Photoprotective and antiphotomutagenic effect of Baccharis dracunculifolia optimized extract in the alternative Saccharomyces cerevisiae model

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Research Article

Keywords: Baccharis dracunculifolia, antiphotomutagenic, photoprotective, phenolics, green sunscreens, Saccharomyces cerevisiae

Posted Date: March 5th, 2024

DOI: https://doi.org/10.21203/rs.3.rs-3967453/v1

Additional Declarations: No competing interests reported.

Abstract

The recent discovery of hazardous side effects associated with sunscreen ingredients has prompted the search for natural alternatives that can effectively absorb UV rays. This study focuses on evaluating the chemical profile and potential photoprotective and antiphotomutagenic effects of an ethanolic extract from Baccharis dracunculifolia using the Saccharomyces cerevisiae model. An analysis conducted using ESI-QToF-LC-MS confirmed the presence of flavonoids and phenylpropanoids in the B. dracunculifolia extract, including compounds such as apigenin, hydroxycinnamic acid derivatives, caffeoylquinic acids, and chlorogenic acid. In the yeast model, the extract demonstrated the ability to protect S. cerevisiae yno1 strain cells against cytotoxic lesions induced by high Solar Simulated Light (SSL) irradiation exposure. Furthermore, the extract exhibited antioxidant properties, as evidenced by its protection against SSL-induced oxidative damage in the yno1 strain, which is an indicator of oxidative damage. Additionally, the extract showed potential as an antiphotomutagenic agent, as the yno1-treated strain displayed a lower frequency of photomutagenicity in terms of CanR/107 mutant cells, thereby reducing both lethal cytotoxic lesions induced by SSL and protecting the cells against SSL-induced oxidative mutagenic lesions. These findings suggest that the B. dracunculifolia extract holds promise as a natural and effective photoprotective ingredient, offering a green alternative to traditional sunscreens by reducing the need for harmful chemical filters.

Introduction

Given the mounting concern over the association between organic filters and coral bleaching, alongside the potential for endocrine changes demonstrated through the absorption of photoprotective formulations (Matouskova and Vandenberg, 2022; Ko et al. 2021), plant extracts rich in phenolic substances have been studied as a green alternative (Addor et al. 2021; Da Silva et al. 2019). In this way, it is possible to reduce the amount of organic filters and minimize the possibility of stimulating allergenic or irritating pathways, and reduce the potential for damage to marine ecosystems, thereby ensuring greater safety for their use (He et al. 2020; Velasco et al. 2008; Da Silva et al. 2022a).

Despite being adequate and predictive, the assessment of the photoprotective potential *in vitro* has some detection limitations (Bendová et al. 2007; Dimitrovska et al. 2017; Hedayat et al. 2020). Thus, new evaluation methods have been studied to predict the value of the sun protection factor. In this context, strains of *Saccharomyces cerevisiae* have shown to be a promising *in vitro* biomodel for application in photobiological studies (Paiva et al. 2020). In spite of being a lower eukaryote, *S. cerevisiae* genome shares various human ortholog DNA repair genes, such as *ogg1* and *yno1* genes, which are involved in UV-induced oxidative damage repair by an enzymatic mechanism or by a repair signaling role, respectively (Rinnerthaler et al. 2012; De Padula et al. 2004). Hence, deficient strains in these genes are promising bioindicator models for efficacy and photosafety assessment of UV filters and antioxidant actives (Paiva et al. 2020).

In fact, the inactivation of *ogg1* repair made the CD138 strain (*ogg1* mutant) a good bioindicator of photomutagenic and antiphotomutagenic potential of chemicals (De Padula et al. 2004). The OGG1 enzyme acts in the Base Excision Repair (BER) mechanism, repairing oxidized DNA and removing 8-hydroxydeoxyguanosine (8-OHdG) lesions (Schuch et al. 2017). The CD138 (*ogg1*) strain had shown an interesting potential for photoprotective and photo/antiphotomutagenic assessment of UV filters (Pinto et al. 2010), novel nanocomposite (Paiva et al. 2014) sunscreen formulation excipients (Hossy et al. 2017), and natural antioxidant actives (Da Silva et al. 2019). Although the CD138 (*ogg1*) strain had shown as good a photobiological model in previous studies (Diniz et al. 2019). *Diniz et al.* (2019) had highlighted a more promising yeast strain at solar simulated light irradiations. The AWP001 (*yno1*) strain is deficient in Yno1 protein, which is a yeast NADPH oxidase protein that is ortholog to the NADPH oxidase (NOX) gene involved in UVB induced stress repair signaling (Rinnerthaler et al. 2012; Glady et al. 2018). In this sense, AWP001 (*yno1*) strain displays a more suitable yeast model for *in vitro* photoprotective and photo/antiphotomutagenicity assessment using irradiation protocols with UV doses and exposure times comparable to environmental sunlight (Diniz et al. 2019).

Baccharis dracunculifolia (Asteraceae), also known as vassourinha or alecrim-do-campo, is a species native to Brazil, popularly used as tea for the treatment of gastrointestinal diseases (Iurckevicz et al. 2021). More recently our research group has evaluated the photoprotective and antioxidant potential *in vitro* and *in silico* for formulations of cutaneous application. This study indicated that the optimized extract obtained from *B. dracunculifolia* demonstrates a sun protection factor and antioxidant activities *in vitro*. These properties were also studied in *in silico* approach and showed the antioxidant and photoprotective mechanism of action of the compounds described for the species *B. dracunculifolia*. The set of results suggest that the different compounds of the extract have synergistic and global action, allowing greater protection and absorption in the solar spectrum (Vilas-Boas et al. 2023). In this scenario, medicinal plants and plant-derived products present therapeutic benefits as a source of ingredients to prepare alternative sunscreens, since they can act on different biological targets and have a lower incidence of side effects.

In this regard, this study aims to evaluate the photoprotective and antiphotomutagenic potential in a yeast model, using AWP001 (*yno1*) mutant of the optimized *B. dracunculifolia* extract to be used as a source of ingredients to prepare alternative sunscreens. In addition, chemical characterization of the plant extract by ESI-QToF-LC-MS was done to confirm the presence of the bioactive phenolics.

Materials and Methods

Collection and chemical characterization of B. dracunculifolia extract

The collection of leaves and branches of *B. dracunculifolia* was donein June 2019, at the Ecological Station of the Federal University of Minas Gerais (Belo Horizonte, Minas Gerais). The leaves were dried in an oven (≤ 40 ºC) and ground in a knife mill. The powder of the leaves (1g) was extracted with 20 mL of

pure ethanol as solvent, under reflux at 60°C, for 20 min (3-fold repetition). The solvent was removed under low pressure and the resulting extract was protected from light and kept in a desiccator until use.

The evaluation of chemical composition of *B. dracunculifolia* extract was carried out by liquid chromatography coupled to electrospray time-of-flight mass spectrometry. The experiment was done at the Analytical Center of the Institute of Chemistry of the University of São Paulo on a Shimadzu CBM-20A chromatograph (Tokyo, Japan) coupled with a Shimadzu SPD-20A ultraviolet detector and MaXis 3G Bruker Daltonics equipment with electrospray ionization (ESI) (Massachusetts, United States). The Supelco Ascentis C18 column (5 µm, 250 x 4 mm, internal diameter) was used in the experiments and the selected UV wavelengths were 270 and 350 nm in a flow rate of 1 mL/min and oven temperature of 30°C. ESI mass spectra, precursor as well as product ions, were acquired in negative ion mode and recorded between m/z 100–1000. The mass spectrometer parameters were maintained constant for all analyses: drying gas flow, 8.0 L/ min; drying gas temperature, 250°C; nebulizer gas pressure, 2 bar; and collision energy between 25 and 75 eV. The analyses were performed using the following elution gradient system: water (A) and acetonitrile (B), both acidified with 0.1% phosphoric acid; elution profile: 0.00–36.00 min (5–95% B, linear gradient), 37.00–39.00 min (95% B, isocratic), and 40.00–45.00 (95 – 5% B, return to initial conditions). The dataset was acquired, processed, and analyzed using the Bruker Compass Data Analysis software (version 4.3). The MS and MS/MS data were tabulated and compared with published data (Kumazawa et al. 2003; Tomazzoli et al. 2021) and databases such as MASST of the Global Natural Products Social Molecular Networking (Wang et al. 2016; Wang et al. 2020) MoNA (Horai et al. 2010), ReSpect for phytochemicals (Sawada et al. 2012), and PubChem (Kim et al. 2021).

S. cerevisiae strain, culture medium and growth conditions

Yeast Strain, Media and Growth Conditions

The AWP001 mutant *(yno1: KanMX6*) strain was used in this study (Diniz et al. 2019). Yeast cultures were grown at 28°C in Yeast Peptone Dextrose (YPD) liquid medium (1% yeast extract, 1% bacto peptone, and 2% glucose–BactoTM, Merck). YPD solid medium (supplemented with 2% agar) was used for growing survival colonies which was an indicator of photoprotection after treatments. The photo/antiphotomutagenitic assessment was based on mutants resistance to canavanine (CanR mutants) as described by Diniz *et al.* (2019) using supplemented Yeast Nitrogen Based Dextrose (YNBD) solid medium (2% glucose, 0.7% yeast nitrogen base without amino acids, 2% agar, and opposite amino acids and bases– Difco TM, Merck) containing canavanine (Sigma–Aldrich) (60 mg/l) (Da Silva et al. 2019; Pinto et al. 2010; Paiva et al. 2014; Hossy et al. 2017; Diniz et al. 2019).

Solar Simulated Light (SSL)

The SSL irradiation protocol was performed as described by Diniz et al. (2019) using a solar simulator (Oriel Model 91192–1000, Newport Corp., USA) emitting 21.7 J/m^2/s UVA and 1.58 J/m^2/s UVB [4,18]. Atmospheric attenuators AMO and 87,066 were used in order to mimic the environmental UVB/UVA ratio (1/16) (Cardoso-Rurr *et al.* 2018). The dosimetry at 1000 W/m^2 was measured using a VLX-3-W

dosimeter (Vilber Lourmat, Marne-la-Vallée, France) with appropriate photocells for UVA (CX-365) and for UVB (CX-312) [4,18]. The dose rates adopted for UVA and UVB irradiation upon SSL were 20 J/m^2/s and 1.2 J/m^2/s, respectively, as previously defined (Bendová et al. 2007). The SSL irradiation was conducted using different irradiation times and doses as described by Da Silva et al. (2019) and Diniz et al. (2019).

In vitro photoprotective efficacy and antiphotomutagenic effect of B. dracunculifolia extract in AWP001 (yno1) strain

The yeast assay was performed using the methodology defined by Diniz et al. (2019). For that, yeast cultures (10 ml) of AWP001 (*yno1*) *S. cerevisiae* strain were grown to a cell density of ~1×10^8 cells/ml at 28 ºC with agitation for 48 h (stationary phase). After this pre-cultivation, cells were harvested by centrifugation, washed twice, and resuspended in bidistilled and deionized sterile water. Cell suspension was adjusted to 1×10^7 cells/ml and transferred to 5.0 cm diameter glass Petri plates with a final volume of 12 ml. Yeast suspension was exposed to increasing SSL doses under agitation in the presence or absence of 100 µg/ml of *B. dracunculifolia* extract, which was established as the maximal nontoxic concentrations of antioxidant samples when yeast cells were incubated under agitation in the dark (data not shown).

After each dose and irradiation time, aliquots were taken, properly diluted in sterile water, plated on YPD medium, and incubated at 28°C for 2 days for photoprotective efficacy assessment. Surviving colonies were counted and plotted on the survival graph. All survival experiments were independently performed at least four times and are expressed as averages with standard errors (Da Silva et al. 2019; Diniz et al. 2019).

After cell treatments, quantification of CanR mutants was performed by plating dilutions of treated cells on selective medium for antiphotomutagenicity assessment. Colonies were counted after 4 days at 28°C. Frequency of induced CanR mutants was calculated in terms of cell survivors and standardized to 10^7 cells. All mutation frequencies were determined and expressed as averages with respective standard errors. For comparison with control, SSL-induced mutagenesis was standardized at 37% lethal dose (LD_{37}) in order to ensure equivalent lethal hits per cell as in our previous studies with *S. cerevisiae* strains (Da Silva et al. 2019; Diniz et al. 2019).

Statistical analysis

For yeast experiments, statistical analyses were performed using GraphPad Prism (version 6.00) (GraphPad Software, La Jolla California USA). The non-parametric Mann-Whitney test was conducted to demonstrate statistical differences ($p < 0.05$).

Results and Discussion

Chemical composition of B. dracunculifolia extract

The chemical composition of the extract of *B. dracunculifolia* was analyzed by ESI-QToF-LC-MS (**Fig. 1**). The identification of substances was carried out using GNPS, MoNA, PubChem, ReSpect databases, and literature data (Kumazawa et al. 2003; Tomazzoli et al. 2021). Dereplicated compounds were listed in **Table 1**, as well as the following data: Retention time RT (min), molecular formula, *m/z* $[M-H]^-$, and major fragments *m/z* (relative abundance).

Figure 1

The analysis of the UV spectra, together with MS and MS/MS spectra, allowed the identification of 18 compounds. Among them, four caffeoylquinic acids, derived from chlorogenic acid, with deprotonated ions $[M-H]^-$ *m/z* 515.1181, *m/z* 515.1199 (two isomers derived from di-caffeoylquinic acid), *m/z* 529.134 (caffeoylferuloylquinic acid, **A)**, and *m/z* 677.1500 (3,4,5-tricapheoylquinic acid, **B**). The differentiation of di-caffeoylquinic acids using mass spectrometry can be found in the works of Clifford *et al.* (2003) and De Maria and Moreira (2004) where it is possible to carry out the assignment of molecules through the presence and abundance of available fragments after MS3 and MS4 sequential fragmentation experiments. The main fragments of these isomers were identified in our study; however, even though the ESI technique is efficient in identifying isomers, it was not possible to assign them to di-caffeoylquinic acids (3,4-; 3,5-; 4,5-). Some works that describe the chemistry of *B. dracunculifolia* show the 3,4- and 4,5-di-caffeoylquinic isomers, which may suggest its presence in the analyzed extract (Kumazawa et al. 2003; Tomazzoli et al. 2021).

The presence of prenylated derivatives of hydroxycinnamic acid (p-coumaric acid) is widely reported for the species, as well as for green propolis obtained from *B. dracunculifolia* (Kumazawa et al. 2003; Tomazzoli et al. 2021; Machado et al. 2016; Chang et al. 2008; De Sousa et al. 2009). Among these derivatives, it was possible to identify 3,4-dihydroxy-5-prenylcinnamic acid (*m/z* 247.0978, **C**), 3,5-diprenyl-4-hydroxycinnamic acid (*m/z* 299.1656; artepilin C, **M**), 3-prenyl-4-hydroxycinnamic acid (*m/z* 231.034; drupanin, **D**), and 3-prenyl-4-dihydrocinnamoyloxycinnamic acid (*m/z* 363.1601; bacarin, **E**). In addition, it was possible to identify some peaks with the UV spectrum (UV_{max} 312 nm) of prenylated derivatives. The UV spectrum profile corresponding to cumaric acid (UV_{max} 309 nm) was verified; however, a corresponding molecular ion was not observed. This leads us to suggest the presence of polar p-coumaric acid derivatives in the optimized extract sample.

Flavonoids were detected and possible tri-substituted flavones (UV_{max} 330 nm), kaempferol derivatives (UV_{max} 365 nm), apigenin (UV_{max} 336 nm), and naringenin (UV_{max} 288 nm) were identified. It was possible to identify nine molecular ions $[M-H]^-$ of the flavononols dihydrokaempferol (*m/z* 287.0561, **F**) and dihydrokaempferide or AME-aromadendrin-4'-methyl ether (*m/z* 301.0723, **G**); the flavonols kaempferide (*m/z* 299.0575, **O**) and velutin (*m/z* 313.0719, **H**); the flavones apigenin (*m/z* 269.0453, **N**); diosmetin (*m/z* 299.0566, **I**); 4',5,7-trihydroxy-3,6-dimethoxyflavone (*m/z* 329.0669, **J**); and the flavanones naringenin (*m/z* 271.0618, **K**) and isosakuranetin (*m/z* 285.0771, **L**). In addition to these flavonoids, the presence of other derivatives of flavones and flavonols were achieved, but the complete assigns were not possible to confirm. Many of these flavonoids have already been reported by Kumazawa *et al.* (2003) and

Tomazzoli *et al.* (2021) and, in addition, other authors have already reported these same constituents in green propolis, which also serves as a guide when assigning the identity of *B. dracunculifolia* compounds (Tomazzoli et al. 2021; Machado et al. 2016; Xu et al. 2020). The **Fig. 2** shows the main chemical compounds identified in *B. dracunculifolia* extract by ESI-QToF-LC-MS.

Table 1

Figure 2

S. cerevisiae photoprotective efficacy and antiphotomutagenic effect of B. dracunculifolia extract in AWP001 (yno1) strain against SSL irradiation-induced cytotoxicity and genotoxicity

Photoprotection potential of *B. dracunculifolia* extract against SSL irradiation-induced cytotoxicity was evaluated upon *S. cerevisiae* AWP001 (*yno1*) strain treated in comparison with untreated AWP001 (*yno1*) strain (control). Figure 3 displays cell survival of AWP001 (*yno1*) strain in the absence (control) and in the presence of the *B. dracunculifolia* optimized extract (100 µg/ml). This extract was able to protect the *S. cerevisiae* AWP001 (*yno1*) strain cells against induced cytotoxic lesions at the highest SSL irradiation exposure (1 h 44’ 9” and 2 h 18’ 52”), when compared to control (p 0.05) (**Fig. 3**). Therefore, it seems to be a promising photoprotective ingredient in case of long-term SSL exposure, where enzymatic DNA repair tends not to be able to deal with the amount of lethal induced lesions. Additionally, the use of an oxidative damage bioindicator strain, such as the AWP001 (*yno1*) strain, suggests that the *B. dracunculifolia* extract proved to be particularly promising due to its antioxidant property as described in literature (Vilas-Boas et al, 2023; De Sousa, 2011; França et al, 2022; Da Silva, 2022b).

Regarding the photomutagenic/antiphotomutagenic potential upon SSL irradiation of the *B. dracunculifolia* optimized extract, the *S. cerevisiae* AWP001 (*yno1*)-treated strain displayed a lower photomutagenicity frequency in terms of CanR/10^7 cells compared to control ($p < 0.05$) (**Fig. 4**). This result indicates that besides presenting photoprotective potential against SSL-induced lethal cytotoxic lesions, this extract also showed antiphotomutagenic potential by protecting cells against SSL-induced oxidative mutagenic lesions.

Figures 3 and 4

The combination of phenolic substances identified in the extract of *B. dracunculifolia* that has known absorption in the ultraviolet and visible spectrum (Vilas-Boas et al, 2023) could be related to the photoprotection provided by the extract. In the light of this study, it was also possible to verify that this particular combination of flavonoids, phenolic acids, and their derivatives protects cells even in conditions similar to those that occur in human skin, in which there is cumulative sun exposure, through redox therapeutic response which prevents the formation of DNA damage. Phenolic acids are related to the increase of antioxidant and anti-inflammatory mediators and the activation of pro-survival signals (Caruso et al, 2022). On the other hand, flavonoids modulate the cellular response by regulating the oxidative system, contributing to cellular homeostasis (Sarkar et al, 2022). Similar results were observed

for *B. dracunculifolia* extract in rat hepatoma cell line culture (HTC). The researchers concluded that the extract has the ability to reduce DNA damage and has no mutagenic or genotoxic effects (Roberto et al, 2016). Thus, the use of this extract as an active ingredient would be an alternative with a dual mechanism: prevention of oxidative damage and absorption of sun rays.

Conclusion

In conclusion, in this study the results allowed us to assign the presence of phenolic compounds and their derivatives such as apigenin, naringenin, caffeoylquinic acids, and cumaric acid derivatives in the *B. dracunculifolia* extract. The unique combination of these molecules may be involved in the observed photoprotective and antiphotomutagenic potential against SSL. In addition, the use of a strain with a potential bioindicator of photobiological events of an oxidative nature, such as the *S. cerevisiae* AWP001 (*yno1*) strain, also allowed us to corroborate the antioxidant and photoprotective properties of the tested optimizes plant extract, which proved to be particularly relevant in protecting against cytotoxic lesions and also induced-mutagenesis upon exposure to SSL radiation, indicating its promising use in multifunctional and eco-friendly photoprotective formulations.

Abbreviations

SSL -Solar Simulated Light, YPD- yeast peptone dextrose, CanR-canavanine-resistant mutants; UV-ultraviolet rays.

Declarations

Acknowledgments We thank D.Sc Marcelo de Padula and Raiane Diniz for providing the yeast strain and for the experiments with *Saccharomyces cerevisae*. This study was funded by grants and fellows from CAPES (Coordination for the Improvement of Higher Education Personnel), UFRJ (Universidade Federal do Rio de Janeiro), and UFMG (Universidade Federal de Minas Gerais), Brazil.

Authors' contribution Isis Tavares Vilas-Boas (M.Sc.) was responsible for phytochemical investigation experiment, formal analysis, writing original draft; Raiane Rosales Diniz (PhD) was responsible for *Saccharomyces* strain investigation experiment; Marina Scopel (PhD) and André Augusto Gomes Faraco (PhD) supervised the phytochemistry experiment; Marcelo de Pádula (PhD) supervised *Saccharomyces* strain investigation experiment and critical reading of the manuscript. Rachel Oliveira Castilho (PhD) conceptualization, methodology, writing - Review & Editing, supervision, project administration and funding acquisition. All the authors have contributed to the final manuscript writing and approved submission.

Funding CNPq (National Council for Scientific and Technological Development) 303757/2019-1 and 311875/2022-0, FAPEMIG (Fundação de Amparo à Pesquisa do Estado de Minas Gerais) APQ-00901-21 and RED-00099-21.

Declaration of interests The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Data is available on request. Please contact rocastilho40@gmail.com

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Table 1

Table 1 Dereplication of *Baccharis dracunculifolia* extract using ESI-QToF-LC-MS.

I.D.	RT (min)	Proposed nomenclature (Molecular Formula)	*m/z* $[M-H]^-$	Main fragments *m/z* (relative abundance)
1	12.1	N.I.	519.1718	399.1309(33); 339.1067(20); 309.0997(90); 279.0870 (100); 111.0442(14)
2	13.7	Di-caffeoylquinic derivative ($C_{25}H_{24}O_{12}$)	515.1181	353.0882(100); 335.0796(16); 191.0542(20); 179.0346(20); 173.0463(29); 135.0460(37)
3	13.7	N.I.	489.1602	369.1212(49); 309.0997(100); 279.0874(84)
4	14.2	Di-caffeoylquinic derivative ($C_{25}H_{24}O_{12}$)	515.1199	353.0887(100); 191.0556(26); 173,0345(15); 135.0459(9)
5	16.3	N.I.	379.1759	379.1759(100); 317.1745(84); 259.1683(45); 243.1789(52); 126.0314(66)
6	16.3	Dihydrokaempferol ($C_{15}H_{12}O_6$)	287.0561	259.0617(100); 125.0239(83)
7	16.3	Caffeoylferuloyl Quinic Acid ($C_{26}H_{26}O_{12}$)	529.1341	367.1032(90); 353.0952(100)
8	17.2	3,4,5-Tricaffeoylquinic acid ($C_{34}H_{30}O_{15}$)	677.1500	515.1195(100); 353.0866(15); 191.0599(17); 179.0345(27); 173.0453; 135.0433(23)
9	18.7	N.I.	361.1662	317.1758(100); 287.1642(11); 243.1762(58); 126.0324(13)
10	20.1	Apigenin ($C_{15}H_{10}O_5$)	269.0453	269.0453(100); 151.0039(75); 119.0502(56)
11	20.3	N.I.	361.1656	317.1760(100); 287.1653(75); 243.1767(70)
12	20.3	Diosmetin ($C_{16}H_{14}O_6$)	299.0566	284.0334(100); 117.0346(4)
13	20.3	Naringenin ($C_{15}H_{12}O_5$)	271.0618	151.0033(100); 119.0502(61)
14	20.7	Isoramnetin derivative	315,0511	300,0275; 272,0333; 165,9895
15	21.1	N.I.	345.1714	315.1625(21); 271.1729(100); 97.0288(22)
16	21.1	Dihydrokaempferide / AME ($C_{16}H_{14}O_6$)	301.0723	284.0309(19); 227.0703(4); 152.0121(9)
17	22.3	3,4-Dihydroxy-5-prenylcinnamic acid ($C_{14}H_{16}O_4$)	247.0978	203,1087(100); 148.0508(95)
18	22.8	N.I.	499.2547	455.2672(28); 409.2612(32); 379.2486

				(100)
19	23.2	Drupanin ($C_{14}H_{16}O_3$)	231.1034	187.1133(100); 176.0469(12); 132.0590(49)
20	23.2	Derivative of propenoic acid	315.1605	315.1605(100); 253.1606(82)
21	23.9	N.I.	347.1861	347.1861(100); 317.1755(80)
22	25.5	Flavone derivative	673.3368	389.1969(68); 343.3557(100); 285.0772(40); 164.0121(9); 117.0348(41)
23	25.5	Isosakuranetin ($C_{16}H_{14}O_5$)	285.0771	285.0771(100); 268.0381(28); 243.0667(6); 164.0112(15)
24	25.9	Velutin ($C_{17}H_{14}O_6$)	313.0719	298.0482(100); 283.0248(34); 117.0345(16)
25	25.9	Kaempferide ($C_{16}H_{12}O_6$)	299.0575	299.0575(100); 284.0335(66)
26	26.5	4',5,7-trihydroxy-3,6-dimethoxyflavone ($C_{17}H_{14}O_7$)	329.0669	314.0438(79); 2 71.0249(100)
27	26.5	Flavone derivative	299.0572	284.0323(100)
28	28.6	Tricine derivative	329.1764	299.1658(100); 255.1748(7)
29	28.6	Coumaric acid derivative	267.1033	163.0395(100); 145.0261(37); 119.0484(65); 117.0357(55)
30	30.2	Artepilin C ($C_{19}H_{24}O_3$)	299.1656	255.1761(100); 232.1108(3); 200.1210(13)
31	32.8	N.I.	641.3478	329.1760(100)
32	32.8	N.I.	447.2181	297.1503(42); 149.0611(100)
33	33.8	Baccarin ($C_{23}H_{24}O_4$)	363.1601	187.132(100); 149.0609 (38)
34	34.4	N.I.	517.3540	471.3482(100)
35	40.2	N.I.	555.4423	277.2181(100)
36	41.2	N.I.	911.7124	501.3588(7); 455.3538(100)

N.I. – Not identified

Figures

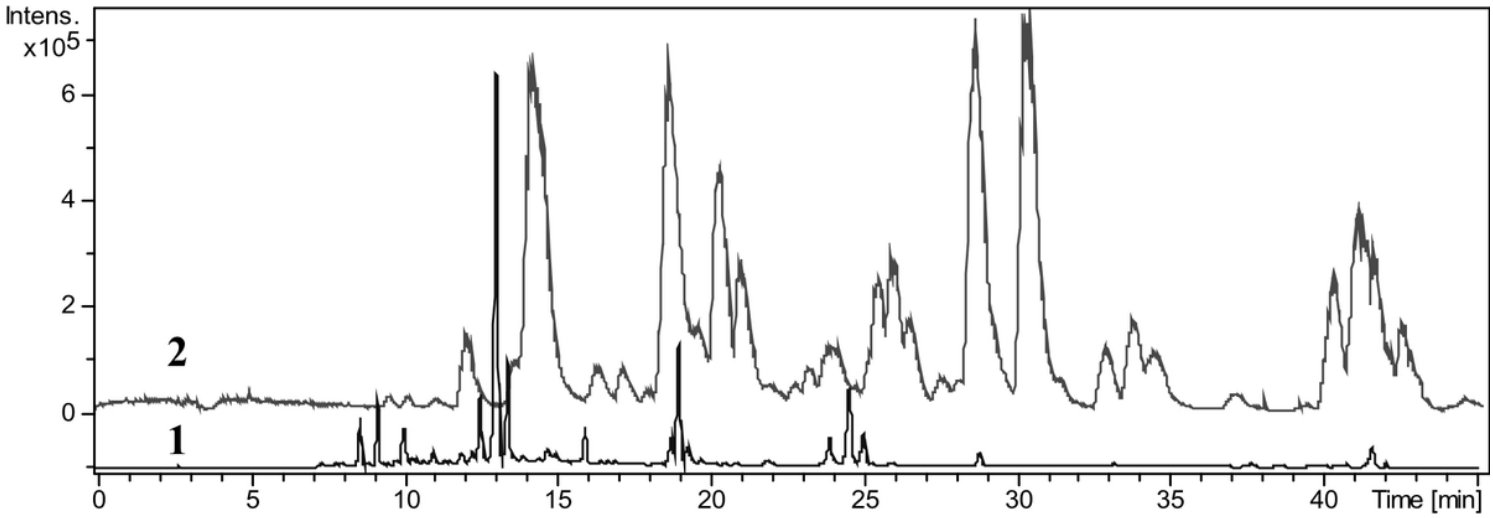

Figure 1

Chromatograms of the optimized extract of *Baccharis dracunculifolia* obtained in the ESI-QTOF-LC-MS/MS experiment. **1**: Chromatogram UV/vis in l350nm: black. **2**: Chromatogram of total ion (BPC – base peak chromatogram): gray. Mass spectra was acquired in negative ion mode and recorded between m/z 100-1000 and collision energy between 25 and 75 eV.

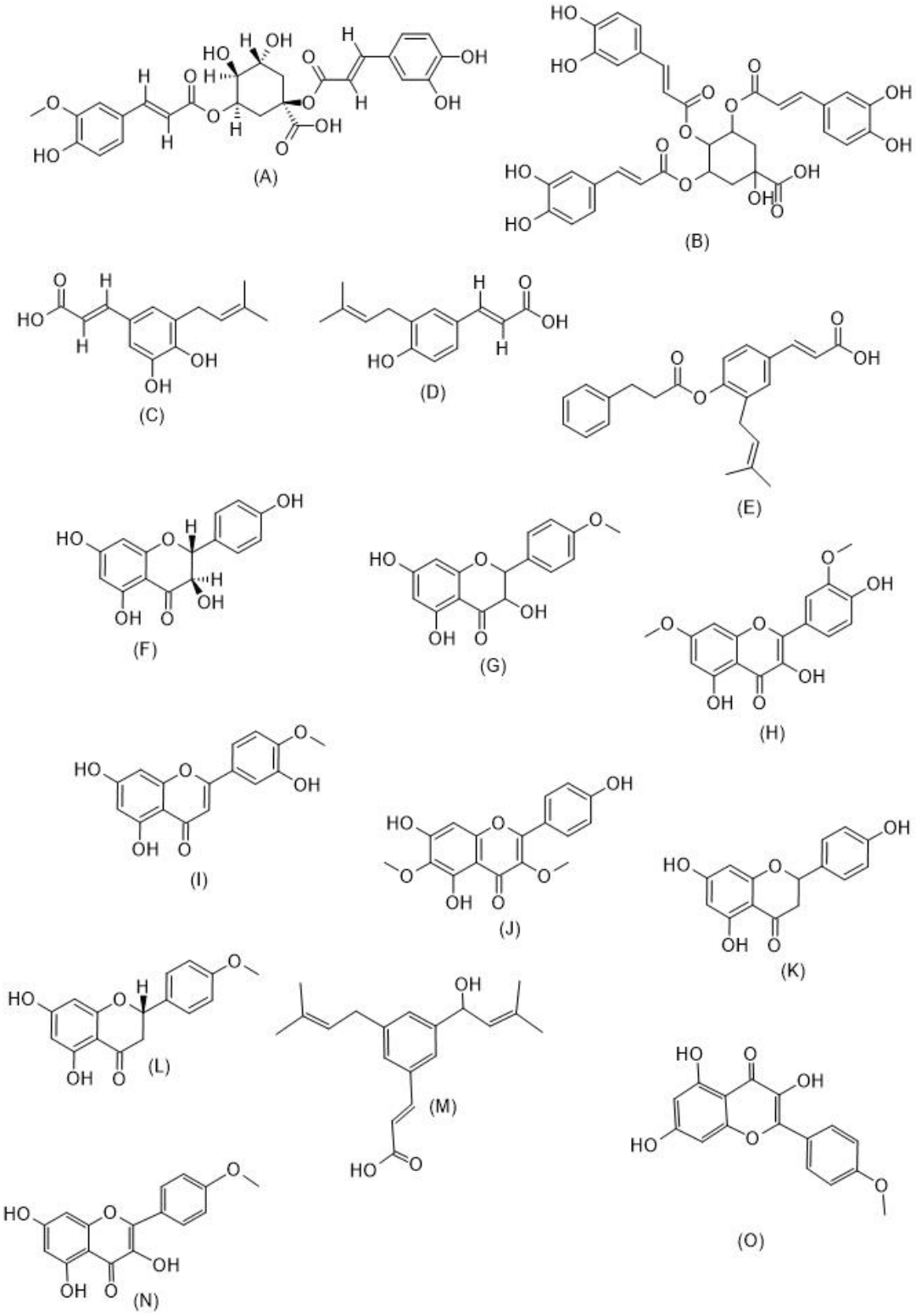

Figure 2

Chemical compounds identified in *B. dracunculifolia* extract by ESI-QToF-LC-MS. Caffeoylferuloylquinic acid, **A**, 3,4,5-tricapheoylquinic acid, **B**., 3,4-dihydroxy-5-prenylcinnamic acid, 3-prenyl-4-hydroxycinnamic acid (drupanin), **D**), and 3-prenyl-4-dihydrocinnamoyloxycinnamic acid (bacarin), **E**, dihydrokaempferol, **F**, dihydrokaempferide, **G**; velutin, **H,** diosmetin, **I,** 4',5,7-trihydroxy-3,6-dimethoxyflavone, **J**, naringenin, **K,**isosakuranetin, **L**, 3,5-diprenyl-4-hydroxycinnamic acid (artepilin C), **M**, apigenin, **N**, kaempferide, **O**.

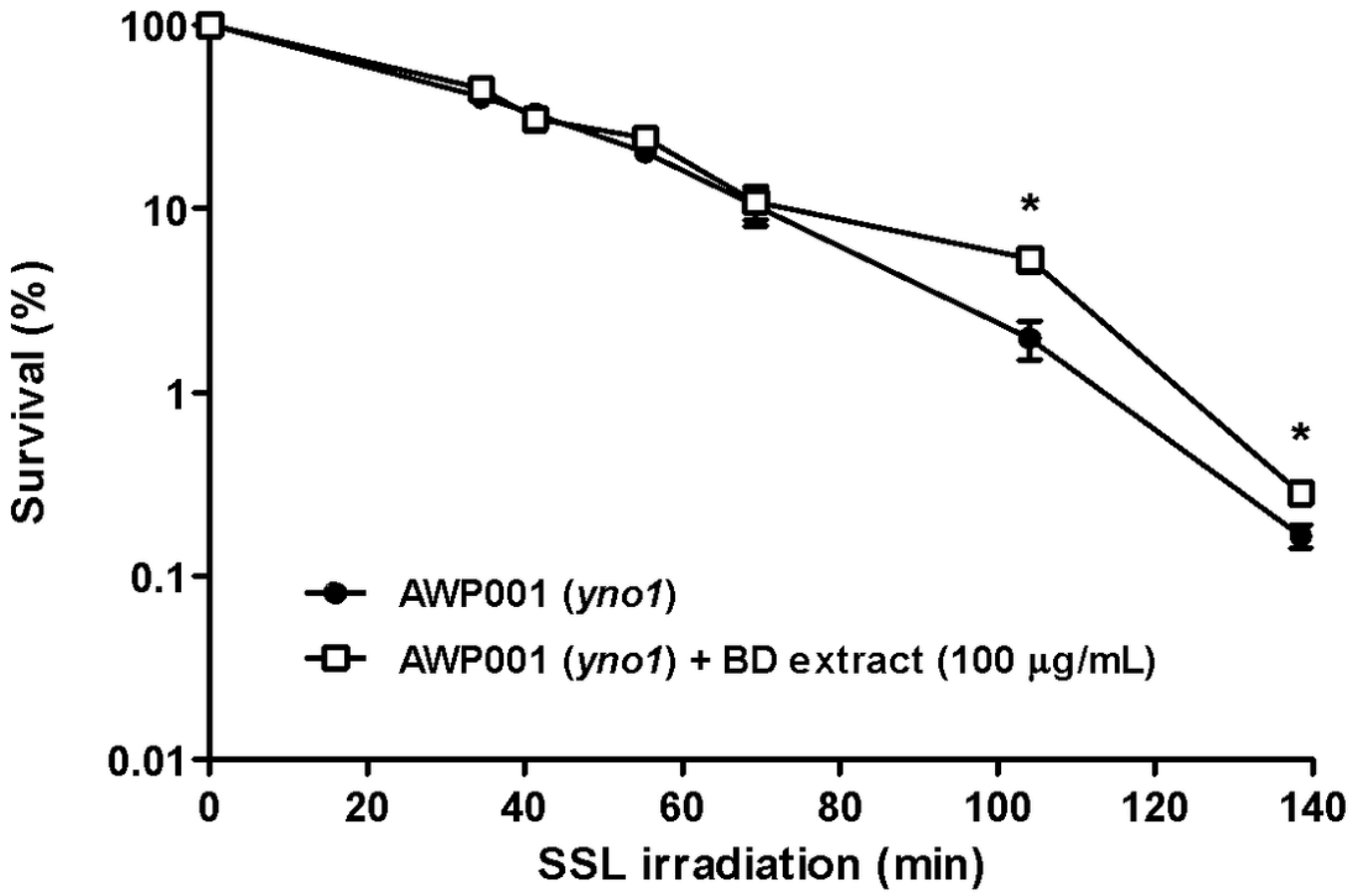

Figure 3

Survival (%) of *S. cerevisiae* AWP001 mutant (*yno1*) strain to SSL radiation treatment in the absence (●) (control) and in the presence of 100 µg/ml of *B. dracunculifolia* (BD) extract (□). * Statistically different (p<0.05) from control (AWP001 strain plus SSL). Data represent means ± SE.

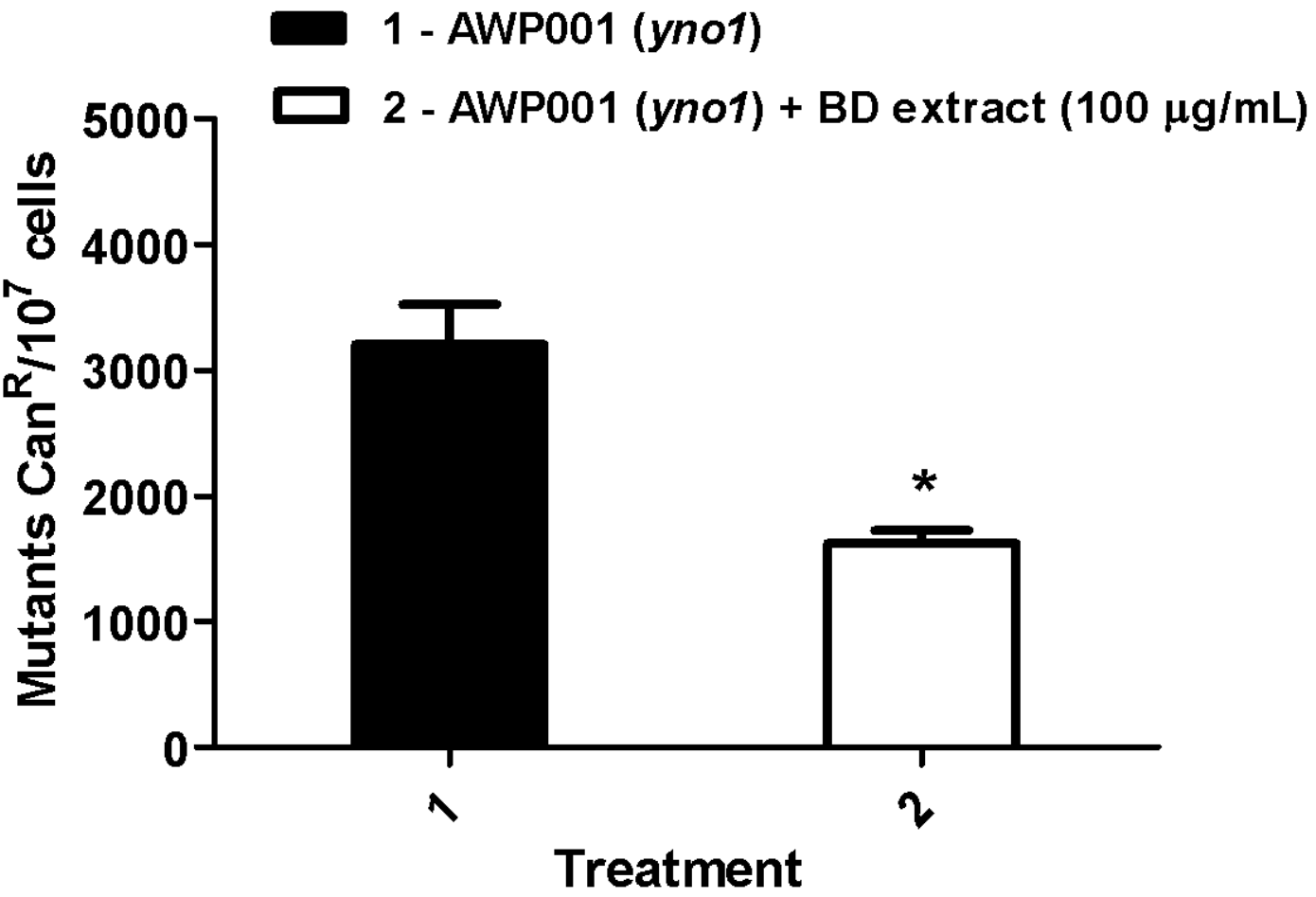

Figure 4

SSL-induced mutagenesis of *S. cerevisiae* AWP001 mutant (*yno1*) strain to SSL radiation in the absence (1) and in the presence of 100 µg/ml of *B. dracunculifolia* extract (2). Data represents means ± SE. * Statistically different (p <0.05) from control (AWP001 strain plus SSL).