellular protein extracted from treated and untreated bacteria is shown in Figure 7. Induction of some new protein bands due to the treatment with some concentrations of *R. stricta* leaves extract was noticed in treated *E. coli and S. aureus* as indicated by the arrows (Figures 7A and B). These results together, strongly point out the mutagenicity and lethality effects of the leaves extract of *R. stricta* on the treated bacteria.

DISCUSSION

R. stricta is commonly used in folk medicine for the treatment of many diseases. In the present study, the antimicrobial activities of the leaves aqueous extract of *R. stricta* against the microorganisms were examined and its potency was assessed by the presence or absence of inhibition zones and zone diameter. In addition, it was found in that dose and exposure time to *R. stricta* leaves extract is inversely proportionate to growth turbidity, which means that an increase in the dose and exposure time is met by a decrease in survival of treated microbe strains, whereas tropical effect increased as dose and exposure time increased. All these results are in general agreement with the rule mentioned by Fincham et al.

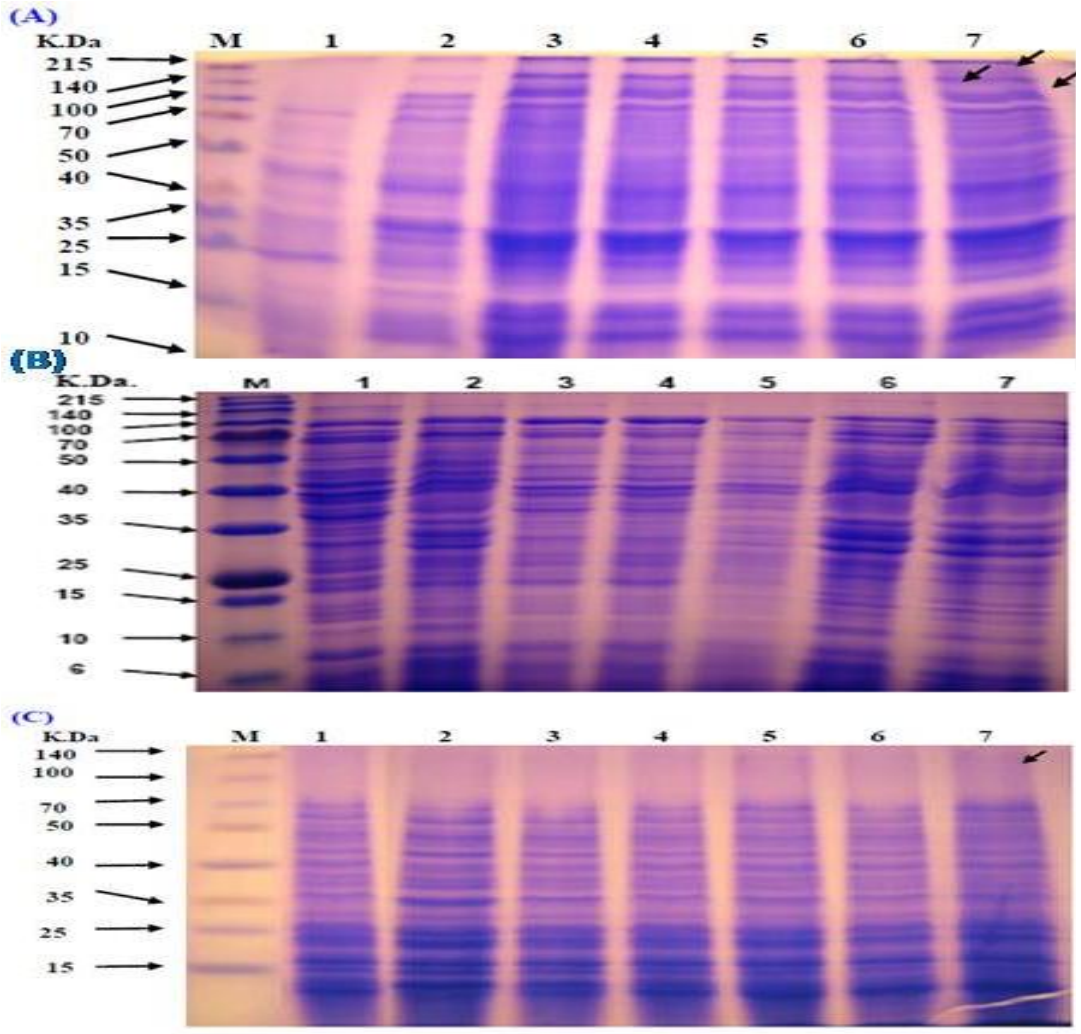

Figure 7. Protein banding pattern as reveled by SDS-PAGE of the three bacterial strains after the treatment with different concentrations of the leaves aqueous extract (LE) of *Rhazya stricta*; whereas; **A)** *E. coli*; **B)** *S. aureus* and **C)** *A. hydrophila*. 1, Untreated; 2, treated strains with 0.10% LE; 3, treated with 0.30% LE; 4, treated with 0.70% LE; 5, treated with 1.0% LE; 6, treated with 1.5% LE and 7, treated with 2.0 % LE.

(1979), who stated that by using chemical mutagens, there was a constant relation between the dose and mutation percentage which increases to a certain limit with the increase in dose.

The genetic effect of the *R. stricta* leaves extract as a mutagenic agent was confirmed by RAPD and RFLP. The results of both analyses revealed a polymorphic banding pattern when comparing between the untreated bacteria and those treated with different concentrations of the aqueous leaves extract (Figures 5 and 6). This observation gives good evidence to the ability of *R. stricta* extract to induce point mutation as a result of deletion compromising at least one nucleotide as revealed by the disappearance of many genetic bands and change in restriction endonucleases sites as compared with untreated bacteria. These results suggesting molecular changes as a deletion in one or more loci which affect gene expression and interruption in biochemical pathways of DNA and protein synthesis consequently as Alkaloids in *R. stricta* leaves extract often do. These results are consistent with the results obtained by Adam et al. (2000); Morita et al. (2005) and Gilani et al. (2006). Some of the components of *R. stricta* may act as intercalation agent or generates free radicals which are interacted with plasmid DNA to account for the observed deletions, as suggested by similar results obtained by Ansah et al. (2005) and Baeshin et al. (2008) in their study with *Cryptolepis sangvinolehta* and *Aspergillus terreus.*

Based on the obtained results of SDS-PAGE protein profile and concentration, the decrease of total protein concentration and induction of the expression of new

proteins as revealed by appearance on new bands due to the treatment could be explained if we consider the ability of *R. stricta* leave extract to apply a stress on the treated bacteria. Under this stress, treated bacteria could probably respond with increase of the expression level of some proteins and induction of others. The occurrence of frame shift mutations due to the stress in studied strains could be considered too. However, these speculations need more confirmation. In fact, our data provides additional evidence for the mutagenicity of this extract that demonstrated before for the first time by Baeshin et al. (2005) in *S. cerevicae*.

Moreover, the reported damage showed by the SEM images indicated the lethal effect of the high concentration of the leave extract on treated bacteria. Similarly, Burt and Reinders (2003) found that oregano and thyme essential oil exhibit strong antimicrobial properties against *E. coli* O157:H7 and observed cells were damaged when treated with essential oil using SEM and the damage in the cells was similar. Burt (2004) tried to explain the mechanism of action for essential oil components in bacterial cells. It was thought to be the degradation of the cell wall damage to cytoplasmic membrane proteins, the binding of proteins, leakage of cell contents, and coagulation of cytoplasm and depletion of the proton motive force.

In a conclusion, the leaves extract of the medicinal plant, *R. stricta,* proved to have antimicrobial activities against three bacterial species. This finding indicates the possibility of using the *R. stricta* leave extract as a source of antibacterial compounds for treatment of infections caused by multi-drug resistant (MDR) bacterial pathogens. The antimicrobial activities of the leaves extract of *R. stricta* could be explained as, under low concentration treatment, it has some compounds that work as a mutagenic agent that cause likely deletion mutations, and probably frame shift mutations, in the treated bacteria. Increase of the concentration and duration of the treatment are lethal for the treated bacteria as the consequences of cytoplasmic membrane damages.

ACKNOWLEDGEMENTS

We would like to acknowledge the support provided by King Abdulaziz City for Science and Technology (KACST) for funding this work through project # 08-Bio-201-11, as part of National Science, Technology and Innovation Plan.

REFERENCES

Adam SE, AL–Farhan AH, AL–Yahya A (2000). Effect of combined Citrullus colocynthis and *Rhazga stricta* use in Najdi sheep. Am. J. Chin. Med. 28:385-390.

Ahmad H, Ghulam RB, Latif A (2004). Medicinal flora of the Thar Desert- an overview of problems and their feasible solutions. Zonas Áridas. 8:1-11.

Ali BH, Al-Qarawi H, Mousa HM, Bashir AK (2000). Concentration of amino acids in brains of mice treated with the traditional medicinal plant *Rhazya stricta.* Dence. Indian J. Pharmacol., 32:253-254.

Al-yahya MA, Al-Meshal IA, Mossa JS, Al- Badr AA, Tariq M (1990). Saudi Plants: A Phytochemical and Biochemical Approach. King Saud University Press, Riyadh, Saudia Arabia.

Ansah C, Khan A, Gooderham NJ (2005). *In vitro* genotoxicity of the West African anti-malarial herbal Cryptolepis sanguinolenta and its major alkaloid cryptolepine. Toxicol. 1:208 (1):141-7.

Atalay S (2001). Antiviral and cytotoxic activities of extracts from the cell cultures and respective parts of some Turkish medicinal plants. Turk. J. Biol., 25:343-350.

Badreldin H, Ali A, Al-Qarawi K, Ahmed O, Bashir MT (2000). Photochemistry, pharmacology and toxicity of *Rhazya stricta* Decne: A review. Photother. Res. 14(4):229-234.

Baeshin NA, Qari SH, Sabir JSM, ALhejin AM (2008). Biochemical and Molecular Evaluation of Genetic Effects of *Rhazya stricta* (Decne) Leafs Extract on *Aspergillus terreus*. Saudi J. of Biol. Sci. 15:25-33.

Baeshin NA, Twaty N, Al-Hebshi A (2005). Evaluation the genotoxicity of Rhazya stricta leves extract by the *Saccharomyces cerveisiae* auxotrophic mutats test. Egyp. J. Nat. Toxi. 2:87-100.

Bradford MM (1976). A rapid and sensitive method for quantitation of microgram quantities of protein was utilizing the principle of protein–dye binding, Ann. Biochem. 72:248-254.

Burt SA (2004). Essential oils: their antibacterial properties and potential applications in foods- a review. Int. J. Food Microbiol., 94:223-253.

Burt SA, Reinders RD (2003). Antimicrobial activity of selected plant essential oils against *Escherichia coli* O157:H7. Lett. Appl. Microbiol. 36:162- 167.

Fincham JRS, Day PR, Radford E (1979). Fungal Genetics". University of California Press. Berkeley and Loss Angeles.

Gilani SA, Kikuchi A, Shinwari ZK, Khattak ZI, Watanab KN (2006). Phytochemical, pharmacological and ethnobotanical studies of *Rhazya stricta* Decne. Phytother. Res. 21:301-307.

Khan S, Khan GM (2007). *In vitro* antifngal activity of *Rhazya stricta*. Pak. J. Pharm. Sci., 20 (4):279-284.

Lambert J, Sang S, Dougherty A, Colby G, Mayers O, Timmermann B (2005). Cytotoxic lignans from L area Iridentata. Phytochem. 66:811-815.

Morita H, Awang K, Hadi AH, Takeya K, Itokawa H, Kobayashi J (2005). Conformational analysis of rhazinilam and three-dimensional quantitative structure-activity relationships of rhazinilam analogues. Bioorg. Med. Chem. Lett. 15 (4):1045-50.

Mukhopadhyay S, Handy GA, Funayama S, Cordell GA (1981). Anticancer indole alkaloids of *Rhazya stricta*. J. Nat. Prod. 44:696-697.

Rahman AU, Qureshi MM (1990). Applications of modern 2D-NMR techniques in structure elucidation of natural products. Pure Appl. Chem. 62:1385-1388.

Rahman, MA, Mossa JS, Al-Said MS, Al-Yahya MA (2004). Medicinal plant diversity in the flora of Saudi Arabia 1: A report on seven plant families. Fitoterapia, 75:149-161.

Sharaf M, El–Ansari M, Maltin S, Saleh N (1997). Four flavonoide glucosides from *P. harmala* L. Phytochem. 44:533-356.

Szabó FL, (2008). Rigorous Biogenetic Network for a Group of Indole Alkaloids Derived from Strictosidine. Molecules, 13:1875-1896.

Tanira MO, Ali BH, Bashir AK, Dhanasekaran S, Tibirica E, Alves L (2000). Mechanism of the hypotensive action of *Rhazya stricta* in rats. Pharmacol. Res., 41 (3):369-387.

Vukovic N, Milosevic T, Sukdolak S, Solujic S (2007). Antimicrobial Activities of Essential Oil and Methanol Extract of *Teucrium montanum*. eCAM ;4(S1)17-20.

Xü Y, Chen H, Liu W, Gu Z, Liang H (1998). Two sapogenins from *Tribulus terrestris* L. Phytochem. 49:199-201.