Antiradical Activities and Cytotoxic Effects of *Phlomis brevibracteata* Turrill on SK-HEP-1 Adenocarcinoma Cell Line [†]

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Abstract: The present study investigates the total phenol amount, radical scavenging and anticancer activity of methanolic extract of *Phlomis brevibracteata* Turrill (PBT) leaves; an endemic plant of North Cyprus. The antiradical activity and cytotoxicity of 70% methanolic extract of PBT leaves were analyzed by the implementation of DPPH, ABTS radical scavenging activity tests and MTT assay, respectively. Our results showed significant antiradical scavenging activity in the total phenolic content of the PBT leaves. MTT assay proved potent cytotoxic activity against SK-HEP-1 cell line not only for 70% methanolic extract of PBT, but also for gallic acid and caffeic acid constituents of the total phenolic extract.

Keywords: *Phlomis brevibracteata* Turrill; antiradical; cytotoxicity

1. Introduction

Medicinal plants have gained an increased attention to treat complications such as cancer by isolating the right active ingredients [1]. A recent estimation has claimed that out of 250,000 discovered plant species, one thousands of them show significant anticancer potency. Beside all the available anticancer medication in the market, the need of discovering novel medications with an improved efficacy and side-effect profile is still felt. The genus *Phlomis* includes up to 100 species that are distributed in Euroasia [2]. Of these species, *Phlomis brevibracteata* Turrill (PBT) is a plant from North-Cyprus which belongs to the family of Lamiaceae. The plant occurs in 16 places, it is classified as endemic and it is also in the Red List of Threatened Species [3]. *P. brevibracteata* grows between 100 and 1100 m above sea level in Mediterranean climate. In traditional medicine *Phlomis* plants have been used to treat diabetes, gastric ulcer, haemorrhoid, inflammation and to heal wounds [3]. *Phlomis* species are also known to have antidiabetic effects by reducing the oxidative stress, which in turn explains their hepatoprotective characteristics [4,5]. It is believed that flavonoids, iridoids, phenyl ethanoid/propanoid glycosides, essential oils, lignans, diterpenoids and alkaloids are the main phytochemicals responsible for the majority of the above-mentioned effects [4]. The aim of this study is to investigate radical scavenging activities and cytotoxicity effects of PBT on SK-HEP-1 adenocarcinoma cell line by (DPPH●), ABTS+● and MTT assay, respectively.

2. Materials and Methods

2.1. Material Collection and Extract Preparation

Air-dried PBT leaves were cut into small pieces and extracted with 70% methanol (x 3) at room temperature. Methanol was evaporated under vacuum (40 °C). Extracts were lyophilized and stored at +4 °C until further analysis.

2.2. Total Phenolics

Total phenols were estimated as GAE, per gram of extract [5].

2.3. 1,1-Diphenyl-2-Picrylhydrazyl Radical (DPPH●) Scavenging Activity

Extracts were dissolved in aqueous methanol, solutions were prepared between 0.01 and 1 mg/mL concentrations. Each extract dilution was mixed with Tris-HCl buffer than DPPH● in MeOH was added to all tubes. After 30 min absorbance was recorded at 517 nm. The percentage inhibition was calculated using Eqn.1 (%inhibition = $[(A_{\text{control}} - A_{\text{sample}}) / (A_{\text{control}})] \times 100$). Estimated IC₅₀ values were presented as the mean value of triplicate analyses.

2.4. ABTS+● Radical Scavenging Activity

The ABTS+● radical and K₂S₂O₈ solution prepared. The absorbance was adjusted to 0.700 with water at 734 nm. Extracts were prepared at 3 and 6 mg/mL concentrations. Standard Trolox stock solution was prepared at 0.1, 0.5, 1, 1.5, and 2 mM concentrations. After ABTS+● solution was added to the sample, the absorbance was measured at 734 nm, at first and 30th minutes. Results are expressed as the Trolox equivalent antioxidant capacity (TEAC, mM Trolox).

2.5. Cell Culture and MTT Assay

Human Hepatic Adenocarcinoma cell line SK-HEP-1 were maintained in DMEM supplemented with 10% FBS, 100 U/mL penicillin, 2 mM L-glutamine, 100 mg/mL streptomycin and 1X NEAA at 5% CO₂ at 37 °C. MTT assay was used to determine the cytotoxic effects of methanolic extracts of PBM, gallic acid and caffeic acid on SK-HEP-1 cancer cell line for 48hrs. Briefly, 5×10^3 cells were seeded in a 48 well plate, after overnight incubation. The, cells were then incubated with different concentrations of extract and standart containing medium. Following a 48-h treatment, MTT was added to the medium. Than formazan crystals were dissolved with DMSO and absorption was measured at 570 nm. The cell viability was calculated as viable cell percentage.

2.6. Statistical Analysis

Experiments have been independently carried out three times, and all samples were presented as mean $\pm$ SE from six measurements for cytotoxicity analysis, and as mean $\pm$ SD for antioxidant assays. One way ANOVA test was used for analysis. $P < 0.05$ was considered as statistically significant. IC₅₀ (the median growth inhibitory concentration) values have been calculated from the dose response inhibition curve. All the analyses were performed using GraphPad Prism 5 software.

3. Results

3.1. Antiradical Activity of PBM

The PBT leaves were extracted using 70% methanol as a solvent and the yield of extract was found 11.79%. Total phenol amount of PBT leaf extract was calculated as gallic acid equivalent (GAE) per gram extract. The total phenol amount of the extract was recorded 24.360 mgGAE/gextract $\pm$ 0.296 (mean $\pm$ SD, $n = 6$). Depending on the concentrations, the extract managed to scavenge the DPPH● radical at physiological pH. For both Figure 1a,b, linear correlations were found between concentration and absorbance/inhibition ($r^2 = 0.9907$). IC₅₀ value of 70% MeOH extract was also

calculated using Equation 1 as 0.556 mg/mL. Antiradical activity was found high since the 70% MeOH extract is rich in phenolic components. PBM was also tested in ABTS+• assay, and the results are 2.29 ± 0.10 and 0.81 ± 0.31 TEAC in 3 mg/mL and 6 mg/mL concentrations, respectively. The results revealed a significant antiradical activity mainly because of the high total phenolic content of PBM.

Figure 1. DPPH• scavenging activity analysis (a) Absorbance values of different concentration of extract (b) Inhibition values of different concentration of PBM in DPPH• scavenging assay.

3.2. Cytotoxicity Effect of PBM on SK-HEP-1 Cell Line

Cytotoxicity effect of PBM significantly increased in a concentration-dependent manner. At concentrations over 1000 $\mu\text{g/mL}$, the cytotoxicity increased drastically as shown in Figure 2. ($p < 0.0001$). The IC_{50} value of PBM was determined as 1056 $\mu\text{g/mL}$.

Figure 2. Cytotoxicity effect of different concentrations of PBM on SK-HEP-1 cell line. (***) $p < 0.0001$

3.3. Cytotoxicity Effect of Gallic acid and Caffeic Acid on SK-HEP-1 Cell Line

As depicted in Figure 3a, each of the applied concentrations of gallic acid has a different level of cytotoxicity potency. Starting from 100 $\mu\text{g/mL}$, the cytotoxicity became significant (** $p < 0.001$, *** $p < 0.0001$). In case of caffeic acid, cytotoxicity became significant after 250 $\mu\text{g/mL}$ as shown in Figure 3b (** $p < 0.001$, *** $p < 0.0001$). IC_{50} values were determined as 179 $\mu\text{g/mL}$ and 319 $\mu\text{g/mL}$ for gallic acid and caffeic acid, respectively.

Figure 3. Cytotoxicity effect of gallic and caffeic acid on SK-HEP-1 cell line. (a) The effect of different concentrations of gallic acid (b) The effect of different concentrations of caffeic acid. (** $p < 0.001$, *** $p < 0.0001$)

4. Discussion

Although information about anticancerogenic and antiradical effects of *Phlomis* is available in to the best of our knowledge this is the only study analyzing the antioxidant activity and cytotoxicity effects of Cyprus endemic PBT [6]. Our findings have similarities with the literature in terms of antioxidant and cytotoxicity activities of Cyprus endemic *Phlomis*. We believe that the antioxidant and anticancerogenic effects of PBT on SK-HEP-1 cell line are closely related to the phenolic constituents. In future studies, we plan to investigate the active constituents and molecular mechanisms of anticancerogenic effects. Overall, our results suggest that PBT is a good phytotherapy candidate.

Author Contributions: I.K. and M.K. conceived and designed the experiments; I.K, M.K, N.Z., K.R. and M.D. performed the experiments; and I.K. and M.K. analyzed the data; I.K, M.K. and N.Z. wrote the manuscript.

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