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# Comparison of antioxidant and antimicrobial activities of plant extracts Centaury (*Hypericum montbretii* and *Hypericum bupleuroides*) and Blackthorn (*Paliurus spina-christi* Mill) in Artvin, Giresun region of Turkey

## *Türkiye'nin Artvin ve Giresun bölgelerindeki Kantaron (Hypericum montbretii ve Hypericum bupleuroides) ve Karaçalı (Paliurus spina-christi Mill) bitkilerinin antioksidan ve antimikrobiyal aktivitelerinin karşılaştırılması*

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**Anahtar kelimeler:**
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**Abstract**
Antioxidant activity 13 different samples of medicinal and aromatic plants of Centaury (*Hypericum montbretii),* Centaury (*Hypericum bupleuroides*), Blackthorn (*Paliurus spina-christi* Mill), including leaf, flower and stem, was investigated using DPPH, FRAP and CUPRAC methods. Total flavonoid, total phenolic amount and the antibacterial features of extracts from these herbs were also determined. According to the obtained of antioxidant datas, except for the result of total polyphenol assay, the dried leaves of Centuary *(Hypericum montbretii)* had the best antioxidant property that was realized in all results. Accordingly test of total polyphenol, the content of yellow flower of Centuary *(Hypericum montbretii)* herb was measured as of 22.48±0.66 mg GAE /g dry herb sample and so this herb had the highest total phenolic content. Antimicrobial activity tests were carried out using disc diffusion methods with 12 microbial species and the most of them displayed good-moderate antimicrobial and antifungal activity.

**Özet**
Tıbbi ve aromatik bitkilerden olan Kantaron (*Hypericum montbretii* ve *Hypericum bupleuroides*) ve karaçalı (*Paliurus spina-christi* Mill) bitkilerinin yaprak, çiçek ve kök gibi kısımlarından oluşan 13 farklı örneğin antioksidan aktivitesi DPPH, FRAP ve CUPRAC yöntemleri kulanılarak incelenmiştir. Aynı zamanda bitki ekstraktların toplam fenolik ve toplam flavonoid miktarları da ölçülmüş ve antimikrobiyal özellikleri de belirlenmiştir. Antioksidan aktivite sonuçlarına göre tüm analizler içinde (toplam polifenol miktar analizi hariç) en iyi aktivite Kantaron (*Hypericum montbretii*) bitkisinin kuru yaprağında olduğu bulunmuştur. Toplam polifenol analizine göre ise en yüksek aktivitenin 22.485±0.656 mg GAE /g değeri ile Kantaron'un (*Hypericum montbretii*) çiçek kısmına ait olduğu görülmüştür. Antimikrobiyal analizler ise disk düfüzyon yöntemi kullanılarak yapılmış ve bitki ekstraktlarının 12 tane test mikrorganizması üzerine etkisi incelenmiştir. Bu sonuçlara göre bitkilerden çoğunun bakterilere ve mantar suşlarına karşı iyi derecede aktivite gösterdiği gözlenmiştir.

## INTRODUCTION

Medical herbs have been significant resources for the cure of different illnesses from ancient ages. The WHO (World Health Organization) has forecasted that nearly 80% of the more than 4 billion citizens of the world trust on conventional drugs for their basic healthcare requirements, and it can safely be supposed that a large piece of conventional remedy includes the utilize of herb extracts (Fransworth et al. 1985). Medical herbs are broadly utilized in daily lifetime as piece of public medical cures in Turkey. The vegetation in Turkey is extraordinary for its variety and it is a wealthy resource of medical herbs (Demiray et al. 2009). Accordingly researches of WHO, there are nearly 20.000 herbs utilized for medicinal objectives and 9000 herp kinds

have been listed from the vegetation of Turkey (Ilcım and Digrak 1998).

The ordered antibacterial remedy for a contaminated sufferer can provide the distinction among treatment and demise or long-dated disablement. Unhappily, the usage and misusage of antibacterials has caused the brutal enlargement of durable bacteries the loss of effect of these "miracle drugs" (Organization 2001). Hence there is a requirement to evolve alternate antibacterial medicines for the remedy of contagious illnesses. One of the approaches is to research regional medical herbs for feasible antibacterial features. Medical plants symbolize a wealthy resource from that new antifungal and antimicrobial chemotherapeutic drugs may be acquired (Katalinic et al. 2006). Plants have ability to produce certain bioactive molecules, like phenols, flavonols, coumarins, quinones, flavonoids, phenolic acids, flavones and tannins (Cowan 1999). These components with phenolic structures are highly active against the pathogen and can inhibit bacterial or fungal growth (Das et al. 2010).

Reactive oxygen species (ROS) are created frequently as a piece of ordinary aerobic living and include free radicals. ($O_2$·–, ·OH, $H_2O_2$, $^1O_2$·) One of the top common toxical efficacy of oxygen radicals is harm to cellular systems which is launched by a operation given as lipid peroxidation. They may also cause damage DNA, inducing DNA mutations leading to cancer, aging and degenerative diseases (Devasagayam et al. 2004, Finkel and Holbrook 2000, Klaunig and Kamendulis 2004). The equilibration between antioxidants and oxidants is thought to be a crucial notion protecting a healthful biologic mechanism. Therefore, the significance of the antioxidant components of herb samples in the care of health and conservation from illnesses is also growing attention between man of sciences, nutrient producers, and users as the tendency of the future is acting against to all-duty nutrient with particular health efficacy. (Kähkönen et al. 1999). The kind *Hypericum* L., a branch of the Guttiferae (*Hypericaceae*) class contains, at the most recent count, 469 species. *Hypericum perforatum* L., one of the best- proverbial branches of the type, is a significant medical plant of that extracts are taken for their offered efficiency opposite light to middle depression (Crockett and Robson 2011). Thus, there are numerous researches that have been directed with *H. perforatum*, yet many finited researches for other kinds such as *H. bupleuroides* and *H. montbretii*. While *H. montbretii* can grow generally in the western Tukey, *H. bupleuroides* can be found in the eastern black sea region. In present article, we focused on antioxidant and antimicrobial properties of these species used as medicinal plant in Turkey. *Paliurus spina-christi* is kinds of Paliurus local to the Mediterranean zone and southwest and central Asia (Brantner et al. 1996). No research could be discovered the antimicrobial activity of *Paliurus spina-christi Mill* grown in Turkey.

Countless research has indicated that medical herbs are resources of different food and nonfood substances, much of that have antimibacterial and antioxidant contents that can preserve the man system opposite pathogens and cellular oxidation mechanisms. Hence it is significant to symbolize diverse kinds of medical herbs for their antibacterial and antioxidant contents. The aim of this paper was to determination the antibacterial and antioxidant contents of the stem, flower and leaf supplied from non-wood forest products such as Centaury (Hypericum montbretii and Hypericum bupleuroides), and Blackthorn (Paliurus spina-christi Mill), which they have been utilized for medicinal aims in the Eastern Anatolia Zone (Artvin and Giresun).

## MATERIALS AND METHODS

### The Chemicals

Methanol, 2,4,6-tripyridyl-s-triazine(TPTZ), Folin-Ciocalteu's phenol reagent, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were gotten by Sigma Chemical Co. (St. Louis, MO, USA). Neocuproine (2,9–dimethyl-1,10 phenanthroline), acetic acid, ammonium acetate, aluminium nitrate nonahydrate and sodium carbonate, were gotten from Merck Chemical Co. (Darmstadt, Germany). The substances were analytic rate.

**The Plant Material**

*Hypericum bupleuroides* were obtained from two distinct areas (Murgul-Tiryal, Giresun-Kümbet), *Hypericum montbretii* was obtained in Artvin-Hatila. *Paliurus spina-christi* Mill was collected from two different regions (Artvin-Seyitler, Giresun- Kale). Gathered herb samples were dried before analysises (40°C). Almost 10 g of herb of was utilized to get methanolic samples for every kind. These extracts were utilization to define antioxidant activities and process was made three times. Spectrophotometric techniques were used for antioxidant analyzes and also total flavonoids, polyphenols. These techniques are constantly utilization for the native substances.

**Total Phenolic Assay**

Amount for samples was detected from utilizing the Folin-Ciocalteu analysis (Slinkard and Singleton 1977). Standard was Gallic acid (1, 0.5, 0.25, 0.125, 0.0625 and 0.03125 mg/mL) in this work. Shortly, 0.5 N and 400 µL Folin-Ciocalteu tests, 20 µL methanolic samples (1 mg/mL), 680 µL of distilled water and 20 µL of gallic acid (diverse concentrations) were stirred and the solution was stirred. After 3-minute waiting, 400 µL of $Na_2CO_3$ (10%) mixture was annexed and again vortexed. Then the solution was waited about 2 h. Following time, absorbances of the solutions were determined for 760 nm. Total phenolic compounds from concentrations were determined for dry weight of sample as mg of gallic acid.

**Total Flavonoid Assay**

Amount was determined with utilizing aluminum chloride analysis (Chang et al. 2002). Standard was Quercetin. 0.1 mL 1 M $NH_4CH_3COO$, 4.3 mL methanol, 0.5 mL of Quercetin (0.03125; 0.0625; 0.125; 0.25; 0.5 and 1mg/mL), and 0.1 mL 10% $Al(NO_3)_3$ were put in the tubes and then they were vortexed. Mixtures were waited about 40 minutes. Absorbances were determined for 415 nm following time. Flavonoid amounts for herbs were defined for dry weight of sample as mg quercetin.

**The Determination of Antioxidant Activity**

Contents antioxidant for plants were calculated utilizing by FRAP and CUPRAC techniques. FRAP test was utilized calculated antioxidant activity. The technique is reduction of $Fe^3$+-TPTZ compound to the $Fe^{2+}$ -TPTZ compound with electron giving material this situation (Benzie and Szeto 1999). The 100 µL of the test extract or the blank and 3 mL of FRAP test (including TPTZ, $FeCl_3$, and acetate buffer) were annexed and vortexed. The absorbance rates on 593 nm were determined for at 25°C about 4 min. Standard graph for 100-1000 µmol/L was compared by the last absorbance. The result was explained for dry sample as µmol $FeSO_4.7H_2O$.

CUPRAC technique is contained of stirring the antioxidant mixture and ammonium acetate aqueous (pH 7), a neocuproine alcoholic solution and copper (II) chloride solution, after calculating the advanced absorbance on 450 nm later 60 minutes (Apak at al. 2004). 1mL $NH_4Ac$ (1M), 1mL Neocuproine (7.5 mM) and 1mL CuCl2 (10) mM were annexed, after that 0.9 mL $H_2O$ and 0.2 mL sample annexed and vortexed. Latest amount was 4.1 mL. Later, the last absorbance value was calculated on 450 nm. Conclusions were appraised by Trolox ® equivalent antioxidant activity (TEAC).

The radical cleaning capacity of samples opposite DPPH● radical (2,2-diphenyl-1-picrylhydrazyl) was defined on 517 nm in spectrophometer. Analysis is situated on the color alter of the DPPH mixture. DPPH radical is deactivated from the antioxidants (Pokorny et al. 2001). Shortly, variety concentrations for 0.75 mL of each extracts were vortexed together with DPPH in methanol (0.1 mM, 0.75 mL). The radical deactivating ability was determined with utilizing Trolox (standard) and results are explained as $IC_{50}$ (concentration for samples that causes 50% cleaning of DPPH● radical).

**The Biological Materials**

12 microorganisms were utilized in this research (Table 1). All test microorganisms acquired from Karadeniz Technical University, Farabi Hospital, Trabzon, Turkey where the organisms were clinically isolated from patients. The microorganisms were stored at -80 °C in

the Microbiology laboratory, Faculty of Science at the Karadeniz Technical University, Trabzon, Turkey where the antibacterial tests were carried out. The strains were prepared for 24 h (37°C) on Mueller-Hinton agar before use. The nutrient-related microorganisms were utilized because they are often existed in nutrient.

**Table 1**. The name and ATCC numbers of microorganisms used in the experiments

| The Name | ATCC Numbers |
|---|---|
| **G+** | |
| ***Bacillus subtilis*** | ATCC 6633 |
| ***Enterococcus faecalis*** | ATCC 29212 |
| ***Staphylococcus aureus*** | ATCC 25923 |
| ***Staphylococcus epidermidis*** | ATCC 12228 |
| **G-** | |
| ***Escherichia coli*** | ATCC 25922 |
| ***Klebsiella pneumonia*** | ATCC 13883 |
| ***Pseudomonas aeruginosa*** | ATCC 27853 |
| ***Proteus vulgaris*** | ATCC 13315 |
| ***Salmonella typhimirium*** | ATCC 14028 |
| ***Yersinia pseudotuberculosis*** | ATCC 911 |
| ***Enterobacter cloaceae*** | ATCC 13047 |
| **Eukaryote** | |
| ***Candida albicans*** | |

## The Antimicrobial Activity

### Disc-diffusion assay

At first the antibacterial capacity of the samples was detected by means of the disc diffusion process which is widely used for quick screening of natural products (Ozer et al. 2007, Amelia et al. 2006, Murray et al. 1995). All extracts were dissolved in solvent (methanol); the final concentration was 10 µg/disc. Cultures of every bacteria were instilled to Muller-Hinton agar and waited for 16 hours (37° C), then their concentration adjusted to 0.5 McFarland standard turbidity (approximately 1 x $10^7$ - 1 x $10^8$ CFU/mL) with sterile 0.09% isotonic solution. One hundred micro liter of each bacterial suspension was put over the top of Mueller-Hinton agar in a 60-mm plate and extended with a Drigalski tip. The disc (6 mm in diameter) was embed with extracts and put on vaccinated Mueller-Hinton agar. Negative check was arranged utilizing the identical solvent (methanol) used to obtain the samples. Kanamycin was used as positive reference at 10 µg/disk (Sigma). The inoculated plaques were waited for 24 h for clinical microbial strains (37 °C) and at 35 °C for 48 h for yeast. The inhibition values were determined by a caliper thinking the all diameters. Experiment was performed in triplicate. The bacteria, inhibition zone in diameter ≥ 6 mm around the disks impregnated with methanol extract, were used for minimal inhibitory concentration (MIC).

### Minimal inhibition concentration (MIC)

The MIC rates were detected for the organism strains which were susceptible to the artificial extract in the disk diffusion process. Inoculum of the organism strains were obtained by 12 hours agar ethos and solutions were regulated to 0.5 McFarland control blur. Methanol added in extracts, were primary subtilized to the largest variety concentration (500 µg/mL) and after consecutive 2-layer subtilize were done to get a concentration value from 500 µg/mL to 0.49 in 1 mL sterile plates including Muller-Hinton broth. The MIC values of the artificial extracts apposite organism strains were detecting on the ground of a micro-well dilution process (Ozer et al. 2007, Amelia et al. 2006, Murray et al. 1995). Five hundred microliters by the existing mixtures of artificial extract got at the 5000 µg/mL was annexed into the primary sterile tube containing 4500 µl Muller-Hinton broth. Then, 2500 µL by the serial dilutions was carried into the eleven tubes. The last tube (twelve) is having 2500 µL of Muller-Hinton broth without substance. The final amount was 2500 µL. Kanamycin at a value of 500-0.49 µg/mL was obtained in Muller-Hinton broth and utilized as a standard medicine for positive check and with the vaccine on each band was utilized as a negative check. 96 plaques were made from applying 200 µL of Muller-Hinton broth having the diluted compound into every bore, and 5 µL of 0.5 McFarland from 12 h agar cultures were added into every bore. The plaque was veiled with a sterile plaque sealer. The amounts of every bore were waited (37°C) for 24 hours. MIC was obtained as the lowest concentration of sample to limit the large of bacterium.

## RESULTS AND DISCUSSION

It is known that flavonoids and are phenolic acids antioxidant substances. When the value of these substances is abundant, the antioxidant capacities of

herbs increase (Al-Mamary et al. 2002, Robards et al. 1999). Phenolic substances contribute to nutritional value and quality in terms of aroma and taste and also in supplying health helpful efficacy. They also give in herb defense mechanisms to counteract active oxygen species (ROS) for keep alive and forestall chemical harm and destruction of herbivores, insects and microorganisms (Vaya et al. 1997).

The total flavonoid amounts and total phenolic amount, CUPRAC and FRAP values were indicated in Table 2.

**Table 2.** Conclusions of FRAP, CUPRAC, phenolic and flavonoid contents for Centaury and Blackthorn species*

| Samples* | Total phenolics (mg GAE/g) | Total flavonoid (mg QE/g) | FRAP (µmol $FeSO_4.7H_2O$ /g) | CUPRAC (mmol TEAC/g) |
|---|---|---|---|---|
| H. bup. fgk | 14.96±0.42 | 97.45±2.20 | 103.90±1.99 | 1.07±0.14 |
| H. bup. lgk | 18.88±6.26 | 141.89±3.33 | 185.72±1.93 | 1.082±0.08 |
| H. bup. sgk | 6.05±0.62 | 40.37±0.58 | 55.3±1.45 | 0.54±0.02 |
| H. bup. fmt | 13.95±2.49 | 62.06±2.86 | 96.72±4.74 | 0.64±0.16 |
| H. bup. lmt | 5.93±4.71 | 101.48±4.82 | 164.80±12.38 | 1.12±0.13 |
| H. bup. smt | 8.32±0.97 | 32.75±0.85 | 62.29±0.47 | 0.16±0.01 |
| H. mon. fah | 22.48±0.66 | 25.84±2.21 | 57.98±2.57 | 0.24±0.02 |
| H. mon. lah | 21.77±3.28 | 168.45±1.52 | 380.92±4.78 | 2.25±0.08 |
| H. mon. sah | 3.01±0.10 | 26.23±1.11 | 54.46±0.52 | 0.23±0.05 |
| P. spi. fsgk | 4.35±0.25 | 9.73±0.97 | 35.09±1.85 | 0.19±0.05 |
| P. spi. lgk | 10.14±0.94 | 67.72±1.88 | 92.89±3.10 | 0.791±0.09 |
| P. spi. fsas | 8.24±5.99 | 11.74±2.61 | 59.41±1.32 | 0.45±0.06 |
| P. spi. las | 14.63±0.42 | 97.45±2.20 | 103.90±1.99 | 1.07±0.14 |

* H. bup. fgk: Flowers of *Hypericum bupleuroides* (Giresun-Kümbet), H. bup. lgk: Leaves of *Hypericum bupleuroides* (Giresun-Kümbet), H. bup. sgk: Stem of *Hypericum bupleuroides* (Giresun-Kümbet), H. bup. fmt: Flowers of *Hypericum bupleuroides* (Murgul-Tiryal), H. bup. lmt: Leaves of *Hypericum bupleuroides* (Murgul-Tiryal), *H.* bup. smt: Stem of Hypericum *bupleuroides* (Murgul-Tiryal), H. mon. fah: Flowers of *Hypericum montbretii* (Artvin-Hatila), *H.* mon. lah: Leaves of *Hypericum montbretii* (Artvin-Hatila), H. mon. sah: Stem of Hypericum *montbretii* (Artvin-Hatila), P. spi. fsgk: Flower seeds of *Paliurus spina-christi* Mill (Giresun-Kale), P. spi. lgk: Leaves of *Paliurus spina-christi* Mill (Giresun-Kale), *P.* spi. fsas: Flower seeds of *Paliurus spina-christi Mill* (Artvin-Seyitler), P. spi. las: Leaves of *Paliurus spina-christi* Mill (Artvin-Seyitler).

Results indicated that the highest phenolic amount to get from H. mon. fah and H. mon. lah while H. bup. lgk and H. mon. lah showed highest flavonoid contents. Between samples the highest value of flavonoid and polyphenols were obtained Centaury. In addition to these, H. mon. lah founded high capacity in accordance with the FRAP whereas H. mon. lah and H. bup. lmt founded high activity in accordance with the CUPRAC.

The $IC_{50}$ amounts measured from analysis of DPPH were indicated in Fig. 1. Although H. mon. lah and H. bup. lmt had the highest DPPH radical value, the lowest value was acquired by P. spi. las.

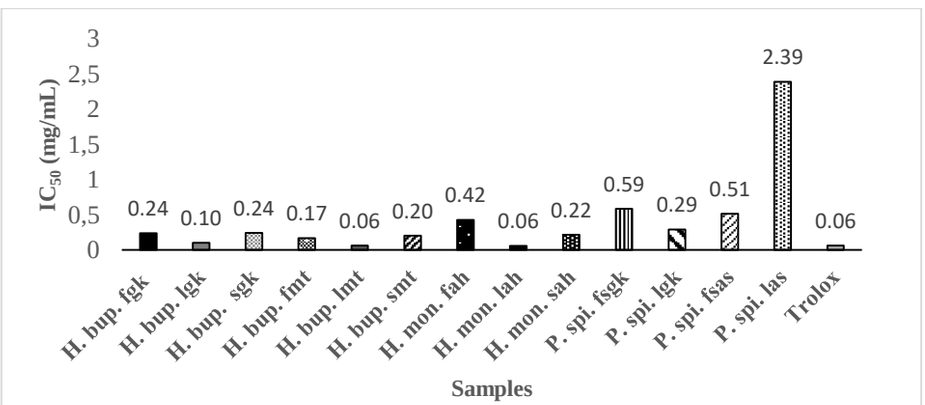


**Figure 1.** The results of DPPH for Centaury and Blackthorn species

The antibacterial properties of herb samples investigated on the microorganisms in the paper were quantitatively and qualitatively detected by appraising the existence, of inhibition regions, region diameter, and MIC rates. The findings of antibacterial property of methanolic extracts are indicated in Table 3.

Results obtained from disc diffusion process, evaluation of minimum inhibitory concentration (MIC), determined that *B. Subtilis* and *C. albicans* were the most sensitive microorganisms showing lowest MIC values 0.20 mg/mL. Extracts of *Hypericum bupleuroides, Hypericum montbretii, Paliurus spina-christi Mill*. showed antimbacterial property aopposite the tested microorganisms, but in flexible value. Findings are comparable to the kanamycine antibiotic, utilized as a positive control.

H. bup. lgk showed antimicrobial activity against 6 out of 12 microorganisms, H. bup. fmt, 5 out of 12 microorganisms and H. bup. fgk, P. spi. fsas, 4 out of 12 and P. spi. fsgk, H. mon. sah, H. mon. lah, H. bup. smt showed antimicrobial activity against 3 out of 12 microorganisms.

And the test samples indicated more potent capacity opposite Gram-positive than Gram negative bacteria.

Also, excellent antimicrobial capacity results were obtained on the test microorganism, yeast like fungi *Candida albicans* (Ca) with the mic values between 0.20 -1.56 µg/mL is better than the standard drug of kanamycine except for extracts of H. bup. sgk, H. bup. lmt, H. mon. fah and P. spi. las.

On the other hand, none of the extracts of plant exhibited the activity on the test microorganisms, *E. faecalis, K. pneumonia, P. aeruginosa, S. typhimirium*, *Y. pseudotuberculosis, E. cloaceae.* Extracts of H. bup. sgk, H. bup. lmt, H. mon. fah and P. spi. las did not shown any antimicrobial activity on the tested microorganisms.

**Table 3.** MIC degrees of herb extracts opposite the microbial strains tested

| Samples | Minimal Inhibition Concentration Values (µg/mL) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Bs | Ef | Sa | Se | Ec | Kp | Pa | Pv | St | Yp | Ec | Ca |
| H. bup.fgk | 0.20 | - | 50 | 12.5 | - | - | - | - | - | - | - | 1.56 |
| H. bup.lgk | 1.56 | - | 1.25 | 12.5 | 100 | - | - | 100 | - | - | - | 0.39 |
| H. bup.sgk | - | - | - | - | - | - | - | - | - | - | - | - |
| H. bup.fmt | 0.20 | - | 25 | 12.5 | 100 | - | - | - | - | - | -- | 0.39 |
| H. bup.lmt | - | - | - | - | - | - | - | - | - | - | - | - |
| H. bup.smt | 0.20 | - | - | - | 50 | - | - | - | - | - | - | 0.39 |
| H.mon.fah | - | - | - | - | - | - | - | - | - | - | - | - |
| H.mon.lah | 0.20 | - | - | 6.25 | - | - | - | - | - | - | - | 0.39 |
| H.mon.sah | 0.20 | - | - | - | 100 | - | - | - | - | - | - | 0.78 |
| P. spi. fsgk | 7.81 | - | 100 | - | - | - | - | - | - | - | - | 1.56 |
| P. spi. lgk | 0.20 | - | - | - | - | - | - | - | - | - | - | 0.39 |
| P. spi. fsas | 0.20 | - | 50 | - | - | - | - | 50 | - | - | - | 0.20 |
| P. spi. las | - | - | - | - | - | - | - | - | - | - | - | - |
| Kanam. | 0.20 | 6.25 | 0.78 | 0.39 | 1.56 | 0.39 | - | 0.19 | 1.56 | 0.78 | 1.56 | - |

Bs: *Bacillus subtilis* ATCC 6633, Ef: *Enterococcus faecalis* ATCC 29212, Sa: *Staphylococcus aureus* ATCC 25923, Se: *Staphylococcus epidermidis* ATCC 12228, Ec: *Escherichia coli* ATCC 25922, Kp: *Klebsiella pneumonia* ATCC 13883, Pa: *Pseudomonas aeruginosa* ATCC 27853, Pv: *Proteus vulgaris* ATCC 13315, St: *Salmonella typhimirium* ATCC 14028, Yp: *Yersinia pseudotuberculosis* ATCC 911, Ec: *Enterobacter cloaceae* ATCC 13047, Ca: *Candida albicans* ATCC 60193, Kanam.: Kanamycine, (—): no activity of test concentrations

In a study on the *Hypericum Perforatum* grown in Iran, the total phenolic content of extract was 505.7±18 mg GAE/g DS, the total flavonoid amount was 23.8±1.6 mg QE/g DS and DPPH activity was 96.0±3.7 mg/mL (Fathi and Ebrahimzadeh 2013). According to our results, *Hypericum bupleuroides* grown in Giresun-Kumbet, Murgul-Tiryal and *Hypericum montbretii* in Artvin-Hatila have more flavonoid contents and better DPPH activities.

Flavonoids are of high attention for their activities, which are essentially interested to their anti-oxidative contents (Côté et al. 2010). In this article, DPPH activities are

highly better than previous studies because our samples have more flavonoid contents. The reason of the differences between these studies can be depended on the growth of plants in different environmental conditions such as temperature, climate, rainfall, sunshine hours, the angle of sunlight and altitude.

In a study conducted in 2007, DPPH was used in order to determine antioxidant capacity and they reported that *Hypericum bupleuroides* and *Paliurus spina-christi* were promising plants as a source of natural antioxidant (Kırca et al. 2008). In parallel with the literature, as a result of the DPPH test performed on blackthorn extracts of P. spi. fsas grown in Artvin-Hatila have a good degree of DPPH activity. Also we found that P. spi. fsgk and P. spi. lgk obtained from Giresun have good-moderate antioxidant activity.

It was reported that the methanolic herb samples of *Paliurus spina-christi* had a total flavonoid amount of 0.16% in fruits, 0.45% in flowers and 0.66% in leaves (Brantner and Males 1999). However, there is not a comprehensive study on all antioxidant parameters of *Paliurus spina- christi Mill* extracts. We showed the DPPH activity, total phenolic and flavonoid contents of flower seeds, stem and leaves of *Paliurus spina- christi* Mill grown in different areas of eastern black sea region in detail. As a result, centaury species and blackthorn have effective antioxidant and antimicrobial activity results. Nevertheless, centaury species have better results than blackthorn in this article.

In literature, the antimicrobial capacity of some *Hypericum* extracts has been well-documented (Dall'Agnol et al. 2003, Reichling et al. 2001, Sakar and Tamer 1990). Researchers showed that *H. caprifoliatum* among six *Hypericum* species was the most effective opposite *S. aureus*, followed by *H. myrianthum.* They also reported that *H. polyanthemum* and *H. ternum* samples were effective opposite *B. Subtilis* but the significant difference between the extracts and the negative controls was observed in the assay with *S. epidermidis, E. coli* and *S. Cerevisiae* (Dall'Agnol et al. 2003). Although there have been several researches on the antimicrobial activity of *Hypericum* species, the antimicrobial activity of *Hypericum bupleuroides* and *Hypericum montbretii* were not reported in detail. We found that the most samples of *Hypericum bupleuroides* and *Hypericum montbretii* have antimicrobial activity with the mic values between 0.196-1.56 µg/mL which is better than the standard drug of kanamycine. A. Brantner et al. reported that *Paliurus spina-christi Mill* has antimicrobial capacity opposite all tested Gram-positive organism (*Streptococcus faecalis*, *Micrococcus luteus* and *Staphylococcus aureus*) by building obvious inhibition areas among 8.5 and 14.0 mm in diameter (Brantner et al. 1996). In this article, we also determined that *Paliurus spina-christi* Mill had antimicrobial activity against *Staphylococcus aureus* and *Proteus vulgaris.*

## CONCLUSIONS

The results of this research show that the herb samples of Centaury and Blackthorn contain compounds with antifungal, antimicrobial and antioxidant property. The changing of synthetical with native antioxidants may be favourable. Based on these findings, it is feasible to finalize which methanolic samples of *Hypericum bupleuroides, Hypericum montbretii, Paliurus spina-christi Mill* can be the potent source of natural antioxidants. The acquired findings may be thought adequate to further researches for the insulation and recognition of the effective guidelines and to appraise of feasible synergism between extract samples for their antimicrobial and antioxidant activity. Researches are in progression to establish the value of toxicity of these herb samples.

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