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Multiple Antioxidant and Bioherbicidal Assays of the Edible Mushroom Species "*Ramaria flava*" in the Amanos Mountains

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ABSTRACT

The phenolic content (TPC) and flavonoids (TFC) of the methanol (MeOH) extract of the edible mushroom species '*Ramaria flava'* in the native flora of Amanos Mountains were 89.0 and 0.97 mg/kg, respectively. *β*-carotene content (TCC) (0.47 mg 100/mL) was higher than that of the lycopene (TLC) (0.19 mg/100 mL). Two-fold dilutions ranging from 1.25 to 5.0 mg/mL of the extract were used for antioxidant assays. The most significant activities were 94.17% for DPPH (2-diphenyl-1-picrylhydrazyl), 64.25% for NO (nitric oxide) scavenging, 2.43 Abs for reducing power (RP) and 1.94 Fe^{+2} mmol/L for Ferric-reducing antioxidant power (FRAP) assays at the upper concentration, respectively. The most prominent result for hydrogen peroxide (H_2O_2) scavenging assay at the following concentrations; 0.0156 to 0.125 mg/mL was 71.68% at 0.125 mg/mL. In the bioherbicidal assay, MeOH extract at 25 mg/mL inhibited the germination (16%), radicle growth (44%), plumule growth (52%), total chlorophyll a and b (78 and 88%), and carotenoid (70%) of *Cucumis sativus*. To conclude, FRAP, NO, and H_2O_2 scavenging activities of the MeOH extract of *R. flava* is the first report.

Keywords: *phenolic content, flavonoids, Ramaria flava, Amnanos Mountains, antioxidant, mushrooms.*

1. INTRODUCTION

Secondary compounds, are produced by fungi for their growth and development stages, which could be regarded as the largest classes of natural products [1]. In addition, the phytoconstituents of this kingdom play vital ecological functions during their life span e.g. interacting with plant roots, regulating the symbiotic life, inhibiting and/or stimulating the other prokaryotic and eukaryotic living beings [1, 2].

Consumers tend to buy naturally grown and/or enriched products in order to care and protect their health from many side effects of the synthetic chemicals. Furthermore, scientist and industrialist in the world have been also focused on readily available bio-originated sources in nature because of their environmentally friendly properties as well as less or no toxic effect on organisms. Among those natural products, many mushroom species, as the significant sources of the secondary compounds, have been consumed popularly by people in both rural regions as well as in the cities around the world [3].

The oxidation is a crucial step for many organisms to produce energy; however, reactive oxygen types produced from the oxidation steps may lead to several chronic diseases [4, 5]. The consumption of food with antioxidative compounds is known to be a great support for mankind to lessen damages originated from oxidation [3, 6, 7, 39].

Various techniques are currently present for the antioxidant potentialities of the extracts from different plants and food, which are either hydrogen (HAT) or single electron transfer one (SET). HAT-derived technique use competetive reactions between antioxidant and substrate for peroxyl radicals occur with degradation of many compounds. SET based methods (DPPH, FRAP, ABTS, FC, TEAC, CUPRAC) determined antioxidant capacity with colour change result in reduction of oxidants by antioxidants [8]. Although methods of radical quenching or reducing are based on transer of electron or hydrogen, the reaction mechanism differed from each other. These differences depend on reaction medium, pH of reaction mixture, structure, interactions of antioxidant compounds and solubility of antioxidant compounds in solvents [9].

Of the some important phytocompounds, e.g β-carotene, is known to be a precursor of Vitamin A, which has also some important derivatives such as lycopene. Both of them function as the crucial antioxidants. In addition, phenolic compounds in different kingdoms of life such as plantae and fungi are being intensively studied for their protective health effects on human beings and also their significant sources of pharmaceutical potentialities [10].

Research on the phytotoxic asssays are considered as the feasible tool for sustainable agriculture [11]. Secondary metabolites reported significant allelopatic effects on wild and agricultural plants [12, 13]. Bioherbicidal power of the metabolites derived from macroscobic fungi have been assayed on some plants in different countries as well [1, 14, 15]. The stimulatory effects of those metabolic products on plants have been observed by inhibiting the seed germination and retardation of the seedling growths of some plant species [16, 17, 18]. It has been suggested

that I) allelochemicals of fungal species are the possible candidates for the developments of many novel products which are alternatives to currently used synthetic compounds [14]; II) metabolites of mushroom could be used with the synthetic chemicals to increase their bioherbicidal potential [19]. Nonetheless, it seemed that this study area still deserves more attention and needs to be more explored when compared to the higher plants. There are reports on different extracts of *R. flava* in relation to the phenolics, flavonoids, β-carotene and lycopene contents as well as anticancer, antibiotic, antioxidant activities [20, 21, 22, 23].

In the Amanos Mountains (Turkey), *R. flava* is one of the most commonly consumed fungal species by the local habitants owing to its delicious taste as well as its nutritonal constituents. As of date, biochemically important some phytoconstituents of *R. flava*, and bioactivities of this mushroom species (antioxidant and bioherbicidal) have not being explored in the flora of Amanos Mountains. Therefore, the current study is to assess (I) phenol, flavonoid, β-carotene and lycopene contents (II) antioxidant activities including more spesifically NO, FRAP and H_2O_2 scavenging activities and (III) bioherbicidal effects of the MeOH extract from *R. flava* on *Cucumis sativus*. MeOH extract of *R. flava* using multiple antioxidant assays including NO, FRAP and H_2O_2 scavenging activites as well as bioherbicidal assays on *C. sativus* could be regarded as the first report.

2. EXPERIMENTAL SECTION

2.1. Sample collection and extraction. On November 2014, *R. flava* was collected from Cebel region (37º01'27" N, 36º22'24" E, 975 m) of the Amanos Mountains (Osmaniye, Turkey). The samples were dried in dehydrator for 48 h and then powdered. The extraction of the pulverized samples (50 g) were made in 0.4 L of MeOH (72 h/60 °C). The yield (%) was calculated on a dry weigth basis. Extract was retained at +4°C before experimens.

2.2. Antioxidant assays. Total phenolic (TPC) of *R. flava'*MeOH extract was measured by the Folin-Ciocalteu reagent [24]. Gallic acid was used to obtain a calibration curve (ranging from 0 to 100 mg/mL), and the results were expressed as gallic acid equivalents (mg GAE/kg). (Absorbance=0.0009x+0.0019: R^2=0.9948). Total flavonoid content (TFC) of the extract was measured by using the $AlCl_3$ method [24]. TFC was expressed as quercetin equivalents in milligram per kilogram (mg QE/ kg).

Total β-carotene (TCC) and lycopene content (TLC) in *R. flava* was estimated [25]. TCC and TLC (mg 100/mL) was determined by using the Equation 1 and Equation 2 as follows;

$$\text{Lycopene(mg/100mL)}=-0.0458A_{663}+0.204A_{645}+0.372A_{505}\ 0.0806A_{453} \quad (1)$$

$$\beta\text{-carotene(mg/100mL)}=0.216A_{663}-1.22A_{645}\ 0.304A_{505}+0.452A_{453} \quad (2)$$

Where; A is absorbance value at stated wavelength.

Reducing power assay (RP) of the extract was measured [26]. Absorbance of the solution was determined at 700 nm. BHT was used as a reference. DPPH radical assay (DPPH) was determined [40]. DPPH was calculated in the Equation 3;

$$\text{Scavenging activity of DPPH (\%)}=100\times(A_{control}-A_{sample})/A_{control} \quad (3)$$

Where; $A_{control}$ and A_{sample} are absorbance values of DPPH solution and sample at stated wavelength, respectively.

Nitric oxide scavenging activity (NO) was measured by using Griess reaction [28]. Scavenging of NO was calculated in the below Equation 4 [27].

$$\text{Scavenging activity of NO (\%)}=100\times(A_{control}-A_{sample})/A_{control} \quad (4)$$

Where; Acontrol and Asample are absorbance values of blank solution without sample and sample at stated wavelength, respectively.

Ferric reducing ability antioxidant power (FRAP) was determined [29]. The calibration curve was generated with the solution of $FeSO_4.7H_2O$ (0.2-2 mmol/L). Hydrogen peroxide (H_2O_2) scavenging activity of the extract at different concentrations (0.0156 to 0.125 mg/mL) were assayed [30]. Absorbance of the test was determined spectrophotometrically at 230 nm. BHT as a positive control was also assayed. The results are recorded using the following Equation 5 ;

$$\text{Scavenging activity of } H_2O_2\ (\%)=100\times(A_{control}-A_{sample})/A_{control} \quad (5)$$

Where; $A_{control}$ and A_{sample} are absorbance values of blank solution without sample and sample at stated wavelength, respectively.

2.3. Bioherbicidal assay. Seeds of *Cucumis sativus* were dipped into the sodium hypochlorite solution (1.5%) for 15 min [31]. Sterilized seeds (n=15) were transferred into petri dish including double-layered filter papers. The following concentrantions (1.56, 3.12, 6.25, 12.5 ve 25.0 mg/mL) of the MeOH extract of *R. flava* were prepared by adding distilled water. Distilled water was used as the control during the assay. 10 mL of each solution were pipetted to glass petri dish with test seeds and then, surrounded by the stretch film thoroughly. All treatments were incubated for 7 days (12 h at dark, 12 h at light) at 24 ºC and 80% humidity. Seed germination, radicle lengths, plumule lengths, fresh and dry weight of the seedlings were recorded and calculated as described earlier [32].

Total chlorophyll and carotenoid contents of *C. sativus* were determined [34, 35]. 100 mg of *C. sativus* leaves were thoroughly crushed with 80% acetone solution (10 mL) in porcelain mortar and placed into centrifuge tubes. Extracts were incubated at 4 °C for two days in the dark and then, centrifuged at 4000 g for 10 minutes. The absorbance of supernatant was measured at 480, 510, 645 and 663 nm wavelength in spectrophotometer (UV 1800 Shimadzu). 80% acetone solution was used as a blind. Chlorophyll a, b and carotenoid contents were calculated in following Equation 6, 7 and 8, respectively.

$$\text{Chlorophyll a (mg/g)}=(11.75\times A_{663}-2.35\times A_{645})\times 0.1 \quad (6)$$

$$\text{Chlorophyll b (mg/g)}=(18.61\times A_{645}-3.96\times A_{663})\times 0.1 \quad (7)$$

$$\text{Carotenoid (mg/g)}=[(1000\times A_{480})-(2.27\times\text{Chlorophyll a})-(81.4\times\text{Chlorophyll b})]/227 \quad (8)$$

Where; A is absorbance value at steted wavelength.

3. RESULTS SECTION

3.1. Antioxidant assays. Antioxidant and bioherbicidal activities of the MeOH extract of *R. flava* were investigated and the results were presented in Figure 1 and 2. The yield of the MeOH extract from *R. flava* was 8.59% (w/w). As shown in Table 1, the contents of the TPC (gallic acid, mg/kg), and TFC (quercetin equivalents (mg/kg), TCC and TLC (mg/100 mL) of the MeOH extract of *R. flava* were 89.0 0.97, 0.47 and 0.19, respectively. In previous reports, TPC (pyrocatechol µg/mg) and TFC (quercetin µg/mg) of ethanol extract from *R. flava* in the region of Kayseri (Turkey) was 39.83 and 8.27, respectively [20]. In another study, TPC and TFC values (µg GAE/mg extract) of MeOH extracts of *R. flava* were 10.51 and 0.50, respectively [21]. TPC (mg GAE g^{-1}) of ethanol extract of *R. flava* collected from Aershan region (Mongolia) was 12.95 [22]. TPC, TFC, TCC and TLCs (mg/g) were 9.84, 0.92, 0.14, and 0.06 in the baked samples and 5.90, 0.86 and 0.004 in fresh samples of *R. flava'* MeOH extract, respectively. Unlike baked samples, lycopene was not determined in fresh samples [25].

RP of the MeOH extract of *R. flava* at 1.25, 2.5 and 5 mg/mL increased with concentration when compared with BHT as the control. RP of the MeOH extract was 0.852 at 1.25 mg/mL, 1.562 at 2.5 mg/mL and 2.437 at 5 mg/mL, respectively. In a previous report, RP of the MeOH extract of *R. flava* collected from Muğla (West Mediterranean Part of Turkey) was found as 1.913 at 20 mg/mL [21]. Different RP values in the extracts of assayed mushroom species may be related to amounts of reductors that react with free radicals [33].

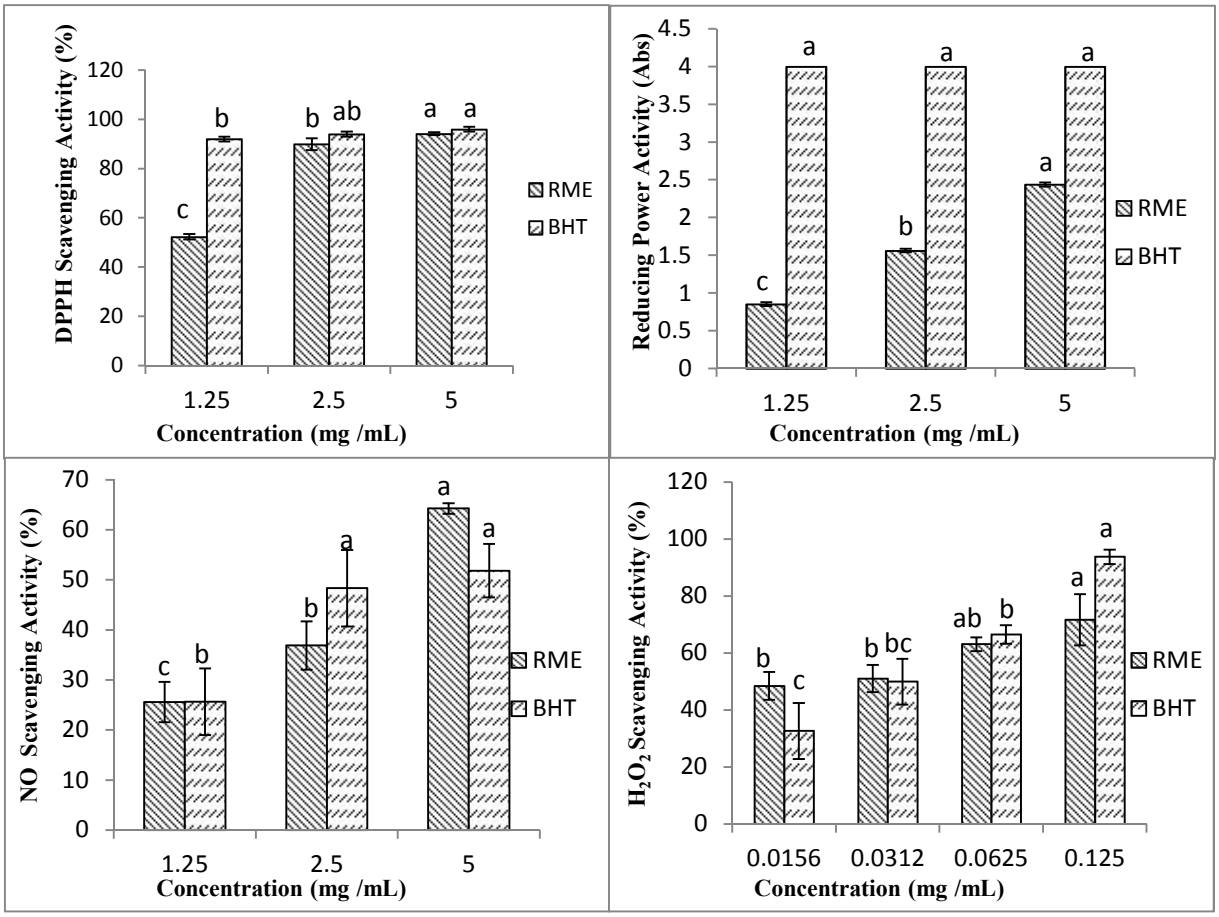

Figure 1. Antioxidant activities of *Ramaria flava*' MeOH extract.

Each value is expressed as mean ± standart deviation. Bars with different letters are significantly different among concentrations at $P \leq 0.05$ (ANOVA followed by LSD test). Abs: Absorbance, C: Control, RME: *R. flava'* MeO extract, BHT: Butylated hydroxytoluene.

DPPH scavenging activities (%) of MeOH extract of *R. flava* were assayed at 1.25, 2.5 ve 5 mg/mL. The result of this assay was found as 52.30% at 1.25 mg/mL, 89.92% at 2.5 mg/mL, and 94.14% at 5 mg/mL. BHT was used as the control with DPPH activity of 96% at 5 mg/mL. In previous studies, DPPH scavenging activities (%) of ethanol extract of *R. flava* was 94.7 in Kayseri [20] and MeOH extract of *R. flava* was 94.78 at 12 mg/mL in Muğla [23]. In a recent report, DPPH scavenging activities (IC_{50}, µg/mL) of the baked MeOH extract of *R. flava* was 0.17 and those of the fresh MeOH extract of *R. flava* from local markets in different regions of Anatolia-Turkey was 4.63 [23]. NO scavenging activities *R. flava's* extract (%) were 25.57, 38.88 and 94.25 at 1.25, 2.5 and 5 mg/mL, and those of BHT were 25.66, 48.33 and 51.83 at 1.25, 2.5 and 5 mg/mL, respectively. It is apparent that higher concentration of the MeOH extract of *R. flava* revealed higher NO activity than that of BHT. The present result of NO assay of *R. flava* indicated that the MeOH extract of *R. flava* include significant amount phytoconstituents.

FRAP capacity of the MeOH extract of *R. flava* increased with the concentration. At 1.25, 2.5 and 5 mg/mL, FRAP capacity (mmol Fe^{+2}/L) was 0.759, 1.372 and 1.942 respectively.

H_2O_2 scavenging activities (%) of *R. flava* extract and BHT (0.125 mg/mL) were 71.68 and 93.76 at the upper doses, respectively. According to obtained results, MeOH extract from *R. flava* extract had high level of H_2O_2 scavenging activities and therefore, the extract of *R. flava* eleminates the toxic effect of H_2O_2.

3.2. Bioherbicidal effects of *R. flava*. The secondary metabolites of the fungi have been reported as some inhibitory effects on either supressing the seed germination of the plants and/or the retardation of the seedling growth [36, 37, 38]. In the present study, MeOH extract at different concentrations (1.56 to 25.0 mg/mL) of *R. flava* has been assayed on the seeds of *C. sativus* for its bioherbicidal potential and the results of those assays were shown in Figure 2. Upper dose (25.0 mg/mL) when applied on *C. sativus*'seeds inhibited the germination by 16%, radicle growth by 44%, plumule growth by 52%, fresh weigth (mg) by 29%, dry weigth (mg) by 42%, chlorophyll a by 78%, chlorophyll b by 88% and carotenoid by 70%.

Table 1. Contents of total phenol, flavonoid, β-carotene and lycopene in *R. flava* methanol extract.

Substance	Value of *R. flava*
Total phenol (mg/kg)	89.0
Flavonoid (mg/kg)	0.97
β-carotene (mg 100/ mL)	0.47
Lycopene (mg 100/ mL)	0.19

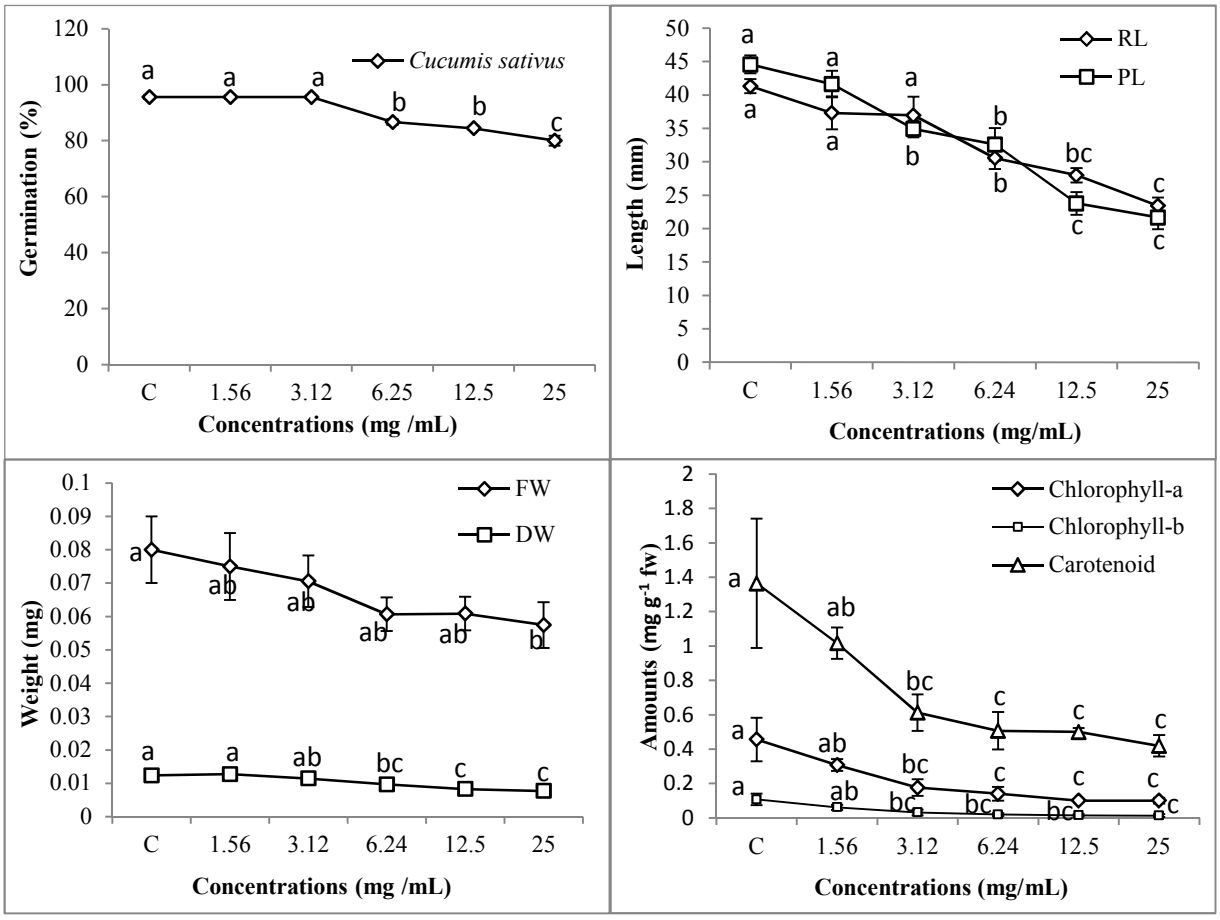

Figure 2. Bioherbicidal effects of *Ramaria flava* on the germination, radicle and plumule length, fresh and dry weight, chlorophyll a, b and carotenoid contents of *Cucumis sativus.*

Mean with different letter in curves is significant at $P \leq 0.05$ (ANOVA followed by LSD test). C: Control, RL: Radicle Length, PL. Plumule Length, FW: Fresh Weight, DW: Dry Weight**.**

4. CONCLUSIONS

MeOH extract of *R. flava* collected from Amanos Mountains indicated both significant antioxidant compounds (fenol, flavonoid, β-carotene and lycopene). Antioxidant powers of *R. flava* differs from previous investigations. This could be due to some factors such as various extraction methods, pH of reaction medium, structure and the interaction of antioxidant components in mushroom body, solubility of the antioxidant compounds in reaction mixture, harvesting location and time. The development of natural herbicides could lessen the adverse effects of residues, resistance and environmental pollution of synthetic materials. It is quite apparent that MeOH extract of *R. flava* could be a novel source for the production of the bio-based herbicides for wild plants in the future. However, further research is suggested for I) identification and isolation of specific compounds obtained from

fungal species that is responsible for antioxidant and herbicidal activity, II) determining side effects, feasibility and cost analysis of the extract obtained from *R. flava*, III) testing their potential against crops under field conditions.

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