Journal of Research in Pharmacy

Research Article

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Elucidation of biological properties of some commercial anthraquinones: Mutagenic / antimutagenic and antimicrobial activity approaches

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Received: 31 July 2018 / Revised: 30 October 2018/ Accepted: 31 October 2018

ABSTRACT: Anthraquinones (AQ) are the most common group of naturally occurring quinones. Both natural and synthetic AQs have been used as colorants in food, drugs and cosmetic industries. The aim of this study was to evaluate the mutagenic/antimutagenic potentials of some AQs (Alizarin, quinizarin, purpurin, and 1,8-dihydroxy anthraquinone) with *Salmonella typhimurium* TA98 and TA100 strains by Ames test and antimicrobial activity by broth microdilution method. So, AQs were tested for their toxicity and nontoxic doses of the chemicals were used. The results manifested that none of the chemicals were mutagenic for TA98 and TA100 strains both with and without metabolic activation enzymes (S9 mix). Purpurin and alizarin exhibited strong antimutagenic effects against 4-nitrophenylendiameine and 2-aminoflourene at all test doses (1000, 500 and 250 µg/plate) for TA98; and against sodium azide and 2-aminoanthracene for TA100. Alizarin showed the highest inhibition rate (93%) against sodium azide at a concentration of 1000 µg/plate. While 1,8-dihydroxy anthraquinone and quinizarin revealed strong antimutagenicity at 10000 µg/plate without S9 mix, they exhibited excellent antimutagenic action after addition of S9 enzymes for TA98 strain at all test doses. Similarly, 1,8-dihydroxy anthraquinone and quinizarin were moderate antimutagenic against sodium azide at all test doses without S9 mix, their antimutagenicity increased and they ameliorated the mutagenic action of 2-aminoanthracene by the addition of S9 for TA100. These two chemicals were strong antimutagenic against promutagens activated by S9 mix. Also it was defined that purpurin and alizarin have antimicrobial capacity against MRSA strains.

KEYWORDS: Alizarin; quinizarin; purpurin; 1,8-dihydroxy anthraquinone; Ames test; antimicrobial.

1. INTRODUCTION

With around 300,000 numbers, higher plants are the source of many defined chemical substances. [1]. Because of the chemical substances they have, these plants have become the main focus of herbal drug and drug production. Many bioactive substances with medical properties have been isolated and identified. [2]. These compounds can be grouped into the two classes: primary and secondary metabolites. Especially secondary metabolites, acting as gun against herbivores, pathogen microorganisms and competing plants and as signal molecules, are much more diverse than primary metabolites. [3-5]. Although phenolic compounds are abundant in plant kingdoms, they can also be found in a variety of living groups such as bacteria, fungi and algae. [6]. Phenolics are of special interest related to their activities, such as antioxidants [7], antimutagenic [8], anti-inflammatory and inhibitions of enzymes associated with important ailments including Alzheimer, diabetes [9, 10]. In this context, new studies performed on plants or plant-derived products are very important for the search of natural and safely functional food ingredients.

Anthraquinones (AQ) are a group of phenolics and are widely distributed in some plant families such as Rubiaceae, Leguminosae and Rhamnaceae [10]. They are a group of chemicals with very wide use and applications. It is known that AQ-containing plants have been used for a long times for remedy of illnesses [11]. Both natural and synthetic AQ are widely used in different industries such as textile, foods, cosmeceuticals and pharmaceuticals. Furthermore, based on the redox potential, they act as catalysts in many chemical processes, such as the reduction of pollutants. [12]. Anthraquinone derivatives have a great

How to cite this article: Uysal A, Gunes E, Durak Y. Elucidation of biological properties of some commercial anthraquinones: mutagenic / antimutagenic and antimicrobial activity approaches. J Res Pharm. 2019; 23 (2): 208-216.

ISSN: 2630-6344

https://doi.org/10.12991/jrp.2019.126

pharmacological potentials including laxative [13], anticancer [14], antiinflammatory [15], antiarthritic [16], antifungal [17], antibacterial [18], antiviral [19], antiplatelet [20], and neuroprotective effects [21], and antimalarial [22] activities.

Thus, the goals of this study were i) to evaluate the mutagenic, antimutagenic potentials of some AQs (Alizarin, quinizarin, purpurin, and 1,8-dihydroxy anthraquinone) with histidine dependent mutant *Salmonella* strains (*S. typhimurium* TA98 and TA100) by Ames test ii) antimicrobial activities of them against standard bacteria and methicillin resistant *Staphylococcus aureus* (MRSA) strains obtained from several clinical specimens.

2. RESULTS

2.1. Mutagenic and Antimutagenic Evaluation

Table 1 shows the possible mutagenic action of AQs, observed in *S. typhimurium* TA98 and TA100 with and without S9 mix. In Ames assay, positive control mutagens increased the number of His^+ revertant colonies in two mutant strains, in the presence and absence of metabolic activation system. In order to investigate whether there was a dose relationship between doses, three different doses of four AQ were examined and the samples did not induced two-fold increase of spontaneous revertants at all test concentrations. Therefore alizarin, quinizarin, purpurin, and 1,8-dihydroxy anthraquinone did not show mutagenicity for both test strains in the condition both with and without metabolic activation system. These results suggest that these chemicals may be safe for use in humans and should be considered for further medical development studies.

Table 1. Mutagenicity of some anthraquinones towards *S. typhimurium* TA98 and TA100 strains with and without S9.

Concentration (µg/plate)		TA 98		TA 100	
		S9-	S9+	S9-	S9+
Negative Control [a]	100 µl/plate	42±6	48±3	164±2	175±3
Positive Control [b]		453±91	3100±109	2022±82	5243±557
	0	36±4	32±2	154±8	171±6
Purpurin	1000	21±3	26±1	133±38	151±19
	500	33±9	45±4	179±2	153±8
	250	36±1	29±2	219±20	202±15
Alizarin	1000	26±6	38±4	243±26	210±22
	500	32±1	37±3	250±31	198±16
	250	28±2	22±0	228±10	175±8
1,8-dihydroxy anthraquinone	10000	47±1	49±2	142±23	215±12
	5000	44±1	45±2	182±10	224±10
	1000	35±3	42±3	171±9	145±7
Quinizarin	10000	21±4	37±0	176±2	159±12
	5000	25±3	27±1	183±7	191±4
	1000	26±3	35±2	146±20	165±8

[a] 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

[b] Positive controls:
2-Aminofluorene (7.5 µg/plate) was used as positive indirect mutagen in the presence of S9 mix; 4-nitro-*o*-phenylenediamine (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.

Because of test chemicals did not reveal any mutagenicity in assay, antimutagenic potentials of these AQs against well-known mutagens were tested towards *S. typhimurium strains* TA98, TA100, both with and without S9 mix. The revertant colony numbers and inhibition rates were presented in Table 2.

Table 2. Antimutagenicity and inhibition ratios of some anthraquinones towards *S. typhimurium* TA98 and TA100 strains with and without metabolic activation (S9) against direct and indirect mutagens.

	Concentration (µg/plate)	TA 98				TA 100			
		S9 (-)	% Inhibition	S9 (+)	% Inhibition	S9 (-)	% Inhibition	S9 (+)	% Inhibition
Negative Control [a]	100 µl/plate	42±6		48±3		164±2		175±3	
Positive Control [b]		453±91	0	3100±109	0	2022±82	0	5243±557	0
Purpurin	1000	185±7	63	321±12	91	397±36	87	856±43	86
	500	195±6	61	654±33	80	648±23	74	1141±54	81
	250	262±5	45	945±21	71	1054±68	52	1925±31	65
Alizarin	1000	161±6	69	512±19	85	284±80	93	587±15	92
	500	169±7	67	921±15	71	451±38	84	895±36	86
	250	172±3	66	1457±39	54	438±31	85	2167±112	61
1,8-Dihydroxy anthraquinone	10000	276±53	42	668±12	80	1359±55	36	1453±410	75
	5000	309±31	34	745±21	77	1357±16	36	1505±78	74
	1000	354±12	23	864±25	73	1371±19	35	1964±35	65
Quinizarin	10000	272±60	43	617±9	81	1401±181	33	1135±157	81
	5000	270±14	43	875±45	73	1388±23	34	1403±29	76
	1000	297±28	37	1542±68	51	1418±296	32	2443±33	55

[a] 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

[b] 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.

According to the table, purpurin can be considered as robust antimutagenic at the concentrations of 1000, 500 and 250 µg/plate for TA98 with rates of 63%, 61% and 45%, respectively against 4-NPDA. When combined with 2-AF, the purpurin induced the inhibition ratios more than 60%, reaching 71%, 80%, and 91%, respectively with S9 for TA 98 and making them as excellent antimutagenic. Similarly alizarin manifested strong antimutagenicity at all test doses without metabolic activation for TA98 (Table 2). The inhibition of 2-AF increased by addition of metabolic activation enzymes with the rates of 85%, 71%, 54% respectively (Table 2).

1,8-Dihydroxy anthraquinone was significant antimutagenic (42%) at 10000 µg/plate dose against 4-NPDA, while 5000 µg/plate dose was moderate antimutagenic (34%) without S9 mix for TA98. The lowest dose (1000 µg/plate) was not antimutagenic for this strain. After addition of S9 mix, 1,8-dihydroxy anthraquinone revealed strong antimutagenicity against 2-AF at all concentrations for TA98 strain (80%, 77%, 73%).

Quinizarin manifested strong antimutagenicity at doses of 10000 and 5000 µg in the without S9 enzymes. Associated with 2-AF, quinizarin increased the antimutagenicity ratios and were defined as very mighty antimutagenic with ratios of 81%, 73% and 51%, respectively (Table 2).

When the results evaluated for TA100, purpurin and alizarin showed great antimutagenic activities against SA and 2-AA both with and without metabolic activation. For purpurin, the greatest activity was determined against SA at 1000 µg/plate dose with a ratio of 87%. Also alizarin revealed the greatest antimutagenicity against SA with a rate of 93%. These two chemicals showed the greatest activities against 2-AA with S9 mix at 1000 µg dose with rates of 86% (purpurin), 92% (alizarin), respectively.

1,8-Dihydroxy anthraquinone and quinizarin were considered as moderate antimutagenic against SA at all test doses without S9 for TA100. The rates were ranging between 32% - 36%. Associated with 2-AA, 1,8-dihydroxy anthraquinone increased the inhibition rates reaching 75%, 74%, 65% and determined as strong

antimutagenic. Similarly, quinizarin was determined as strong antimutagenic after addition of S9 mix against 2-AA with ratios of 81%, 76% and 55% (Table 2).

2.2. Antimicrobial Activity Evaluation

Antimicrobial properties of tested chemicals obtained from broth microdilution test results were presented in Table 3. Test was performed on seven standard bacteria and one yeast and eight MRSA strains.

Table 3. The minimum inhibitory concentration values of anthraquinones obtained from the test against standard bacteria and MRSA isolates.

Tested Microorganisms	MIC values of Anthroquinone (mg/ml)				MIC value of Gentamicin (µg/ml)	MIC value of Oxacillin (µg/ml)
	Purpurin	Quinizarin	Alizarin	1,8 dihydroxy anthraquinone		
Escherichia coli ATCC 25922	2.5	2.5	2.5	2.5	2.44	
Pseudomonas aeruginosa ATCC 15442	1.25	2.5	2.5	2.5	9.76	
Klebsiella pneumoniae ATCC 70603	1.25	2.5	2.5	2.5	2.44	
Staphylococcus aureus ATCC 43300 (MRSA)	1.25	5	2.5	5	78.12	64
Salmonella enteritidis ATCC 13076	1.25	5	2.5	5	4.88	
Streptococcus pneumoniae ATCC 10015	1.25	5	2.5	5	2.44	
Sarcina lutea ATCC 9341	1.25	2.5	2.5	5	4.88	
Candida albicans	2.5	5	2.5	2.5	312.5	
MRSA strain 2 (ES 25)	0.019	5	0.312	5	312.5	≥128
MRSA strain 7 (ES 75)	1.25	5	2.5	5	156.25	≥128
MRSA strain 8 (ES 93)	0.625	5	1.25	5	78.12	≥128
MRSA strain 9 (ES 100)	1.25	5	2.5	5	78.12	≥128
MRSA strain 10 (ES 107)	0.019	5	0.625	5	156.25	8
MRSA strain 11 (ES 110)	1.25	5	2.5	5	156.25	≥128
MRSA strain 12 (ES 123)	0.039	5	0.625	5	78.12	16
MRSA strain 13 (ES 124)	1.25	5	2.5	5	78.12	≥128

According to Table 3, it was determined that purpurin showed weak antimicrobial activity against standard bacteria and Candida. MIC value s were defined as 2.5 mg/ml for *E. coli* and Candida, while it was observed as 1.25 mg/ml dose for remaining standard bacteria. But it was seen that purpurin has remarkable anti-MRSA potential against isolates. 0.019 mg/ml MIC values were defined for strain number 2 and 10. For 12 numbered strain MIC was detected as 0.039 mg/ml. Except for these MRSA strains others were more resistant to purpurin. It can be said purpurin have great potential for combating with resistant bacteria such as MRSA.

When quinizarin were evaluated, it manifested weak antimicrobial action against microorganisms. MIC values was ranging between concentrations of 2.5 to 5 mg/ml. Similarly, these concentrations were defined for 1,8 dihydroxy anthraquinone While standard bacteria were resistant to alizarin at 2.5 mg/ml concentration MIC value, MRSA strains affected from this chemical at doses changing between 0.312 to 2.5 mg/ml. As a result it was defined that purpurin and alizarin were effective against MRSA strains.

3. DISCUSSION

Anthraquinone derivatives used for not only colorant but also medical treatment of some disorders caused by pathogens and inflammation [12]. However, there are some concerns about anthraquinones, especially toxicity causing to cell destroy, despite their variable pharmacological effects [23]. Moreover, it is thought that the structural similarity of anthraquinone to the toxic anthracene is the reason of the toxicity of these chemicals [24]. So some of the anthraquinone derivatives have been widely investigated for their potential hazardous activities. In this study mutagenic/antimutagenic potentials of four anthraquinones commercial were determined by Ames test system. The results showed that they have no mutagenicity at the test concentrations. Moreover, alizarin, quinizarin, purpurin, and 1,8-dihydroxy anthraquinone had great potential of antimutagenicty against direct and indirect mutagens in Ames test. Some hydroxyanthraquinones

such as dantron showed mutagenicity at doses of 50–100 µg/plate in Ames test [25]. In another study, dantron, emodin, aloe-emodin were determined as mutagenic in lymphoma cells by inhibition of topoisomerase II in mice (mammalian cells) [26]. Nevertheless, chrysophanol was mutagenic while physcion has no genotoxic capacity. Biotransformation of emodin and chrysophanol into mutagenic substances by metabolic activation has been determined by Mueller *et al*. [27]. The studies mentioned above were employed as in vitro. When the animal studies were evaluated, dantron and 1-hydroxyanthraquinone caused to cancer in rodents and they caused to carcinoma cases in hepatocytes and colon [12] Barnard *et al*. [19] determined genotoxicity of Reactive Blue-2 (an anthraquinone dye) in the Comet test. On the contrary, Venturini and Tamaro [28] showed that Reactive Blue-2 has no mutagenic capacity in *Salmonella*/microsome assay. AB-25 has no mutagenicity in Ames test, too. When the two forms of RB-2 was evaluated for their genotoxic and mutagenic properties, they were nongenotoxic in Alkaline Commet test [29]. Ninety different anthraquinone substances were tested for their mutagenicity in Ames test. While, some of them as like 1-phenylaminoanthraquinone had no mutagenic capacity; 1,4- diaminoanthraquinone caused to frameshift mutation [30]. In a study conducted by Laham *et al*. [31] 1-aminoanthraquinone induced adenoma in glands of mammary in female rats.

Opposite to these mutagenic potency of quinones some hydroxyanthraquinones derived such as emodin has an anticancer potential in cancer cell lines [32, 33]. Also inhibition of pancreatic tumour cell growth by emodin was reported by Lin *et al*. [34]. Anticancer features of aloe-emodin were demonstrated by some authors [35-38]. This anti-tumor activity was explained by apoptosis. In our study 1,8-dihydroxy anthraquinone manifested very strong antimutagenicity against 2-AF and 2-AA with S9 mix. Inhibition rates were determined as 80%, 77% and 73% for TA98, 75%, 74% and 65% for TA100. Also alizarin and purpurin have excellent inhibition rates against standard mutagens tested in this study while quinizarin exhibited very high inhibition rates.

Overall, it can be stated from the study that S9 metabolic enzyme system increased the inhibition rate, reaching 92% at some concentrations, of mutagenic effects of 2-AF and 2-AA both for TA98 and TA100 strains. A possible cause of this can be explained by the following way: The antimutagenic response is activated by invoking the competitive inhibition by liver glycosides of P450 isoenzymes [39]. To investigate the mechanism of this inhibition, Takahashi *et al*. [40] examined the effects of purpurin and alizarin pigments on enzymes that metabolize xenobiotics. It was determined that purpurin and alizarin strongly inhibited the activities of CYP1A1, CYP1A2 and CYP1B1. These were potent inhibitors of CYP1B1 because the affinity of these inhibitors to the enzyme is stronger than that of the substrate. This results showed that inhibition of mutagenic substrates, which needs metabolic activation, such as 2-AA and 2-AF in our study can be attributed to inhibition of cytochrome P450 isoenzymes. Also Charehsaz *et al*. [41] and Mocan *et al*. [42] indicated that protective effects of plant extracts increased in the presence of S9 metabolic activation system by inhibiting cytochrome P450 monooxygenases.

Moreover in antimicrobial evaluation it was seen that purpurin and alizarin had significant anti-MRSA activity against clinical MRSA isolates. It can be suggested that some anhtroquinone molecules could be modified for battling with pathogen microorgansims.

It is the fact that, anthraquinones have wide pharmacological properties because of their chemical structure and they have great importance for their medicinal application such as anticancer drugs [12]. Studies regarding with safer use of anthraquinones in pharmacology and medicine should be done and developed.

4. CONCLUSION

In this study, the results manifested that none of the chemicals were mutagenic for TA98 and TA100 strains both with and without metabolic activation enzymes (S9 mix). Purpurin and alizarin exhibited strong antimutagenic effects against 4-nitrophenylendiameine and 2-aminoflourene at all test doses (1000, 500 and 250 µg/plate) for TA98; and against sodium azide and 2-aminoanthracene for TA100. Alizarin showed the highest inhibition rate (93%) against sodium azide at a concentration of 1000 µg/plate. While 1,8-dihydroxy anthraquinone and quinizarin revealed strong antimutagenicity at 10000 µg/plate in the absence of S9 mix, they exhibited excellent antimutagenic action after addition of S9 enzymes for TA98 strain at all test doses. Similarly, 1,8-dihydroxy anthraquinone and quinizarin were moderate antimutagenic against sodium azide at all test doses in the absence of S9 mix, their antimutagenicity increased and they ameliorated the mutagenic action of 2-aminoanthracene by the addition of S9 for TA100. These two chemicals were strong antimutagenic against promutagens activated by S9 mix. Also it was defined that purpurin and alizarin have antimicrobial capacity against MRSA strains.

5. MATERIAL AND METHODS

5.1. Chemicals

Alizarin, quinizarin, purpurin, and 1,8-dihydroxy anthraquinone, D-G-6-P, β-NADP, D-biotin were commercially obtained from Sigma-Aldrich. Sodium azide, 2-aminoflourene, dimethyl sulfoxide (DMSO), L-histidine-HCl monohydrate were obtained from Merck (Darmstad, Germany). Nutrient broth was purchased from Oxoid, S9 rat liver enzymes were obtained from Moltox (Molecular Toxicology Incorporated, USA).

5.2. Bacterial strains

Strains of *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 15442, *Klebsiella pneumoniae* ATCC 70063, *Staphylococcus aureus* (MRSA) ATCC 43300, *Salmonella enteritidis* ATCC 13076, *Streptococcus pneumoniae* ATCC 10015, *Sarcina lutea* ATCC 9341, *Candida albicans* were used for the determination of antimicrobial activities. For determining of growth inhibition of MRSA strains by AQs, methicillin resistant *Staphylococcus aureus* strains were used. Standard bacteria and yeast were obtained from the microorganism culture collection of Microbiology Laboratory of Vocational School of Health Services (Selcuk University).

5.3. Determination of toxic dose levels

Cytotoxic doses of AQs were defined according to Dean *et al*. [43]. Concentrations of 10000, 5000, and 2500 µg/plate of the purpurin and alizarin were determined as toxic, while 1,8-dihydroxy anthraquinone and quinizarin were not toxic at the same doses. Nontoxic doses of the chemicals were used in the assays (1000, 500 and 250 µg/plate for purpurin and alizarin; 10000, 5000, and 2500 µg/plate doses for 1,8-dihydroxy anthraquinone and quinizarin).

5.4. Mutagenicitiy Assay by Ames test

In this experiment, mutagenic activity was evaluated by the *Salmonella*/microsome assay described by Maron and Ames [44]. Two His^- mutant strains of *Salmonella typhimurium* TA98 and *S. typhimurium* TA100 were obtained from The Research Laboratory of Microbiology, Science Faculty, Selcuk University. At the beginning of the assays, standard mutations of the *S. typhimurium* strains were tested and revertant colony numbers were calculated [45]. The modified plate incorporation method was performed with and without S9 mix [46]. 5 µg/plate 4-NPDA for TA98 and 5 µg/plate SA for TA100 were used without of S9 mix; 7.5 µg/plate 2-aminoflourene for TA98 and 5 µg/plate 2-aminoantharecene for TA100 were employed with S9 mix as positive control chemicals. DMSO were used for negative control.

The chemicals determined as nontoxic and no mutagenic were subject to antimutagenicity experiment using the Ames test [46]. The formula presenting below was used to evaluate the inhibition rates of mutagenicity:

$$[(A-B)/(A-C)]\times 100 \qquad \text{(Eq. 1)}$$

where A = No. of his. revertants in the absence of sample, B = No. of his. revertants in the presence of sample, C = spontaneous revertants [46].

The antimutagenicity was evaluated as 'strong' > 40%, 'moderate' when the rates were ranging between 25–40% and 'weak' when the ratios of inhibition less than 25% [47].

5.5. Broth microdilution test

The broth microdilution method was used for the definition of the lowest concentration of the chemicals that inhibits the macroscopic growth of microorganisms (MIC) [46]. The turbidity of bacterial cultures were adjusted to 10^8 CFU/ml (0.5 McFarland). The inoculums used in the test were adjusted to 10^5 CFU/ml. 100 µl of Mueller-Hinton Broth was distributed to each well of micro plates. A 100 µl from the AQs was added into the first wells. Then, 100 µl from first wells was transferred to 7 consecutive wells for dilution and then, the chemical-broth medium in microplate was inoculated with equal amount of each bacterium (100 µl) and was incubated at 37°C for 24 h. Solutions of the tested chemicals were prepared at concentrations of (5-0.0024) mg/ml. Gentamicin was used as control antibiotic. For the assignation of microbial growth, 20 µl of 2,3,5-Triphenyl-tetrazolium chloride (0.5%) was added to each well and incubated for 30 minute again at same temperature.

Author contributions: Concept – A.U., E.G.; Design – A.U., E.G.; Supervision – Y.D.; Materials – A.U., E.G.; Data Collection and/or Processing – A.U., E.G.; Analysis and/or Interpretation – A.U., E.G., Y.D.; Literature Search – A.U., E.G., Y.D.; Writing – A.U.; Critical Reviews – A.U., E.G., Y.D.

Conflict of interest statement: The authors declared no conflict of interest in the manuscript.

REFERENCES

[1] Fiehn O. Metabolomics - the link between genotypes and phenotypes. Plant Mol Biol. 2002;48(1-2):155-171. [CrossRef]

[2] Hamilton AC. Medicinal plants, conservation and livelihoods. Biodivers Conserv. 2004;13(8):1477-1517. [CrossRef]

[3] Hattenschwiler S, Vitousek PM. The role of polyphenols in terrestrial ecosystem nutrient cycling. Trends Ecol Evol. 2000;15(6):238-243. [CrossRef]

[4] Kutchan TM. Ecological arsenal and developmental dispatcher. The paradigm of secondary metabolism. Plant Physiol. 2001;125(1):58-60. [CrossRef]

[5] Lattanzio V, Kroon PA, Quideau S, Treutter D. Plant phenolics - Secondary metabolites with diverse functions. Rec Adv Polyphen Res. 2008;1:1-35.

[6] Lattanzio V. Phenolic Compounds: Introduction. In: Ramawat K, Mérillon JM. (Eds). Natural Products. Springer, Berlin, Heidelberg 2013, pp. 1543-1580.

[7] Carocho M, Ferreira ICFR. A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. Food Chem Toxicol. 2013;51:15-25. [CrossRef]

[8] Birosova L, Mikulasova M, Vaverkova S. Antimutagenic effect of phenolic acids. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2005;149(2):489-491.

[9] Orhan I, Kartal M, Tosun F, Sener B. Screening of various phenolic acids and flavonoid derivatives for their anticholinesterase potential. Z Naturforsch C. 2007;62(11-12):829-832.

[10] Zengin G, Locatelli M, Ceylan R, Aktumsek A. Anthraquinone profile, antioxidant and enzyme inhibitory effect of root extracts of eight Asphodeline taxa from Turkey: can Asphodeline roots be considered as a new source of natural compounds? J Enzyme Inhib Med Chem. 2016;31(5):754-759. [CrossRef]

[11] Monks TJ, Hanzlik RP, Cohen GM, Ross D, Graham DG. Quinone chemistry and toxicity. Toxicol Appl Pharm. 1992;112(1):2-16. [CrossRef]

[12] Malik EM, Muller CE. Anthraquinones as pharmacological tools and drugs. Med Res Rev. 2016;36(4):705-748. [CrossRef]

[13] Van Gorkom BAP, De Vries EGE, Karrenbeld A, Kleibeuker JH. Review article: anthranoid laxatives and their potential carcinogenic effects. Aliment Pharm Therap. 1999;13(4):443-452.

[14] Shrestha JP, Subedi YP, Chen LH, Chang CWT. A mode of action study of cationic anthraquinone analogs: a new class of highly potent anticancer agents. MedChemComm. 2015;6(11):2012-2022. [CrossRef]

[15] Khan K, Karodi R, Siddiqui A, Thube S, Rub R. Development of anti-acne gel formulation of anthraquinones rich fraction from *Rubia cordifolia* (Rubiaceae). Int J Appl Res in Nat Prod. 2012;4(4):28-36.

[16] Davis RH, Agnew PS, Shapiro E. Antiarthritic activity of anthraquinones found in Aloe for podiatric medicine. J Am Podiat Med Assn. 1986;76(2):61-66. [CrossRef]

[17] Wuthiudomlert M, Kupittayanant P, Gritsanapan W. *In vitro* evaluation of antifungal activity of anthraquinone derivatives of *Senna alata*. J Health Res. 2010;24(3):117-122.

[18] Fosso MY, Chan KY, Gregory R, Chang CWT. Library synthesis and antibacterial investigation of cationic anthraquinone analogs. Acs Comb Sci. 2012;14(3): 231-235. [CrossRef]

[19] Barnard DL, Fairbairn DW, ONeill KL, Gage TL, Sidwell RW. Anti-human cytomegalovirus activity and toxicity of sulfonated anthraquinones and anthraquinone derivatives. Antivir Res. 1995;28(4): 317-329. [CrossRef]

[20] Seo EJ, Ngoc TM, Lee SM, Kim YS, Jung YS. Chrysophanol-8-O-glucoside, an anthraquinone derivative in Rhubarb, has antiplatelet and anticoagulant activities. J Pharmacol Sci. 2012;118(2): 245-254. [CrossRef]

[21] Jackson TC, Verrier JD, Kochanek PM. Anthraquinone-2-sulfonic acid (AQ2S) is a novel neurotherapeutic agent. Cell Death Dis. 2013;4: 1-12. [CrossRef]

[22] Winter RW, Cornell KA, Johnson LL, Ignatushchenko M, Hinrichs DJ, Riscoe MR. Potentiation of the antimalarial agent rufigallol. Antimicrob Agents Chemother. 1996;40(6):1408-1411.

[23] Chesis PL, Levin DE, Smith MT, Ernster L, Ames BN. Mutagenicity of quinones - Pathways of metabolic-activation and detoxification. P Natl Acad Sci-Biol. 1984;81(6): 1696-1700. [CrossRef]

[24] Sendelbach LE. A review of the toxicity and carcinogenicity of anthraquinone derivatives. Toxicology. 1989;57(3): 227-240. [CrossRef]

[25] Brown JP. A review of the genetic effects of naturally occurring flavonoids, anthraquinones and related compounds. Mutat Res. 1980;75(3):243-277.

[26] Muller SO, Eckert I, Lutz WK, Stopper H. Genotoxicity of the laxative drug components emodin, aloe-emodin and danthron in mammalian cells: Topoisomerase II mediated? Mutat Res. 1996;371(3-4):165-173.

[27] Mueller SO, Stopper H, Dekant W. Biotransformation of the anthraquinones emodin and chrysophanol by cytochrome P450 enzymes - Bioactivation to genotoxic metabolites. Drug Metab Dispos. 1998;26(6):540-546.

[28] Venturini S, Tamaro M. Mutagenicity of anthraquinone and azo dyes in Ames' Salmonella typhimurium test. Mutat Res. 1979;68(4):307-312.

[29] Leme DM, de Oliveira GAR, Meireles G, dos Santos TC, Zanoni MVB, de Oliveira DP. Genotoxicological assessment of two reactive dyes extracted from cotton fibres using artificial sweat. Toxicol in Vitro. 2014;28(1): 31-38. [CrossRef]

[30] Brown JP, Brown RJ. Mutagenesis by 9,10-anthraquinone derivatives and related compounds in *Salmonella typhimurium*. Mutat Res. 1976;40(3):203-224.

[31] Laham S, Grice H, Sinclair J. Studies in chemical carcinogenesis, III. Alpha-aminoanthraquinone. Toxicol Appl Pharmacol. 1966.

[32] Hsu S-C, Chung J-G. Anticancer potential of emodin. Biomedicine. 2012;2(3):108-116.

[33] Jelassi B, Anchelin M, Chamouton J, Cayuela ML, Clarysse L, Li J, Goré J, Jiang LH, Roger S. Anthraquinone emodin inhibits human cancer cell invasiveness by antagonizing P2X7 receptors. Carcinogenesis. 2013;34(7): 1487-1496. [CrossRef]

[34] Lin SZ, Wei WT, Chen H, Chen KJ, Tong HF, Wang ZH, Ni ZL, Liu HB, Guo HC, Liu DL. Antitumor activity of emodin against pancreatic cancer depends on its dual role: Promotion of apoptosis and suppression of angiogenesis. Plos One. 2012; 7(8). [CrossRef]

[35] Chiu TH, Lai WW, Hsia TC, Yang JS, Lai TY, Wu PP, Ma CY, Ho CC, Lu HF, Wood WG, Chung JG. Aloe-emodin induces cell death through s-phase arrest and caspase-dependent pathways in human tongue squamous cancer SCC-4 cells. Anticancer Res. 2009;29(11):4503-4511.

[36] Guo JM, Xiao BX, Liu Q, Zhang S, Liu DH, Gong ZH. Anticancer effect of aloe-emodin on cervical cancer cells involves G2/M arrest and induction of differentiation. Acta Pharmacol Sin. 2007;28(12): 1991-1995. [CrossRef]

[37] Kuo PL, Lin TC, Lin CC. The antiproliferative activity of aloe-emodin is through p53-dependent and p21-dependent apoptotic pathway in human hepatoma cell lines. Life Sci. 2002;71(16): 1879-1892. [CrossRef]

[38] Pecere T, Gazzola MV, Mucignat C, Parolin C, Dalla Vecchia F, Cavaggioni A, Basso G, Diaspro A, Salvato B, Carli M, Palù G. Aloe-emodin is a new type of anticancer agent with selective activity against neuroectodermal tumors. Cancer Res. 2000;60(11): 57-77. [CrossRef]

[39] Edenharder Rv, Von Petersdorff I, Rauscher R. Antimutagenic effects of flavoniods, chalcones and structurally related compounds on the activity of 2-amino-3-methylinidazo [4, 5-*f*] quinoline (IQ) and other heterocyclic amine mutagens from cooked food. Mutat Res Fund Mol Mech Mut. 1993;287(2): 261-274.

[40] Takahashi E, Fujita K, Kamataki T, Arimoto-Kobayashi S, Okamoto K, Negishi T. Inhibition of human cytochrome P450 1B1, 1A1 and 1A2 by antigenotoxic compounds, purpurin and alizarin. Mutat Res. 2002;508(1-2): 147-156.

[41] Charehsaz M, Sipahi H, Giri AK, Aydin A. Antimutagenic and anticlastogenic effects of Turkish Black Tea on TA98 and TA100 strains of *Salmonella typhimurium* (*in vitro*) and mice (*in vivo*). Pharm Biol. 2017;55(1):1202-1206. [CrossRef]

[42] Mocan A, Zengin G, Mollica A, Uysal A, Gunes E, Crisan G, Aktumsek A. Biological effects and chemical characterization of *Iris schachtii* Markgr. extracts: A new source of bioactive constituents. Food Chem Toxicol. 2018;112:448-457. [CrossRef]

[43] Dean BJ, Brooks TM, Hodsonwalker G, Hutson DH. Genetic toxicology testing of 41 industrial-chemicals. Mutat Res. 1985;153(1-2):57-77.

[44] Maron DM, Ames BN. Revised methods for the Salmonella mutagenicity test. Mutat Res. 1983;113(3-4):173-215.

[45] Mortelmans K, Zeiger E. The Ames Salmonella/microsome mutagenicity assay. Mutat Res-Fund Mol Mech Mut. 2000;455(1-2):29-60.

[46] Zengin G, Uysal A, Gunes E, Aktumsek A. Survey of phytochemical composition and biological effects of three extracts from a wild plant (*Cotoneaster nummularia* Fisch et Mey.): A potential source for functional food ingredients and drug formulations. Plos One. 2014;9(11). [CrossRef]

[47] Negi PS, Jayaprakasha GK, Jena BS. Antioxidant and antimutagenic activities of pomegranate peel extracts. Food Chem. 2003;80(3):393-397.