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New Perspectives on *Asphodeline lutea* from Bulgaria and Turkey: Anti-mutagenic, Anti-microbial and Anti-methicillin Resistant *Staphylococcus aureus* (MRSA) Activity

Ahmet Uysal[1], Irina Lazarova[2], Gokhan Zengin[3*], Erdogan Gunes[3], Abdurrahman Aktumsek[3] and Reneta Gevrenova[4]

[1]*Deparment of Medicinal Laboratory, Vocational School of Health Services, Selcuk University, Turkey.*
[2]*Department of Chemistry, Faculty of Pharmacy, Medical University of Sofia, Bulgaria.*
[3]*Deparment of Biology, Faculty of Science, Selcuk University, Turkey.*
[4]*Department of Pharmacognosy, Faculty of Pharmacy, Medical University of Sofia, Bulgaria.*

Authors' contributions

This work was carried out in collaboration between all authors. Authors AU, IL, GZ and EG designed the study and prepared the manuscript. Authors AA and RG managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: In this study, methanol extracts of *Asphodeline lutea* roots from Bulgaria (ALB) and Turkish (ALT) origin were evaluated for their anti-microbial, anti-MRSA properties and they were also screened for the potential of mutagenic and anti-mutagenic activities.
Methodology: The broth micro dilution method was performed for the anti-microbial activities. For mutagenicity and anti-mutagenicity screening of the extracts, plate incorporation method of Ames test was employed.
Results: *Sarcina lutea* was the most sensitive bacterium against ALB and ALT extracts at doses of 1.56 and 0.78 mg/ml, respectively. Both extracts exhibited similar activity against methicillin

**Corresponding author: E-mail: gokhanzengin@selcuk.edu.tr;*

sensitive *Staphylococcus aureus* (MSSA) and *Salmonella enteritidis* with MIC value 6.25 mg/ml. Based on the results obtained from Ames test, no mutagenic activity was found for frame shift mutation (TA98) and base pair substitution (TA100) in all concentrations of *A. lutea.* Strong anti-mutagenic properties with rates of 77% and 75% were observed at the highest concentrations of both extracts against 2-aminofluorene-induced mutagenicity on TA 98 with the presence of metabolic activator S9 system.

Conclusion: As a result, the extracts revealed significant anti-MRSA activity with MIC values 6.25 mg/ml against MRSA strains isolated from infections and manifested strong anti-mutagenic activity against known mutagens; it may be used in drug formulations against MRSA infections and may be used as a natural anti-mutagenic agent in the pharmacology and food industries.

Keywords: Asphodeline lutea; anti-MRSA; anti-mutagenic; natural agent.

1. INTRODUCTION

Asphodeline lutea (L.) Rchb. is a wild plant traditionally used as a food in the Mediterranean region but its benefits of medicinal properties are not well studied [1]. Phytochemical investigation revealed that the roots are source of antharquinones, naphthalenes, flavonoids and phenolic acids [2,3]. Recently, 1-hydroxy-8-methoxy-3-methylanthraquinone, 1,5,8-trihydroxy-3-methylanthraquinone, 1,1',8,8',10-pentahydroxy-3,3'-dimethyl-10,7'-bianthracene-9,9',10'-trione, 2-acetyl-8-methoxy-3-methylnaphthoquinone, 2-acetyl-1-hydroxy-8-methoxy-3-methylnaphthalene and 2-acetyl-1,8-dimethoxy-3-methylnaphthalene were evidenced for the first time in the *A. lutea* roots, together with the known chrysophanol and asphodeline [3]. The antioxidant activity of 2-acetyl-1-hydroxy-8-methoxy-3-methylnaphthalene, isolated from *A. lutea* chloroform extract, was found during oxidation of triacylglycerols [4].

Previous investigation revealed stronger antioxidant potential of *A. lutea* methanol-root extract from Bulgarian origin as compared with the Turkish sample [2]. It exhibited higher ferric and cupric reduction ability, metal chelating and radical scavenging activity. Anthraquinones, naphthalenes, phenolic acids and flavonoids were determined in both *A. lutea* root extracts. A naphthalene derivative (2-acetyl-1,8-dimethoxy-3-methylnaphthalene) was the major compound in Bulgarian accession (0.96±0.03 mg/g), while caffeic acid was the main analyte in Turkish one (0.42±0.06 mg/g). [5] The alcoholic extracts from *A. lutea* roots revealed anti-cholinesterase, anti-tyrosinase, anti-amylase, anti-glycosidase activity and anti-proliferative effect towards MCF-7 and MCF-10A cell lines. In the present study, we aimed at investigating the anti-mutagenic and anti-microbial and anti-methicillin resistant *Staphylococcus aureus* (MRSA) activity of *A. lutea* root extracts from Bulgarian and Turkish origins.

2. MATERIALS AND METHODS

2.1 Plant Materials

The plant material from Bulgarian origin was collected near the town of Pernik. The voucher specimens (SOM 1312) were deposited at Institute of Biodiversity and Ecosystems Research, Bulgarian Academy of Sciences, Sofia, Bulgaria. *A. lutea* from Turkish origin were collected from Konya: between Cevizli and Beysehir. The voucher specimens (GZ 1002) were deposited at Department of Biology, Faculty of Science, Selcuk University, Konya Turkey. Both Bulgaria and Turkish plant materials were collected at identical season (flowering stage (May to June, 2013)) due to avoid differences of the active principles. The roots of Bulgaria and Turkish plant materials were separated and dried at ambient conditions in the dark until powdered process.

2.2 Preparation of the Methanolic Extracts

To produce solvent extracts, the air-dried samples (10 g) of the roots of *A. lutea* were macerated with 250 mL of methanol at room temperature for 24 h. The extracts concentrated under vacuum at 40℃ by using a rotary evaporator. Extracts were stored at +4℃ in dark until use. The extraction yields were 12.5% for ALB (*A. lutea* from Bulgaria) and 20.6% for ALT (*A. lutea* from Turkey).

2.3 Anti-microbial Activity

2.3.1 Microorganisms

Strains of *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 3166, *Bacillus cereus*

ATCC 11778, *Bacillus subtilis* RSHM 03013, *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas aeruginosa* ATTC 15442, *Klebsiella oxytoca* ATCC 10031, *Listeria monocytogenes*, methicillin sensitive *Staphylococcus aureus* ATCC 25923 (MSSA), *Klebsiella pneumoniae* ATCC 70603, *Salmonella enteritidis* ATCC 13076, *Streptococcus mutans* NCTC 10449, *Sarcina lutea* ATCC 9341, *Enterococcus faecalis* ATCC 29212, *Candida albicans*, methicillin resistant *Staphylococcus aureus* ATCC 43300 (MRSA) for the determination of anti-microbial activity, and strains of methicillin resistant *Staphylococcus aureus* isolated from clinical samples for the detection of anti-MRSA activities of *Asphodeline lutea* extracts were used. The methicillin resistances of strains were determined by agar screening, oxacillin disc diffusion and broth microdilution methods. The standard microorganisms were obtained from Microbiology Research Laboratory, Department of Biology, Selcuk University, and Konya, Turkey. Standard and isolated strains of bacteria were grown to exponential phase in Brain-Heart Infusion Broth (Merck) at 37℃ overnight with aeration in shaking incubator. The cultures were then plated on Mueller-Hinton Agar (Merck), overnight at 37℃ with aeration. Then, approximately five colonies of cultures suspended in sterile physiologic water. The bacterial suspensions were adjusted to 0.5 McFarland standard turbidity (10^8 CFU/ml). Finally, these suspensions used as inoculums were prepared at 10^5 CFU/ml by diluting fresh cultures at McFarland 0.5 density.

2.3.2 Broth microdilution test

The broth microdilution method was employed for anti-bacterial and anti-MRSA activity tests. Mueller-Hinton Broth (100 µl) was placed into each 96 wells of microplates. The extracts were dissolved in DMSO:PBS (1:1) to a final concentration of 25 mg/ml and sterilized by filtration (0.45 µm, Millipore filters). Extract solutions were added into first rows of microplates at 100 µl volumes and two fold dilutions of the extracts (6.25-0.0030 mg/ml) were made by dispensing the solutions to the remaining wells. Then, 100 µl of culture suspensions were inoculated to each well. Gentamicin was used as positive control. The sealed microplates were incubated at 35℃ for 18 h for bacteria and the microplates containing *Candida albicans* were incubated at 28℃ for 48 h. Microbial growth was determined by adding 20 µL of 2,3,5-Triphenyl-tetrazolium chloride (0.5%) after incubation to each well and incubating for 30 minute at 37℃. Since the colorless tetrazolium salt is reduced to red colored product by biological active bacteria, the inhibition of growth can be detected when the solution in the well remains clear after incubation with TTC. MIC was defined as the lowest sample concentration showing no color change (clear) and exhibited complete the inhibition of growth [6].

2.4 Mutagenicity/Anti-mutagenicity Assay

2.4.1 Strains

The *Salmonella typhimurium* test strains TA98 and TA 100 were obtained from Microbiology Research Laboratory, Science Faculty, Selcuk University. The strains were analyzed for their histidine requirement, biotin requirement, the combination of both, rfa mutation, excision repair capability, the presence of the plasmid pKM101, and spontaneous mutation rate according to Maron and Ames[7]. Two strains of *Salmonella typhimurium* (TA98, TA100), genetically modified and used in the experiments, were responsible for frame shift mutations and base pair substitution, respectively.

2.4.2 Determination of toxic dose levels

For determining of cytotoxic doses of the *Asphodeline lutea* extracts the method described by Dean et al. [8] was used. While 10000, 5000, 1000 µg/plate doses were determined as non toxic doses for ALB, 5000, 1000 and 500 µg/plate doses of ALT were determined as non toxic. The determined non cytototxic doses were used in the experiments.

2.4.3 Mutagenicity assay

Salmonella /microsome test system (Ames test) was performed for determination of mutagenic potencies of the extracts tested. The plate incorporation method was employed according to Maron and Ames [7]. The known mutagens 4-nitro-o-phenylenediamine (4-NPDA, 5 µg/plate) for *S. typhimurium* TA98 and sodium azide (SA) (5 µg/plate) for *S. typhimurium* TA100 were used as positive controls in the absence of S9 metabolic activation enzymes. 2-aminofluorene (2-AF, 7.5 µg/plate) and 2-aminoanthracene (2-AA, 5 µg/plate) were used as positive controls with S9, respectively. Also dimethyl sulfoxide

(DMSO) was used as a negative control in mutagenicity and anti-mutagenicity tests (in duplicate).

In order to test the mutagenicity of the *A. lutea* extracts, 100 µL of an overnight fresh bacterial culture (1-2x10^9 cell/mL), 500 µL of S9 mix (or 500 µL phosphate buffer) and different concentration of the extracts were added to 2.5 mL of melted top agar at 45℃ assay) and mixed gently with a vortex mixer. The mixed solution was poured onto the minimal glucose agar (MGA) plate and rotated immediately. Finally, the plates were placed on a leveled surface for the top agar to solidify and incubated at 37℃ for 48-72 h [6]. After the incubation period revertant colonies were counted and recorded. Also negative control plates were prepared as follow: DMSO (100 µL), S9 mix (or 500 µL phosphate buffer), and 100 µL of overnight bacteria culture were added to top agar and poured onto the MGA plates. The positive controls used for TA98, TA100 and TA98 + S9 mix or TA100 + S9 mix were 4-nitro-o-phenylenediamine, sodium azide and 2-aminofluorene, 2-aminoanthracene, respectively. Samples were tested on triplicate plates in two independent parallel experiments. Mutagenicity of extracts can be indicated using the following criteria: (1) the number of colonies being at least 2-fold or more in 2 consecutive tested dose in comparison with a spontaneous revertant plates; (2) an observable dose-related response with the number of colonies [9].

2.4.4 Anti-mutagenicity assay

Anti-mutagenesis assays were performed according to the method of Maron and Ames (1983) and modified according to Zengin et al[6]. Briefly a 100 µL aliquot of bacterial suspension including 1-2 x10^9 bacteria, 100 µL of different concentrations of extracts, 100 µL of positive mutagen solution, 500 µL of S9 mixture or phosphate buffer (0.1 M) were added to 2.5 mL of top agar containing 10% of histidine/biotin (0.5 mM) for both *Salmonella* strains, mixed by vortex for a few seconds and poured onto the surface of MGA plate. In the anti-mutagenicity test, two *Salmonella* strains were exposed to known mutagenic substance such as 4-NPDA without S9 metabolic activation and 2-AF with S9 for *S. typhimurium* TA 98, SA in the absence of S9 mix and 2-AA in the presence of S9 mix for *S. typhimurium* TA 100 and inhibition of mutagenic action by the plant extract samples were observed. Then, histidine revertant colonies were counted after incubation of the plates at 37℃ for 48-72 h. Each sample was assayed using triplicate plates and the data presented as mean±SD of two independent assays. The number of revertant colonies grown on plates containing the mutagen without plant extract was defined as 100% with 0% inhibition. The anti-mutagenic potential (Inhibition) was determined by equation: $[(A-B)/(A-C)] \times 100$, where A = No. of his. revertants in the absence of sample, B = No. of his. revertants in the presence of sample, C = spontaneous revertants [6]. Anti-mutagenicity was recorded as follows: strong: 40% or more inhibition; moderate: 25–40% inhibition; low/none: 25% or less inhibition [10].

2.5 Statistical Analysis

Except for inhibition rates, all values obtained are expressed as the mean±S.D. The evaluation of statistical significance was determined by one-way ANOVA (SPSS 13.0 for Windows) followed by Tukey HSD and Tamhane tests. The level of significance was set at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Anti-microbial Activity

In this study anti-microbial and anti-MRSA activities of methanol extracts of *Asphodeline lutea* collected from different localities were investigated by broth micro dilution method according to Zengin et al [6]. The obtained results are presented in Table 1 and Table 2.

The MIC values obtained for extracts against the bacterial strains varied among the two plant extracts. As can be clearly seen from the Table 1, ALB revealed antibacterial activity against *S. aureus* (MSSA), *S. enteritidis*, and *S. lutea*. Except for *S. lutea*, the MIC values of the extract were determined as 6.25 mg/ml against these strains. *S. lutea* was the most sensitive bacterium against extract of ALB and the MIC value was determined as 1.562 mg/ml. As distinct from the ALB, ALT was found to be effective against *K. oxytoca* and *S. aureus* (MRSA) at a concentration of 6.25 mg/ml. Moreover it exhibited similar antibacterial activity as other plant against *S. aureus* (MSSA) and *S. enteritidis* at a dose of 6.25 mg/ml. *S. lutea* was the most sensitive bacterium, too. But the ALT extract was more effective than the other against *S. lutea* and MIC value was determined as 0.781 mg/ml. Except from bacteria mentioned above, most of the strains tested were found to be resistant to both plant extracts. Neither ALT nor ALB plant extracts exhibited any activities

against *Candida albicans*. The obtained results showed that the antimicrobial activities of the tested extracts depend on its origin. It may be stated that both two extracts were more effective against some Gram positive microorganisms affected from extracts than Gram negative.

In addition to antimicrobial activity, the extracts of *Asphodeline lutea* were tested against clinical isolates of MRSA. The MIC values of the extracts obtained from the study against MRSA strains presented in Table 2.

Table 1. Antimicrobial activity and MIC values of *Asphodeline lutea* extracts against standard microorganisms

Tested microorganisms	MIC values of *Asphodeline lutea* extracts (mg/ ml)		MIC value of gentamicin (µg/ml)
	ALB	ALT	
Escherichia coli ATCC 3166	-	-	2.44
Escherichia coli ATCC 25922	-	-	2.44
Pseudomonas aeruginosa ATCC 15442	-	-	9.76
Bacillus cereus ATCC 11778	-	-	9.76
Bacillus subtilis RSHM 03013	-	-	9.76
Klebsiella oxytoca ATCC 10031	-	6.25	2.44
Listeria monocytogenes	-	-	
Pseudomonas aeruginosa ATCC 27853	-	-	9.76
Staphylococcus aureus ATCC 25923 (MSSA)	6.25	6.25	2.44
Klebsiella pneumoniae ATCC 70603	-	-	2.44
Staphylococcus aureus ATCC 43300 (MRSA)	-	6.25	78.12
Salmonella enteritidis ATCC 13076	6.25	6.25	4.88
Sarcina lutea ATCC 9341	1.5625	0.7812	4.88
Streptococcus mutans NCTC 10449	-	-	
Enterococcus faecalis ATCC 29212	-	-	2.44
Candida albicans	-	-	312.5

ALB: A. lutea from Bulgaria; ALT: A. lutea from Turkey

Table 2. Anti MRSA activity and MIC values of *Asphodeline lutea* extracts against MRSA strains

Tested microorganisms	MIC values of *Asphodeline lutea* extracts (mg/ml)		MIC value of gentamicin (µg/ml)	MIC value of oxacillin (µg/ml)
	ALB	ALT		
MRSA ATCC 43300	-	6.25	78.12	64
MRSA strain 1 (ES 107)	6.25	6.25	156.25	8
MRSA strain 2 (ES 128)	6.25	6.25	78.12	≥128
MRSA strain 3 (ES 123)	6.25	6.25	78.12	16
MRSA strain 4 (ES 124)	6.25	6.25	78.12	≥128
MRSA strain 5 (ES 110)	6.25	6.25	156.25	≥128
MRSA strain 6 (ES 100)	6.25	6.25	78.12	≥128
MRSA strain 7 (ES 75)	6.25	6.25	156.25	≥128
MRSA strain 8 (ES 25)	6.25	6.25	312.5	≥128
MRSA strain 9 (ES 93)	6.25	6.25	78.12	≥128
MRSA strain 10 (ES 29)	6.25	6.25	312.5	32
MRSA strain 11 (ES 69)	6.25	6.25	312.5	≥128
MRSA strain 12 (ES 68)	6.25	6.25	156.25	≥128
MRSA strain 13(ES 16)	6.25	6.25	156.25	16
MRSA strain 14 (ES 67)	6.25	6.25	156.25	32

ALB: A. lutea from Bulgaria; ALT: A. lutea from Turkey

The results manifested that clinical MRSA strains were equally affected from both plant extract originated in different localities. The MIC values of both two extract were determined as 6.25 mg/ml against tested strains. It may be stated from the table that *Asphodeline lutea* extracts have remarkable anti-MRSA activity and it may be used in drug formulations against MRSA infections.

The crude extracts containing 1,8-dihydroxyanthraquinones or individual compounds are known for their antimicrobial property. 1,8-dihydroxyanthraquinones inhibit enzymes involved in metabolism of microorganisms [11]. Their antibacterial activity is due to inhibition of nucleic acid synthesis [12]. The chemical structures of 1,8-dihydroxyanthraquinones define their antimicrobial activity against some bacteria strains. The decreasing order of growth inhibition of *Staphylococcus aureus* is rhein> emodin> 1,8-dihydroxyanthraquinone [11]. Moreover, oxidized forms of 1,8-dihydroxyanthraquinones exhibit higher anti-bacterial activity then their reduced forms [13]. The various degree of inhibiting activity on the microbial growth and on their enzymes action depends on number and position of hydroxyl groups in anthraquinone molecule.

3.2 Mutagenic/Anti-mutagenic Activity

This study examined methanol extracts of *Asphodeline lutea* from Turkey and Bulgaria for their potential mutagenic and anti-mutagenic activities toward *S. typhimurium* TA98 and TA100. The revertant colony numbers observed in the mutagenicity assay were determined and are given in Table 3.

According to Table 3 spontaneous revertants were within normal ranges in all strains examined. The mean revertant colony numbers in negative control were 32±4 for TA98 and 132±13 for TA100 with S9 and 35±2 and 103±6 without S9, respectively. The extracts (ALB and ALT) neither induced base pair mutations on *S. typhimurium* TA100 nor induced frame shift mutations on *S. typhimurium* TA98. In another word they appeared to be non mutagenic in all experimental conditions. As would be expected, the plates with the known mutagens (SA, 2-AF, 2-AA and 4-NPDA) showed significant increases relative to the spontaneous mutation rate in the two tested strains. The revertant colony numbers were increased in plates exposed to extracts with S9 mix both for two strains (Table 3). But these increases were not significant statistically at $p<0.05$ (Tamhane test). In order to investigate whether there was a dose relationship between doses, 3 different doses of *A. lutea* extracts were examined and the extracts did not induced twofold increase of spontaneous revertants at all test concentrations. Consequently extracts of *A. lutea* from different localities were not found to be mutagenic for TA98 and TA100 in the condition both with and without S9 mix. These results suggest that the methanol extract of *A. lutea* may be safe for use in humans and should be considered for further medical development studies.

Possible anti-mutagenic potential of two plant extracts against known mutagens were also investigated. The revertant colony numbers obtained from the anti-mutagenicity assays and inhibition rates of the extracts were given in Table 4.

ALB extract reduced the mutagenic activity of 4-NPDA at a dose of 10000 µg/plate and it was statistically significant $p<0.05$ and determined as strong anti-mutagenic with the rate of 41% without S9 mix for TA98 strain (Table 4). Although the extract at a concentration of 5000 µg/plate was moderate anti-mutagenic (25%), it had no anti-mutagenic activity (9%) at a dose of 1000 µg/plate for TA98 in the absence of S9 mix. With the addition of S9 metabolic activation enzymes, it was seen that there were increases in the rate of inhibition of mutagenesis induced by premutagen 2-AF, activated with S9 mix, for TA 98. 10000 µg/plate doses manifested strong anti-mutagenic activity with a ratio of 77%, and 5000 and 1000 µg/plate doses of extract revealed moderate antimutagenic activity with rates of 39% and 31%, respectively (Table 4). It is seen that inhibition ratios increased from 41% to 77%, 25% to 39% and 9% to 31% when the S9 mix was added. It can be stated that S9 mix increased the inhibition rates of mutagenesis for TA98 strain.

For ALT extract, dose-dependent anti-mutagenic activity was observed at a dose of 5000 µg/plate (Table 4). It was determined that this concentration had moderate anti-mutagenic capacity against 4-NPDA for TA98 strain in the absence of S9. On the contrary, 1000 and 500 µg/plate doses were found to be weak anti-mutagenic with ratios of 23% and 5%, respectively for TA98. In the assay performed with S9 mix, although 5000 µg/plate dose revealed very strong anti-mutagenic activity against mutagenesis induced by premutagen 2-

AF with a rate of 75%, 1000 and 500 µg/plate doses were not anti-mutagenic against this mutagen. The reduction of 2-AF induced mutations at the highest dose was considered to be dose-dependent and it was statistically significant (Table 4).

It was seen that ALB methanol extract showed moderate anti-mutagenic activity at all test doses (10000, 5000, and 1000 µg/plate) in the absence of S9 mix for TA100 (Table 4). The inhibition rates determined against mutation induced by sodium azide were 32%, 29%, and 27%, respectively. Especially 10000 and 5000 µg/plate concentrations of extract manifested strong anti-mutagenic activity after addition of S9 mix. Considering the whole of study, 10000 µg/plate dose of extract exhibited the strongest activity with 86% inhibition rate against the 2-AA, activated with the S9 mix for TA100 (Table 4). Although the inhibition rate of 5000 µg dose was determined as strong anti-mutagenic (69%), 1000 µg dose of extract reduced the inhibition rate from moderate (27%) to weak anti-mutagenicity (18%) with metabolic activation system enzymes for TA100 strain.

ALT showed similar anti-mutagenic activity as ALB extract without S9 mix. The extract was found to be moderate anti-mutagenic at all test doses (5000, 1000, and 500 µg/plate) with ratios of 35%, 31%, and 25%, respectively (Table 4). In contrast to ALB, the inhibition rates of ALT extract decreased in the presence of S9 mix and determined as weak anti-mutagenic (Table 4).

It can be stated from the study, the anti-mutagenic activities of ALB extract were higher than ALT extract. Except for 5000 µg/plate dose in the presence of S9 mix for TA98, other test doses of ALT revealed weak anti-mutagenicity both for TA98 and TA100 in the presence of S9 mix. The strongest anti-mutagenic activities of ALB were determined as 86% for TA100 and 77% for TA 98 in the presence of S9 mix at a dose of 10000 µg/plate. It was seen that metabolic activation enzymes increased inhibition rates against mutation induced by 2-AF and 2-AA for ALB. This significant effect is not considered toxic and no damage was observed on the background wrought in any of the plates. These results suggest that this extract should be suitable for evaluation concerning CyP450 modulations effects and it could be explained by finding that medicinal plants might contain compounds capable of inhibiting the CyP450 required for activating these mutagens[14]. Buening et al. [15] determined that some plant metabolites are potent inhibitors of cytochrome c (P450) reductase.

Table 3. Mutagenicity of *A. lutea* extracts towards *S. typhimurium* TA98 and TA100 strains with or without S9

	Concentration (µg/plate)	Number of his$^+$ revertants/plate			
		TA 98		TA 100	
		S9 (-)	S9 (+)	S9 (-)	S9 (+)
*Negative control	100 µl/plate	35±2a	32±4a	103±6a	132±13a
®Positive control		2626±29b	2866±57b	1952±169b	2915±56b
ALB	0	36±5a	37±7ac	116±18a	144±13a
	10000	37±6a	50±5ac	102±18a	164±3a
	5000	34±8a	53±4c	134±11a	182±6a
	1000	36±4a	47±ac	129±5a	190±7a
*Negative control	100 µl/plate	35±2a	32±4a	103±6a	132±13a
®Positive control		2626±29c	2866±57b	1952±169b	2915±56b
ALT	0	36±5ad	37±7a	116±18ac	144±13a
	5000	29±5ad	35±7a	157±3c	150±14a
	1000	24±2bd	43±3a	134±13ac	148±6a
	500	22±2bd	37±2a	128±4a	182±9a

[abcd] Differences between groups having the same letter in the same column are not statistically significant (ANOVA, Tamhane, p>0.05)

[*] Negative control: DMSO (100 µl/plate) was used as negative control for S. typhimurium TA98 and TA100 both in the presence and absence of S9

[®] Positive controls:2-Aminofluorene (7.5 µg/plate) was used as positive indirect mutagen in the presence of S9 mix; 4-nitro-O-fenilendiamine (5 µg/plate) was used as positive direct mutagen in the absence of S9 mix for S. typhimurium TA98 strain. 2-Aminoanthracene (5 µg/plate) was used as positive indirect mutagen in the presence of S9 mix; Sodium azide (5 µg/plate) was used as positive direct mutagen in the absence of S9 mix for S. typhimurium TA100

ALB: A. lutea from Bulgaria; ALT: A. lutea from Turkey

Table 4. Anti-mutagenicity of *A. lutea* extracts towards *S. typhimurium* TA98 and TA100 strains with and without metabolic activation (S9)

	Concentration (µg/plate)	Number of His+ revertants/plate							
		TA 98				TA 100			
		S9 (-)	% inhibition	S9 (+)	% inhibition	S9 (-)	% inhibition	S9 (+)	% inhibition
*Negative control	100 µl/plate	31±4a		32±4a		128±9e		138±11e	
®Positive control		1022±86c	0	2783±136d		2424±98f	0	1715±164f	0
ALB	0	35±5a		32±6a		140±11e		144±9e	
	10000	615±40b	41	669±46b	77	1698±74g	32	359±42e	86
	5000	777±8bc	25	1707±16c	39	1765±33fg	29	626±32eg	69
	1000	935±56c	9	1925±45c	31	1806±66g	27	1425±141fg	18
*Negative control	100 µl/plate	31±4a		32±4a		128±9a		138±11e	
®Positive control		1022±86c	0	2783±136c		2424±98c	0	1715±164f	0
ALT	0	35±5a		32±6a		140±11a		144±9e	
	5000	705±50b	32	711±74b	75	1627±198b	35	1619±174f	6
	1000	791±71bc	23	2653±159c	5	1727±126b	31	1542±91f	11
	500	973±48c	5	2712±181c	3	1853±205b	25	1575±121ef	9

[abcd] Differences between groups having the same letter in the same column are not statistically significant (ANOVA, Tukey, p>0.05)
[efg] Differences between groups having the same letter in the same column are not statistically significant (ANOVA, Tamhane, p>0.05)
[*] Negative control: DMSO (100 µl/plate) was used as negative control for S. typhimurium TA98 and TA100 both in the presence and absence of S9
[®] Positive controls:2-Aminofluorene (7.5 µg/plate) was used as positive indirect mutagen in the presence of S9 mix; 4-nitro-O-fenilendiamine (5 µg/plate) was used as positive direct mutagen in the absence of S9 mix for S. typhimurium TA98 strain.
2-Aminoanthracene (5 µg/plate) was used as positive indirect mutagen in the presence of S9 mix; Sodium azide (5 µg/plate) was used as positive direct mutagen in the absence of S9 mix for S. typhimurium TA100.
ALB: A. lutea from Bulgaria; ALT: A. lutea from Turkey

The most significant mechanism in anti-mutagenesis is the scavenging of bio-active molecule [16]. The extracts with high level of polyphenolic compunds display potent antioxidant and free radical scavenging activities [17]. The antimutagenic properties of anthraquinones - aloe-emodin-anthraquinone isolated from *Aloe barborescence* [18] and chrysophanol isolated from *Cassia torra* [19] were described. It is known that *A. lutea* root extract is rich in chrysophanol and chrysophanol based anthraquinones [3]. Among compounds structurally related to anthraquinones, anthrone, acridone and xanthone exerted anti-mutagenicity, anthrone being the most active one. All naphthaquinones were potent anti-mutagens, plumbagin and 2-methyl-5-hydroxy naphthoquinone showed excellent anti-mutagenic activity [20]. Overall properties of the plant extract may be due to synergism of the phenolic sub- classes.

4. CONCLUSION

In our previous investigations, we observed that the ALB extract revealed higher levels of antioxidants, higher level of total anthraquinones, and better antioxidant potential as compared with the Turkish one [2,5]. In addition, naphthoquinone-2-acetyl-8-methoxy-3-methylnaphthoquinone in *A. lutea* root extract from Bulgarian origin may account for the reported effects on the inhibition of mutagenesis for both tested strains. Its most remarkable effect was found to be as an inhibitor of mutagenic induction of premutagens requiring CyP450, such as 2AA and 2-AF. All these findings are with good agreement with higher antimutagenic activity of the ALB extract. This property demonstrates that the use of ALB could provide protection against polycyclic aromatic hydrocarbons which are well known as premutagens and precarcinogens. Also it was seen that ALT and ALB extract was significantly effective against *S. lutea*. Gram positive microorganisms were more sensitive than Gram negative against two extracts. ALT and ALB extracts revealed remarkable anti-MRSA activity against clinical isolates of MRSA and these two extracts may be used in pharmaceutical industry for combating against resistant bacteria and treatment of the infections.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

DISCLAIMER

This manuscript was presented as a poster in the conference "2nd International Conference on Natural Products Utilization:" Available link is "https://www.researchgate.net/profile/Reneta_Gevrenova/publications" date10/2015.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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