TITLE: Antioxidant and antiproliferative potential of fruiting bodies of the wild-growing king bolete mushroom, *Boletus edulis* (Agaricomycetes), from Western Serbia

AUTHORS: Aleksandra Novaković, Maja Karaman, Sonja Kaišarević, Tanja Radusin, Nebojša Ilić

This article is provided by author(s) and FINS Repository in accordance with publisher policies.

The correct citation is available in the FINS Repository record for this article.

NOTICE: This is the author’s version of a work that was accepted for publication in *International Journal of Medicinal Mushrooms*. Changes resulting from the publishing process, such as peer review, editing, corrections, structural formatting, and other quality control mechanisms may not be reflected in this document. Changes may have been made to this work since it was submitted for publication. A definitive version was subsequently published in *International Journal of Medicinal Mushrooms*, Volume 19, Issue 1, January 2017, Pages 27–34.
DOI: 10.1615/IntJMedMushrooms.v19.i1.30

This item is made available to you under the Creative Commons Attribution-NonCommercial-NoDerivative Works – CC BY-NC-ND 3.0 Serbia
Antioxidant and antiproliferative potential of fruiting bodies of
wild-growing mushroom *Boletus edulis* (Bull.) collected in Western
Serbia

Aleksandra Novaković¹*, Maja Karaman², Sonja Kasiarević², Tanja Radusin¹,
Nebojša Ilić¹

¹University of Novi Sad, Institute of Food Technology, Bulevar cara Lazara 1,
21000 Novi Sad, Serbia
²University of Novi Sad, Department of Biology and Ecology, Faculty of Sciences,
Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia

SHORT TITLE: Bioactivity potential of *Boletus edulis*

*Address all correspondence to: Aleksandra Novaković, Institute of Food
Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad,
Serbia; Phone: +381214853770; aleksandra.novakovic@fins.uns.ac.rs
ABSTRACT: *Boletus edulis* is considered to be one among the most known and most delicious mushroom in the world which is commonly consumed and eaten in soups, pasta or risotto. Considering its diverse popularity and use, the aim of this work was to study bioactivity of crude aqueous and ethanolic extracts of *B. edulis* prepared from cap and stipe (BecAq, BesAq, BecEtOH, BesEtOH) of wild-growing sporocarps collected from Prijepolje region (Western Serbia). The bioactivity screening included antioxidant (DPPH•, NO•, SO•, OH• and FRAP) and antiproliferative (human breast MCF-7 cancer cell-line; MTT) assays. In addition, all extracts were primarily characterized by UV-VIS spectrophotometry in order to determine total phenolic and flavonoid contents. The highest anti-DPPH and anti-OH radical activity were observed in BecAq (IC$_{50}$ = 50.97 μg/ml and IC$_{50}$ = 2.05 μg/ml) while the highest anti-NO radical activity was observed in BesAq (IC$_{50}$ = 10.74 μg/ml). The ethanolic extract obtained from the mushroom stipe showed higher anti-SO radical activity (IC$_{50}$ = 9.84 μg/ml) and ferric reducing antioxidant power (22.14mg AAE/g d.w.) compared to aqueous extracts. Total phenolic (TP) content for all extracts was similar but total flavonoid (TF) content was significantly higher in BecAq (4.5 mg QE/g d.w.). All crude extracts showed activity against MCF-7 cell line, with BesEtOH (IC$_{50}$ 56 μg/ml) being the most potent. This is the first report on the antiproliferative effects of crude aqueous and ethanolic extracts prepared from cap and stipe of wild-growing sporocarps of *B. edulis* on human breast MCF-7 cancer cell-line.
KEY WORDS: Boletus edulis, fruiting body, phenolic compounds, antiradical and antiproliferative activity

ABBREVIATIONS: AAE: ascorbic acid equivalents; Aq: aqueous; Bec: cap; Bes: stipe; DMEM: Dulbecco’s modified Eagle’s medium; DMSO: dimethyl sulfoxide; DPPH: 2,2-diphenyl-1-picrylhydrazyl; EtOH: ethanol; FC: FolinCiocalteu; FCS: fetal calf serum; FRAP: Ferric reducing antioxidant power; GAE: gallic acid equivalents; IC_{50}: half-maximal inhibitory concentration values; MCF-7: breast carcinoma cell line; MDA: malondialdehyde; MTT: 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide; NADH: nicotinamide adenine dinucleotide; NEDA: naphthylethylenediamine dihydrochloride; NO: nitric oxideradical; OH: hydroxyl radical; PMS: phenazine methosulfate; ROS: reactive oxygen species; SA: sulfanilamide; SNP: sodium nitroprusside dihidrate; SO: superoxide anion radical; TBA: 2-thiobarbituric acid; TF: total flavonoid; TP: total phenolic
I. INTRODUCTION

Since ancient times, mushrooms have been mainly used as special food, due to their high nutritional value, undoubtedly fine flavors, as well as their pronounced health beneficial properties\(^1,2\).

Basidiomycete family Boletaceae are mushrooms that are mainly known by fleshy context and a tubulose, rarely lamellate or loculate hymenophore. This family has extraordinary diversity which includes about 50 genera and 800 species\(^3\).

According to recent phylogenetic analyses, seven major clades at the subfamily level have been revealed, namely Austroboletoideae, Boletoideae, Chalciporoideae, Leccinoideae, Xerocomoideae, Zangioideae, and the Pulveroboletus group\(^4\). Some of these boletes have great economic, dietary and health value such as *Boletus edulis* sensu lato (Porcini) which is a gourmet mushroom highly prized in many parts of the world, including the Balkans region. This species is widely distributed in across Europe, Asia and North America, but it is also one of the most popular mushroom species in Serbian cuisine. Among the species within genus *Boletus*, there are many species which are consumed in our region such as *B. aereus, B. aestivalis, B. erythropus, B. pinicola, B. queletii* etc\(^5\). Within the local edible species in Serbia, *B. edulis*, stands out due to high consumption and its economic value. The mushroom is low in fat and digestible carbohydrates and high in protein, vitamins, minerals and dietary fiber. Although it is sold commercially, it is very difficult to cultivate due to its mychorizal needs. It is available in wild, and it is most often dried, packaged and distributed worldwide. After drying, it keeps flavor which makes it suitable as milled powder to be used in cooking\(^6,7,8\).
This species produces a variety of nutraceuticals, with a diverse spectrum of biological activity such as anti-oxidant, anticancer, anti-microbial\(^9,10,11\). The bioactivity of Porcini was first confirmed by Lucas in 1957, when he isolated a substance from \textit{B. edulis} which showed a significant inhibitory effect against tumor cells of sarcoma (S-180)\(^12,13\).

Free radicals are reactive oxygen species (ROS) in cells, which are constantly produced in the human body \(^14\). There is a lot of evidence that supports the implication of oxidative stress induced by reactive oxygen species (DPPH•\,NO•, SO•, OH•) in the pathogenesis of several chronic and degenerative diseases such as: Alzheimer's, Parkinson's, atherosclerosis, diabetes mellitus, chronic inflammation and cancer. Therefore, the enhancement of the antioxidant systems for the prevention of cellular oxidative stress via the consumption of antioxidant rich foods is of great interest today\(^14,15,16,17,18,19\).

Since the bioactivity of fungal species is mostly influenced by geographical origin or specificity of the single fungal strain\(^20\) the aim of this study was to evaluate antioxidant and antiproliferative properties of aqueous and ethanolic crude extracts of caps and stipes of autochthonous \textit{B. edulis} species with respect to their total phenolic and flavonoid contents.

Moreover, based on the literature data concerning antioxidant properties obtained for different parts of fruiting bodies (cap and stipe) the aim of this study was also to make a comparison between bioactive properties of cap and stipe of autochthonous mushroom species \textit{B. edulis}. 
To the best of our knowledge, this paper presents the first report concerning antioxidant and cytotoxic potential of the tested species from Western Serbian region of Prijepolje. Furthermore, for the first time ethanolic and aqueous extracts of cap and stipe were analyzed against MCF-7 breast carcinoma cell line.

II. MATERIALS AND METHODS

A. Materials and Chemical

*B. edulis* was collected from Prijepolje region (Western Serbia) during autumn 2011. After its identification, a voucher specimen was deposited at the Herbarium BUNS, Novi Sad, Serbia. The fungal samples were separated to the cap (Bec) and stipe (Bes) parts and frozen at -20°C, prior to freeze-drying procedure (Bio alpha, Martin Christ GmbH, Germany). Freeze-dried samples were ground to a fine powder, wrapped in plastic bags and stored in dark place at room temperature, until further use. Folin-Ciocalteu (FC) reagent, anhydrous sodium carbonate, gallic acid, aluminium (III) chloride hexahydrate, sodium acetate trihydrate, quercetin hydrate, 2,2 – diphenyl – 1-picrylhydrazyl (DPPH*), anhydrous iron(III) chloride (FeCl3), disodium hydrogen phosphate, phenazine methosulfate (PMS), and ß-nicotinamide adenine dinucleotide (NADH), malondialdehyde (MDA), 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), were purchased from Sigma-Aldrich (Steinheim, Germany). Dulbecco’s Modified Eagle’s Medium with 4.5% of glucose (DMEM) and fetal calf serum (FCS) were purchased from PAA Laboratories (Pasching, Austria). Ascorbic acid, hydrochloric acid (HCl), potassium dihydrogen phosphate, sodium nitroprusside dihidrate (SNP), naphthylethylene diamine dihydrochloride (NEDA),
and sulfanilamide (SA) were purchased from Lach-ner (Neratovice, Czech Republic). Deionized water was produced using a Millipore water purification system.

B. Preparation of ethanolic and aqueous extracts from *B. edulis*

The processed caps and stipes of *B. edulis* (10 g) were extracted with distilled water (Aq) and ethanol (EtOH) for 24h, on a shaker (Thermo Fisher Scientific, USA; 120 rpm) at room temperature (25°C). The extracts were filtered through Whatman No. 4, while the solvents (EtOH) were removed by rotary evaporator unit at 40°C (Büchi, Switzerland); the aqueous extract (BecAq, BesAq) was freeze-dried prior to analysis. The obtained extracts (ethanolic -BecEtOH, BesEtOH and aqueous BecAq, BesAq) were stored at +4°C and -20°C, respectively. Relevant dried residues were re-dissolved in 5% DMSO.

C. Total phenolic content (TP)

Total phenolic content (TP) of all analyzed extracts was determined according to method by Singleton et al. \(^\text{22}\), adapted for a 96-well plate reader (Multiskan Ascent, Thermo Electron Corporation, USA). Folin-Ciocalteu reagent (125 µl, 0.1 M) was added to 25 µl of diluted extracts. After 10 min, 100 µl of 7.5% w/v sodium carbonate was added and reaction mixture was incubated for 2 h. Absorbance was read at 690 nm. TP was expressed as mg gallic acid equivalents (GAE)/g of dry weight (d.w.). The experiments were performed in triplicate.

D. Total flavonoid content (TF)
Total flavonoid (TF) content of all extracts was measured spectrophotometrically, in a 96-well plate reader, using a modified method by Chang et al.\textsuperscript{23}. The relevant sample (30 μl) was mixed with 90 μl of methanol, 6 μl of 0.75 M aluminium-trichloride, 6 μl of 1 M sodium acetate and 170 μl of distilled water. Absorbance was measured at 414 nm, after incubation of 30 min. The experiments were repeated three times, while results were expressed as mg quercetin equivalents (QE)/g of dry weight (d.w.).

**E. Determination of DPPH•, NO•, SO• and OH• scavenging activity**

DPPH• scavenging activity was evaluated according to Espin et al.\textsuperscript{24}. The reaction mixture consisted of 10 μl of sample, 60 μl of DPPH• solution and 180 μl of methanol. After incubation of 60 min (dark place, at room temperature), absorbance was measured at 540 nm. Each sample was tested at different concentrations in the range (15 – 600 μg/ml), while results were expressed as IC\textsubscript{50} value.

Nitric oxide scavenging capacity was determined according to the procedure of Green et al.\textsuperscript{25}. The reaction mixtures in test tubes consisted of 30 μl extract, 500 μl SNP, and 500 μl of phosphate buffer, pH=7.4. Control contained equivalent volume of ethanol, while reagents were replaced with phosphate buffer in the correction. Test tubes were incubated at room temperature for 90 min, under light exposure. After incubation, 1 ml of Griess reagent (0.2% solution of NEDA and 2% solution of SA in 4% phosphoric acid in the ratio 1:1 (v:v) was added equally to samples, corrections and control. Aliquots of 250 μl were transferred to the plate, and their absorbance was measured using plate reader at 540 nm. Samples were tested at different concentrations (0.9 – 1477 μg/ml) to obtain IC\textsubscript{25}. 


Superoxide anion radical scavenging capacity of extracts was determined by measuring their ability to neutralize superoxide anion radicals generated during aerobic reduction of nitro blue tetrazolium by NADH mediated by PMS. Total amount of 100 µl of 677 µM NADH, 100 µl of 60 µM PMS, 200 µl of 144 µM NBT and 1,1 ml of phosphate buffer (pH = 8.3) were mixed with 10 µl of extract in the test tube. Control contained ethanol instead of extract, and correction contained 10 µl of extract and 1,5 ml of phosphate buffer. After 5 min, aliquots of 250 µl were transferred to the well plate and their absorbance was measured at 540 nm using plate reader. Five different concentrations of each sample (1.65 – 99.3 µg/ml) were tested to obtain IC₅₀.

The content of OH radicals was determined from the degradation reaction of 2-deoxy-D-ribose into fragments, while the malondialdehyde MDA reaction with 2-thiobarbituric acid TBA reagent was determined spectrophotometrically at 532 nm. Each reaction was performed in triplicate using 10 µL of fungal extract (0.98 – 28.79 µg/mL).

All results were calculated to obtain IC₅₀, and all experiments were performed in triplicate.

**F. Ferric reducing antioxidant power (FRAP)**

FRAP assay was performed according to a modified procedure of Benzie and Strain. The FRAP reagent consisted of 300 mM acetate buffer (pH=3.6), 10 mM TPTZ in 40 mM HCl and 20 mM FeCl₃, in the ratio 10:1:1 (v:v:v). The sample (10 µl), 225 µl of FRAP reagent and 22.5 µl of distilled water were added in a 96-well plate. Absorbance was measured after 6 min at 620 nm. The results were
expressed as mg ascorbic acid equivalents (AAE)/g of dry weight (d.w.). Each analysis was performed three times.

G. Antiproliferative activity

1. Cells

Estrogen dependent MCF-7 cells were grown in DMEM supplemented with 10% FCS. The cells were seeded in a 96-well microplate (5000 cells per well). After incubation for 24 h, the growth medium was replaced with 100 µl of medium containing examined samples (extracts) at four different concentrations (33.3 µg/ml, 100.0 µg/ml, 300.0 µg/ml, and 900.0 µg/ml). Untreated cells served as control, while pure DMSO was used as a positive control. The effects of the extracts on the growth of MCF-7 cells were evaluated by standard colorimetric assay – MTT.

2. MTT Assay

After 24 h and 72 h respectively, the cell viability was determined by the proliferation MTT assay\textsuperscript{29}. This assay is based on the color reaction of mitochondrial dehydrogenase in living cells with MTT reagent. After incubation, MTT reagent was added to each well (50 µg/100 µl/well at 37 °C, in 5% CO\textsubscript{2} for 3 h). The crystals of produced formazan were dissolved in 100 µl of acidified isopropanol (0.04 M HCl in isopropanol). Absorbance was measured at 540 nm and 690 nm on a 96 well plate reader (Multiskan Ascent, Thermo Electron Corporation, USA).

Calculation of cytotoxicity is expressed as: a percentage of corresponding control value (non-treated cells) obtained in a minimum of three independent experiments. The half-maximal inhibitory concentration values (IC\textsubscript{50}), defined as the
concentration that inhibits 50% of cell growth, were calculated from concentration-response curves. IC\textsubscript{50} values were determined in accordance with dose dependent effects and dose-response curves. IC\textsubscript{50} values were expressed as the mean value of a minimum of three repeated experiments performed for each extract.

**H. Statistical analysis**

Statistical analysis was performed using Statistica software system (StatSoft, Inc. version 12.0, 2013)\textsuperscript{30}. Significant differences between two groups were determined by Duncan's multiple range tests. Finally, Pearson correlation was calculated for TP and TF, DPPH\textsuperscript{-}, NO\textsuperscript{-}, SO\textsuperscript{-}, OH\textsuperscript{-}, FRAP and IC\textsubscript{50} values for antiproliferative activity.

**III. RESULTS AND DISCUSSION**

**A. Antioxidant activity and total phenolic and flavonoid contents**

Scavenging effects of examined extracts from *B. edulis* on DPPH\textsuperscript{-} increased with increased extract concentrations. For scavenging ability on DPPH radicals, extracts were effective in the following order of activities: BecAq> BesEtOH >BesAq>BecEtOH. Among the examined extracts, IC\textsubscript{50} value of BecAq stood out (50.97 μg/ml) in DPPH\textsuperscript{-} scavenging assay (Table 1). In general, analysis showed that aqueous extracts of cap and stipe had higher activities in DPPH assay than experimental data obtained for *B. edulis* extracts of cap and stipe originated from Portugal\textsuperscript{21} and lower activities than ethanol extracts of the entire mushroom originated from Istra region of Croatia\textsuperscript{31}. Extracts of *B. edulis* showed good scavenging activity on nitric oxide, where BesAq extract showed the highest
activity expressed as IC\textsubscript{25} at 10.74 µg/ml while all extracts were effective in the
following order: BesAq > BecAq > BesEtOH > BecEtOH. The radical scavenging
activity on SO\textsuperscript{-}, exhibited relatively high level at lower concentration (IC\textsubscript{50}
BesEtOH was at 8.55 µg/ml) while the extracts were effective in the following
order: BesEtOH > BesAq > BecAq > BecEtOH.

All analyzed extracts were able to scavenge hydroxyl radicals (BecAq > BecEtOH
> BesAq > BesEtOH) (Table 1), although BecAq extract showed the highest
activity (IC\textsubscript{50}=2.61 µg/ml). These results showed more potent values than those
reported by previous researches on various extracts from \textit{B. edulis}\textsuperscript{32}. Moreover, all
analyzed extracts showed very good scavenging activity towards SO\textsuperscript{-} and OH
radical, although IC\textsubscript{50} values did not differ significantly between them.

Furthermore the aqueous extract obtained from the mushroom cap showed also the
highest ferric reducing antioxidant power (FRAP) (22.14 mg/g): BecAq >
BesEtOH > BecEtOH > BesAq, although IC\textsubscript{50} did not differ significantly.

Total phenolic (TP) content was in the range of 29.18–36.36 mg/g although it did
not differ significantly (Table 2). These results are in accordance with previous
studies on \textit{B. edulis} samples from India, Taiwan, Turkey and Portugal\textsuperscript{16,33,34,35}. The
examined EtOH extracts in this study had slightly lower TP than ethanolic extracts
of \textit{B. edulis} samples from Istra region of Croatia\textsuperscript{31}. TF contents were found to be
in the range of 0.65 – 4.50mg/g and BesAq extract showed significantly higher
concentration. The highest antioxidant values, including antiradical (the lowest
IC\textsubscript{50} values) were found for the BecAq, which is in agreement with its highest TP
and TF content.

### B. Antiproliferative activity
The antiproliferative activity of extracts from *B. edulis* was screened by the MTT assay on human breast carcinoma cells (MCF-7). It has been previously reported\(^{36}\) that compounds exhibiting cytotoxic effects in cell lines can demonstrate distinct kinetic profiles that fit into three categories: acute (< 1 h to full toxicity), subacute (1 - 40 h), and long term (> 40 h). Guided by these findings, exposure times of 24 h and 72 h have been selected to allow the expression of full cytotoxic potential of investigated fungal extracts.

Under the experimental conditions all extracts showed cytotoxic activity (Table 3). At the highest tested concentration (900 µg/ml), cytotoxicity of EtOH extracts was expressed in the range of 45% - 94%, while for Aq extracts it was in the narrow range of activities between 32% - 84%. Ethanolic extracts from stipe (BesEtOH) elicited very prominent antiproliferative effect (IC\(_{50}\)=59.14 µg/ml). This extract showed significantly higher activity (lower IC\(_{50}\)) than aqueous extract especially for the chronic treatment at 72 h (Table 3). The aqueous extracts from cap (BecAq) induced less pronounced cytotoxicity (with IC\(_{50}\)>900 µg/ml). However, when comparing our results with the findings from the similar studies, antiproliferative potency of *B. edulis* on the human breast cancer cell line (MCF-7) is evident.

The mushroom biopolymers (polysaccharides and glycoproteins) isolated from *B. edulis* hot water extract showed very prominent antiproliferative effect in colon cancer cells (CCD 841 CoTr) and no toxic effect against normal colon epithelial cells\(^{18}\). The novel lectin from *B. edulis* was found to effectively inhibit the proliferation MCF-7 (breast adenocarcinoma), HepG-2 (hepatocellular carcinoma), CaCo-2 (colorectal adenocarcinoma), CFPAC-1 (pancreatic duct adenocarcinoma),
HeLa, SK-MEL-28, U-87 MG cell lines. On the other hand, three non-isoprenoid botryane sesquiterpenoids named boledulins A–C (1–3) isolated from the cultures of *B. edulis* exhibited inhibitory activity against five human cancer cell lines: HL-60, SMMC-7721, A-549, MCF-7 and SW480.

The observed difference in antioxidant and antiproliferative activity of *B. edulis* extracts might depend on their chemical composition. Mushrooms contain a variety of complex compounds derived from secondary metabolism such as phenolic compounds, polyketides, triterpenoids and steroids which are specific to each mushroom and have specific effects on humans. Phenolic compounds in mushrooms and in other sources display a remarkable spectrum of biological activities including antioxidant, antitumor and antimicrobial properties.

Protocatechuic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, cinnamic acid, and quinic acid were previously found in some *B. edulis* extracts from Portugal and Serbia and variagatic acid in extracts from Istra region of Croatia. These phenolic acids possess several bioactivities, such as antioxidant, cytotoxic, antiradical and antitumor. Heleno et al. referred the cytotoxic and antitumor activities of *p*-coumaric acid against breast MCF-7, NCI-H460 and HCT15 carcinoma cell lines and also referred to the antioxidant activities. In addition, anticancer studies *in vitro* and *in vivo* pointed to flavonoids as major compounds, against cancer which prevent carcinogen metabolic activation, antiproliferation, cell cycle arrest, induction of apoptosis, promotion of differentiation, antioxidative activity and modulation of multidrug resistance.
Correlation between TP and TF content and tested antiproliferative activity (IC\(_{50}\) value) among all applied tests showed much higher values (r=0.806, p<0.05) for TF content, especially for treatment at 24 h (Fig. 1) than for TP content (r=0.49, p<0.05), and for DPPH assay (r=-0.80, p<0.05) (Fig. 2). This suggests an important role of flavonoid compounds in demonstrated antiproliferative activities and radical scavenging activities on DPPH.

**FIG. 1:** Correlation between TP and TF content and tested antiproliferative activity (IC\(_{50}\) value).
**IV. CONCLUSIONS**

According to the results of this study, it is clearly indicated that the tested mushroom extracts of *B. edulis*, especially aqueous extract from cap have strong antioxidant activity *in vitro*, including antiradical and FRAP activity. Cytotoxicity assay proved that crude ethanolic extract from stipe possesses the highest antiproliferative activity. According to the results obtained the examined fungal species herein, may be considered as a promising source of natural antioxidants with potential significance for regular human diet. Considering autochthonous origin of wild-growing sporocarps of *B. edulis*, demonstrated activities are even more important for the region and for the further exploitation. The future research work should be primarily directed towards elucidation of chemical profiles of analyzed extracts derived from cap and stipe and their possible mechanism(s) of action.

**ACKNOWLEDGEMENTS**

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (project III 46001) and OI172058.


7. Polese JM, Lamaison JL. The Great Encyclopedia of Mushrooms.1999


TABLE 1: Bioactivity of different extracts of *B. edulis*

<table>
<thead>
<tr>
<th>Antiradical assays IC&lt;sub&gt;50&lt;/sub&gt; (μg/ml)</th>
<th>BecEtOH</th>
<th>BecAq</th>
<th>BesEtOH</th>
<th>BesAq</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPPH&lt;sup&gt;*&lt;/sup&gt;</td>
<td>163.83&lt;sup&gt;c&lt;/sup&gt;</td>
<td>50.97&lt;sup&gt;a&lt;/sup&gt;</td>
<td>95.60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>130.42&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>NO'</strong></td>
<td>1034.88&lt;sup&gt;c&lt;/sup&gt;</td>
<td>433.97&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1212.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.74&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SO'</td>
<td>18.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.55&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>OH'</td>
<td>3.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.28&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Antioxidant assay

| FRAP<sup>***</sup> | 12.59<sup>a</sup> | 15.13<sup>b</sup> | 22.14<sup>a</sup> | 8.43<sup>a</sup> |

*expressed as concentration of extracts that caused 50% activity - IC<sub>50</sub> (μg/ml)

**expressed as concentration of extracts that caused 25% activity – IC<sub>25</sub> (μg/ml)

***Ferric reducing antioxidant power (FRAP) is expressed as mg ascorbic acid equivalents/g extract dry weight (mg AAE/g d.w.)

<sup>a,b,c</sup> Significant differences between groups (columns) were determined by Duncan's test (p<0.05)
**TABLE 2: Chemical composition of different extracts of *B. edulis***

<table>
<thead>
<tr>
<th>Total content</th>
<th>BecEtOH</th>
<th>BecAq</th>
<th>BesEtOH</th>
<th>BesAq</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP*</td>
<td>23.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.73&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TF*</td>
<td>1.61&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.50&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.65&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Total phenolic (TP) content is expressed as mg gallic acid equivalents/g extract dry weight (mg GAE/g d.w.), while total flavonoid (TF) content is expressed as mg quercetin equivalents/g extract dry weight (mg QE/g d.w.)*

<sup>a,b,c</sup> Significant differences between groups (columns) were determined by Duncan's test (p<0.05)
### TABLE 3: Antiproliferative activity of *B. edulis* extracts on MCF-7 cell line

<table>
<thead>
<tr>
<th>Extracts</th>
<th>24h</th>
<th>72h</th>
</tr>
</thead>
<tbody>
<tr>
<td>BecEtOH</td>
<td>397.80±68.42</td>
<td>163.82±4.20</td>
</tr>
<tr>
<td>BecAq</td>
<td>900b±0.00</td>
<td>900d±0.00</td>
</tr>
<tr>
<td>BesEtOH</td>
<td>900b±0.00</td>
<td>59.14a±6.66</td>
</tr>
<tr>
<td>BesAq</td>
<td>481.48a±7.25</td>
<td>435.61c±15.14</td>
</tr>
</tbody>
</table>

* Values with different letters in superscript within a row are statistically different (p<0.05), Duncan’s test.

**FIG. 1:** Correlation between TP and TF content and tested antiproliferative activity (IC$_{50}$ value).

**FIG. 2:** Correlation between TF content and scavenging ability on DPPH radicals (IC$_{50}$ value).