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Research Article

Chemical and bioactive potential of the nests of *Polistes nimpha*, *Polistes dominula*, and *Vespa crabro* (Hymenoptera: Vespidae)

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Abstract: This work was accomplished to establish the chemical components and bioactivity potential of the nest materials of *Polistes nimpha* (Christ), *Polistes dominula* (Christ), and *Vespa crabro* (L.). The biological and chemical compounds of materials and their molecular functionalities were detected using FRAP (ferric reducing antioxidant power) method, DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method, and Energy Dispersive X-Ray Analysis. Finally, the bioactivity potentials of nest extracts were assayed and phenolic components were determined. C, N, O, Na, Ca, K, Mg, Al, Si are the elemental components of the nest materials. All nest extracts of three species had high biological activity against nine bacteria and one fungus causing common infections. The maximum antibacterial and antifungal activity was seen when gram-negative *Pseudomonas aeruginosa*, gram-positive *Bacillus cereus*, and gram-positive *Candida albicans* were exposed to ethanol extracts of nest samples in 25 µL. High antioxidant activity can be found in the nest extracts. These extracts might help researchers find novel antifungal and antibacterial substances.

1. INTRODUCTION

Antioxidants are substances that inhibit oxidation, a chemical reaction that produces free radicals which can damage cells. Antioxidants neutralize these free radicals, thereby preventing or reducing cell damage. Antimicrobial activity refers to the ability of a substance to kill or inhibit the growth of microorganisms, such as bacteria, fungi, and viruses. Antimicrobial agents, including antibiotics, antiseptics, and natural compounds like essential oils, play a critical role in preventing and treating infections. This activity is crucial in both medical and food preservation contexts to control harmful microbial growth. All of the eusocial and solitary wasps that are known belong to the Vespidae family. The exquisite nests that Vespidae species use to manage their colonies draw notice. According to research on the nests of eusocial wasps, these nests typically consist of hexagonal cells dangling from a petiole, with the cells typically facing downward (Reeve, 1991; Wenzel, 1998). These cells serve as a breeding environment

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for the wasp as it grows from an egg to an adult. The female wasps construct and enlarge the nests (Jeanne, 1975). The salivary fluids and chewed-up plant and wood fibers make up the paper nest (Evans & West Eberhard, 1970).

In social wasps, the nest is regularly plastered with salivary by the adult wasp to protect the nest from changes in weather conditions (Kudô, 2001). The adult wasp also coats the inside of the brood cell with saliva. The pupating larva secretes and spins the silk. The silk covers the entrance of the brood cell. However, a sterile room is created inside the cell for the pupa to develop, Vespid larvae are exposed to life-threatening pathogens in closed cells (Kirshboim & Ishay, 2020). The possibility of transmission of infections is high due to close contact between wasps living in the colony. Therefore, colonial immunity has developed. Social insects can also produce antimicrobial agents from glands against pathogens (Bot *et al.,* 2002; Turillazzi *et al.,* 2004). Honey, royal jelly, and propolis as bee products have high antimicrobial activity (Anderson *et al.,* 2011). Venom components of some ants, bees, and wasps exhibited antimicrobial activity (Choi & Lee, 2020; El-Seedi *et al.,* 2020; Yacoub *et al.,* 2020; Wen *et al.,* 2021). Nevertheless, there is only a little evidence available about the antibacterial and antioxidant qualities of Vespidae nesting materials.

Materials from the nests of *V. crabro*, *P. dominula*, and *V. crabro germana* were reported to have antibacterial and antioxidant activities (Erturk & Bagdatli, 2019; Ertürk & Şimşek, 2020). Physicochemical properties of the nests of *Vespa orientalis*, *Vespa crabro* (Bagriaçık, 2011); *Polistes gallicus*, *Polistes dominulus*, *Polistes nimpha* (Bagriaçik, 2012, 2013a); *Dolichovespula media*, *Dolichovespula sylvestris* (Bağriaçık, 2013b); *Dolichovespula saxonica* (Ertürk, 2017); *Vespa crabro* (Ertürk & Bağdatlı, 2018) in Türkiye from different regions were previously determined.

In this study, we sought to explain the chemical characteristics and biological activity of the nest components of three species *P. dominula, P. nimpha*, and *V. crabro* found in Türkiye's Black Sea region, as well as to identify the nests' antimicrobial and antioxidant qualities and bioactive potential.

2. MATERIAL and METHODS

2.1. Collecting of Nesting Materials

The nesting materials of P. *nimpha* and P. *dominula* gathered from the stone wall and greenhouse in Terme district of Samsun province (36° 58′ E, 41° 12’ N) and V. *crabro’s* nest from hazelnut tree branches in Trabzon province (39° 43′ E, 41° 00’ N) in July and August 2019. A single nest from each species was researched. Samsun and Trabzon provinces are located in the Black Sea Region of Türkiye with a warm temperate climate, full humidity, and floras (Kottek *et al.,* 2006). Before starting the study, the nests were purified from eggs, pupae, and larvae and stored in the freezer.

2.2. Investigation of Thin Surface Structure

Surface scanning and elemental composition definition analyses of the surface of the nest materials were accomplished with SEM and SEM/EDX techniques with an instrument of SEM-Hitachi, SU1510'. Fixed samples were sputter-coated with a gold layer. Ten to thirty mA electric current was applied during coating for 1 min. little pieces from the hornet and paper wasps nest walls were observed with a stereomicroscope (Leica S8APO).

2.3. Water Vacuum Capacity (%) Determination

The nest examples were cut into small pieces and engrossed in water for one minute. After the submersion process, the samples were reweighed. Percentage capacity of absorption was calculated with the following equation where m1 is the weight of the dried sample before immersion and m^2 is the weight of the sample after immersion: [(m2-m1) / mL] × 100 (Curtis *et al.,* 2005).

2.4. Plant Material and Oral Secretion (%) Determination

The broken pieces of dried nest ingredients were released in 0.5 N KOH solution at 70 °C for two hours. After the process, the samples were filtered with weighed filter papers and kept in a laboratory oven until dry and then the samples were reweighed and the rate of plant material and oral secretion was identified with the following formula where m1 is the weight of the dry sample before the process and m2 is the weight of the sample after the process: Fiber (cellulose) = (m^2 / mL) × 100 (Yamane *et al.*, 1999).

2.5. Oil Content (%) Determination

To calculate the organic compounds like oil and resin in the nests of P. *nimpha*, P. *dominula*, and V. *crabro*, small pieces of paper from the nest were weighed. Each sample was kept engrossed in the petroleum benzine for one hour after which both small pieces of the nest were retrieved. The distinction between the two weights gives us the amount of oily substance. The computation was modified from Yamane *et al.* (1999).

2.6. Bacterial Strains and Growth Conditions

Strains of bacteria and fungi were obtained from ATCC (American Type Culture Collection). The antimicrobial activity of the nest samples was studied using ten bacteria (five gram-positive: *Pseudomonas aeruginosa* ATCC®27853 Gram (-), *Escherichia coli* ATCC®25922 Gram (-), Klebsiella pneumoniae ATCC®13883 Gram (-), Citrobacter freundii ATCC® 43864 (-),*Bacillus subtilis* B209, Gram, Staphylococcus aureus ATCC 6538 Gram (+),*Yersinia enterocolitica* ATCC®27729 Gram (-), *Bacillus cereus* ATCC®10876 Gram (+), *Enterococcus feacalis* ATCC® 29121(+),Mueller Hinton Agar (MHA, Merck) or Mueller Hinton Broth (MHB, Merck) *Candida albicans* ATCC®10231and Sabouraud Dextrose Broth (SDB, Difco) or Sabouraud Dextrose Agar (SDA, Oxoid) were used for growing bacterial and yeast or fungal cells, respectively. For the definition of antibacterial and antifungal efficiency, the diffusion disk plates method was used (Ertürk, 2006). 25µL volume of 46 mg extract 1 mL^{-1} ethanol was used for the analyses.

2.7. Preparation of Extraction of Samples

3 g of a sample placed in an equal volume (30 mL) of 100% methanol was stirred continuously for twenty-four hours at room temperature with a shaker. A syringe filter and filter paper were used to separate the particles (0.45µm). The final concentration of the samples was determined with 100% methanol. The prepared methanolic extract, which was divided into two, was used in antioxidant test and phenolic compound assay.

2.7.1. *Evaluation of total polyphenolic content*

The total polyphenol content (mg GAE/g sample) in the examples was studied using the Folin - Ciocalteu reagent method by Slinkard & Singleton (1977). Following the addition of 680 µL of distilled water to the samples and standards, 400 µL of the 0.2 N Folin-Ciocalteu Reagent, 20 µL of the extract, and the standard solution were added to the mixture. Finally, 400 µL of Na_2CO_3 (10%) was added and vortexed after three minutes had passed. The combination was then allowed to sit at room temperature for 2 hours, after which a UV spectrophotometer was used to measure the mixture's absorbance at 760 nm. The measurement standard utilized was gallic acid. TPC was measured in milligrams of equivalent gallic acid per gram.

2.7.2. *Evaluation of total flavonoid content*

Using the aluminum chloride colorimetry method described by Fukumoto & Mazza (2000), the total flavonoid content (TFC) of extracts was measured. Following that, 2.15 mL of pure methanol was combined with 0.25 mL of both the extracts and the reference solution. 50µL of 10% aluminum chloride and 50 µL of 1M potassium acetate then were added. The mixture's absorbance at 415 nm was measured after 40 min of incubation at room temperature. The

benchmark used was quercetin. The results were presented as milligram quercetin equivalent per gram of material.

2.7.3. *FRAP assays*

The FRAP test was performed using the Benzie & Strain' method (1999). An iron 2, 4, 6-tripyridyl-s-triazine complex (Fe^{3+} -TPTZ) is reduced to its ferrous, colored form (Fe^{2+} -TPTZ) in the presence of antioxidants to create the technique. The method is based on the reduction of to its ferrous, The FRAP reagent contained acetate buffer (300 µM, pH3.6) a solution of 10 µM TPTZ in 40 µM HCl and 20 µM $FeCl_3$. The reagent was prepared daily. 100 µL of samples were mixed with a 3 mL FRAP reagent. The absorbance of the reaction mixture was spectrophotometrically at 593 nm after incubation for 4 min. Standard solutions of $FeSO_4 7H_2O$ (31.25-1000 µM) were used for the calibration curve and the results were expressed FRAP as µmol $FeSO_4.7H_2O$/g.

2.7.4. *DPPH radical scavenging activity*

The method was used for the DPPH assay (Molyneux, 2004). To make the stock DPPH solution, 4 milligram of DPPH was dissolved in 100 mL of methanol. For each sample, 0.75 mL of the extracts were combined with 0.75 mL of the DPPH solution at six different concentrations, and the mixture was vortexed. The mixture's absorbance was measured at 517 nm after the reaction had been allowed to occur at darkroom temperature for 50 min. Trolox served as the reference point for calculations.

2.8. Determination of the Phenolic Composition of Samples by RP-HPLC-UV

20 mL of methanolic extracts that had been prepared for phenolic analysis were received, and they were evaporated using a rotary evaporator at 40° C until dry. 10 mL of acidified distilled water were used to dissolve the residue (pH 2). With consecutive additions of 5 mL of ethyl acetate and 5 mL of diethyl ether, liquid-liquid extraction was performed (Kim *et al.*, 2006). These phases were then dried by rotary evaporation at 40°C (IKA-Werke in Staufen, Germany). The dried sample was redissolved in 2 mL of methanol, passed through RC-membrane syringe filters (0.45 µm), and then injected into the HPLC.

3. RESULTS

3.1. Plant Material (%), Oral Secretion (%), Water Absorption (%), and Oil (%)

The proportion of the plant material to the oral secretion utilized in the building of the nest materials was determined as a percentage. Samples were dowsed in warm KOH solution, the fibers were independent and the oral secretion dissolved. the samples for the *P. dominula* and *V. crabro* nest materials have an analogous proportion of plant material to the oral secretion of % 19.205 and 28.846%, respectively. The amount of oral secretion in nest material of P. *nimpha* was calculated at 30.322%, the water absorption capacity of *P. dominula* and *P. nimpha* is 242.71% and 154.354% while V. *crabro* nest is 283.01%. In addition, the oil percentages of the samples were also determined. The results show that the oil content of the nest of *P. dominula* (15.562 %) is higher than the nest material of *V. crabro (*10.256%). However, the highest oil content was found in *P. nimpha* 18.064 % (Table 1).

Table 1. Water absorption capacity with plant material, oral secretion and oil content of the nests of *V. crabro* (VC) *P. dominula* (PD) and *P. nimpha* (PN).

	(VC)	(PD)	(PN)
Dry weight (mg)	0.312	0.302	0.310
Water absorption capacity (%) determination	1.195 %283.01	1.035 %242.71	0.804 %154.354
Plant material and oral secretion (%) determination	0.222 % 28.846	0.244 % 19.205	0.216 %30.322
Oil content (%) determination	0.280 % 10.256	0.255 % 15.562	0.254 % 18.064

3.2. Surface Properties

3.2.1. Observation of the surface under a light microscope

The basic constructions of the nests' surfaces were monitored with a stereomicroscope. The nest colors of the samples of *P. dominula* and *P. nimpha* were dark brown, black lines, light brown, beige and white highlights whereas the nest colors of the samples of *V. crabro* were brown, light creamcolored tapes with thick stripes, bright brown spots on a dark brown background and rough structure. The honeycomb cover colors of the samples of three wasp species were bright and off-white color. However, the fibers of the *V. crabro* slot had thin, dense, thin spherical structures between the fibers and grooved filaments. The fibers of the nest of *P. nimpha* and *P. dominula* were longer and thicker, there were gaps between the vegetable fibers, the fibers were cross-linked and wavy. Both nest looked very similar to each other. The honeycomb sheaths of the three wasp nests examined were quite different, the fibers were very prominent, and it was found that the structure was persistent on the ground (Figure 1A, 1B, 1C and 1D).

Figure 1A. The outer surface of the section and thickness of the fibers the nestwall of *P. dominula.*

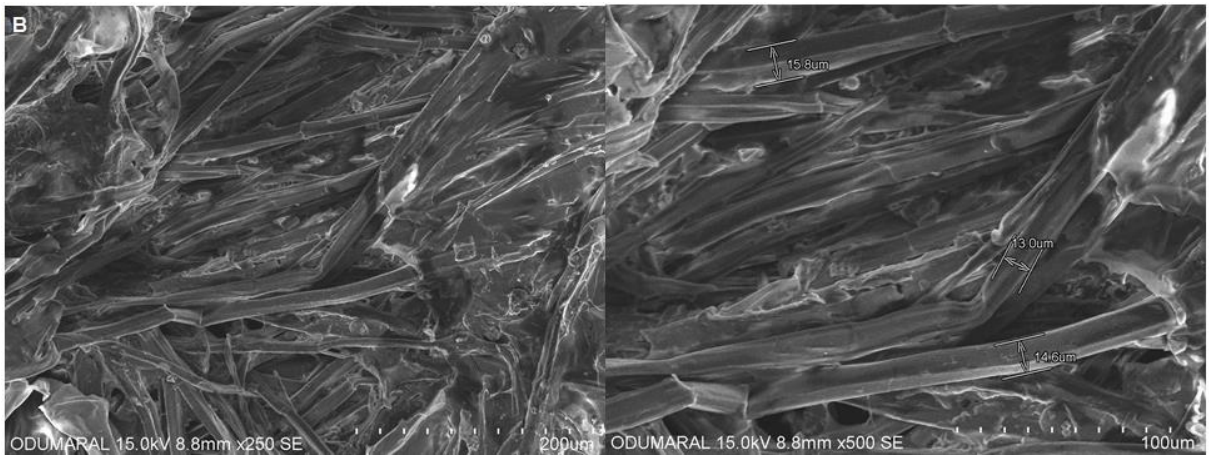

Figure 1B. The inner surface section of the section and thickness of the fibers the nestwall of *P. dominula.*

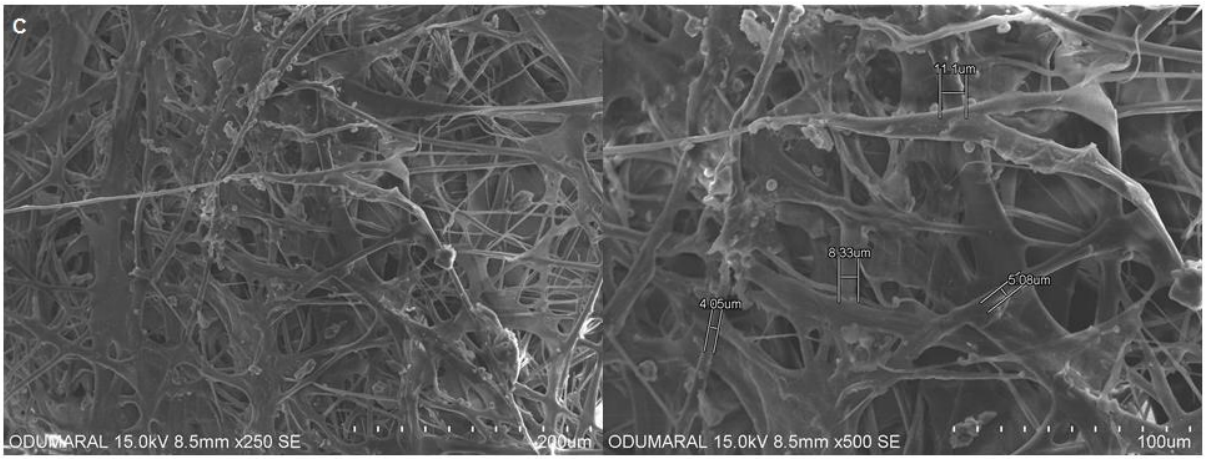

Figure 1C. The thickness of the fibers honeycomb cover of *P. dominula.*

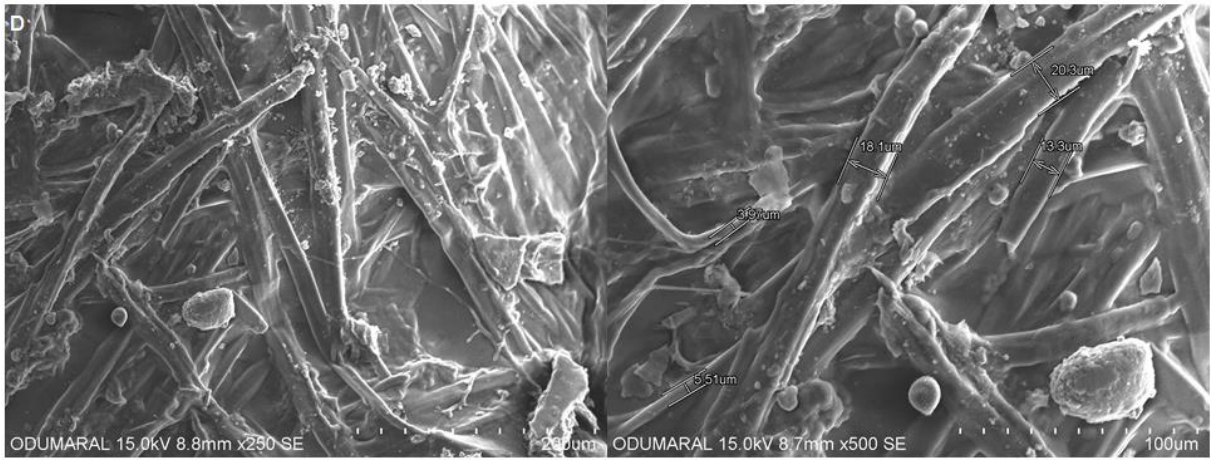

Figure 1D. The outer surface of the section and thickness of the fibers the nestwall of *P. nimpha.*

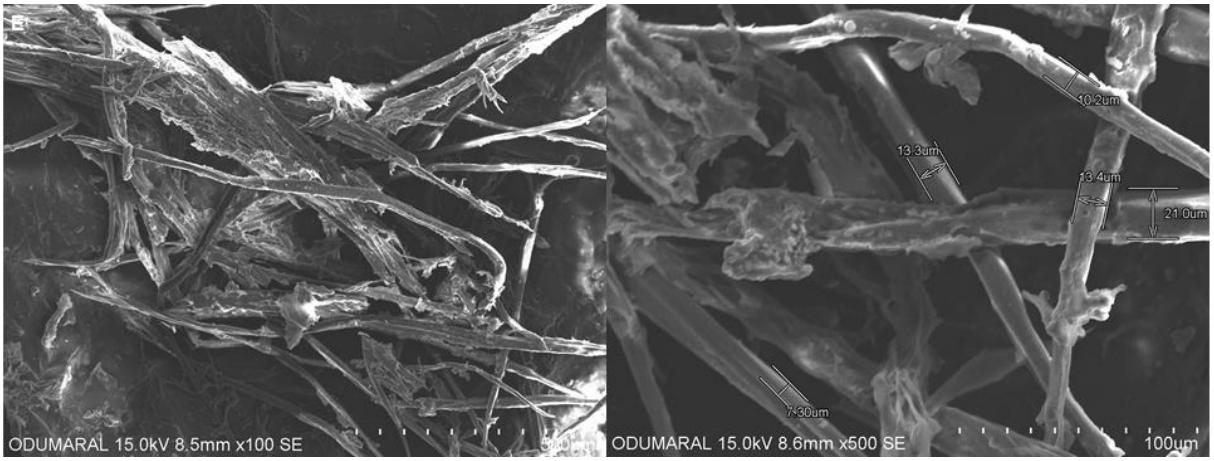

Figure 1E. The inner surface section of the section and thickness of the fibers the nestwall of *P. nimpha.*

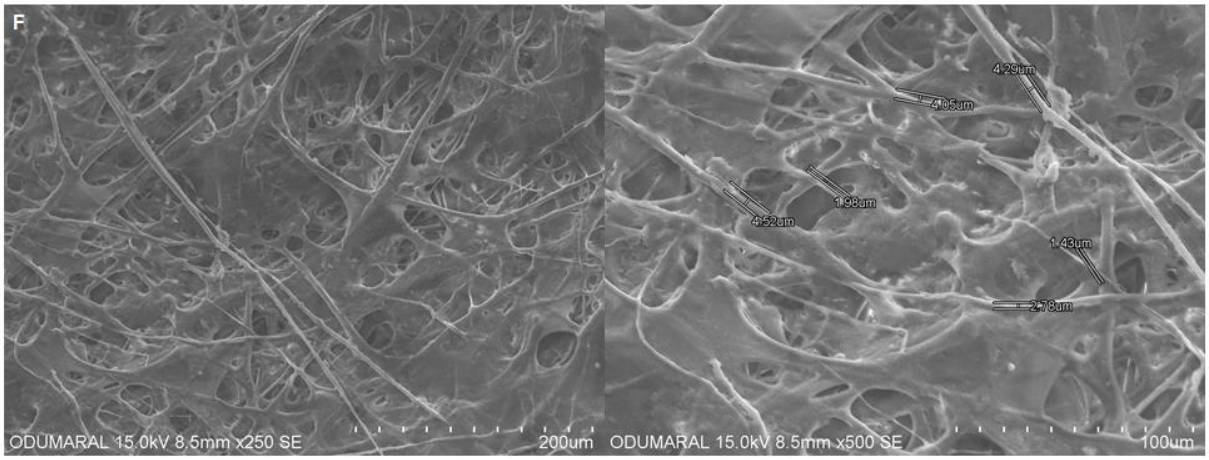

Figure 1F. The thickness of the fibers honeycomb cover of *P. nimpha.*

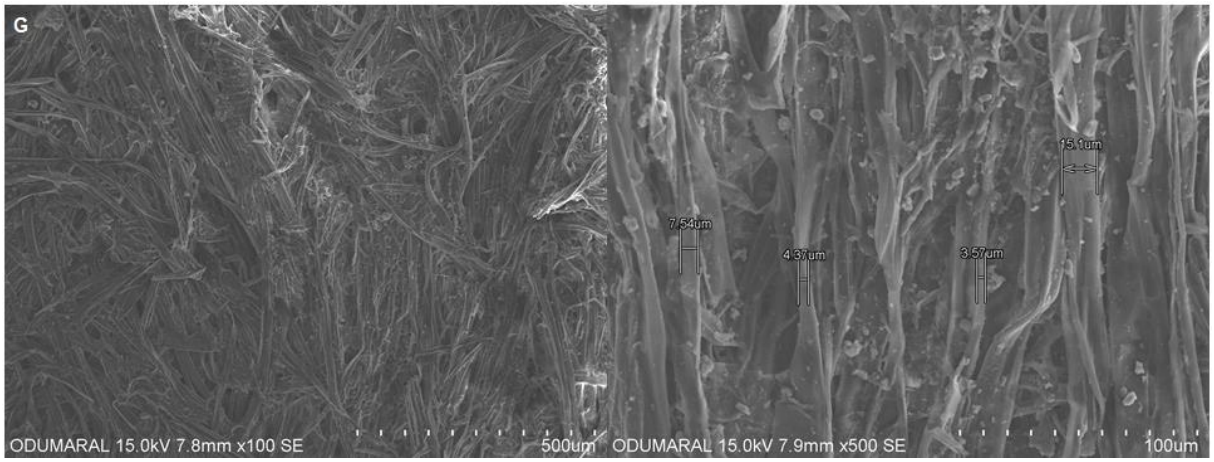

Figure 1G. The outer surface of the section and thickness of the fibers the nestwall of *V. crabro.*

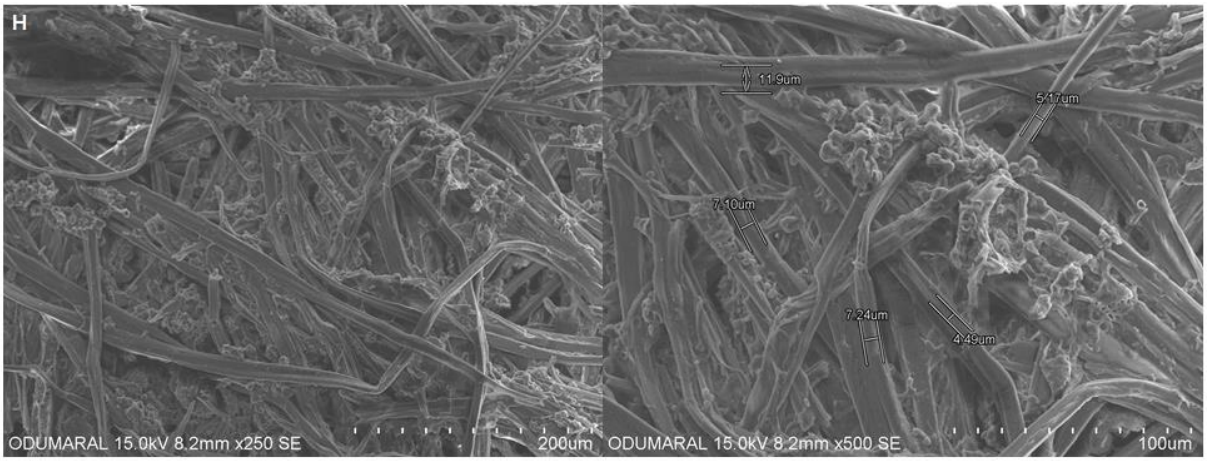

Figure 1H. The inner surface section of the section and thickness of the fibers the nestwall of *V. crabro.*

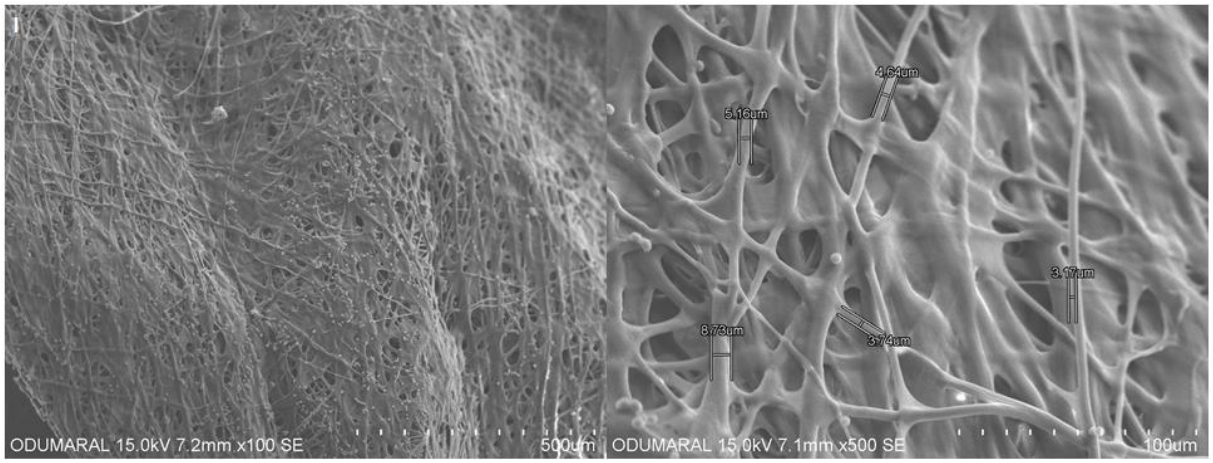

Figure 1I. The thickness of the fibers honeycomb cover of *V. crabro.*

3.2.2. *Observation of surface under SEM*

The oral secretions from *P. dominula*, and *V. crabro* nest were the mixture of salivation and plant fibers was seen as a thin layer. and the saliva including the plant fibers shone as thin varnished beads in the SEM micrographs. Results are as in Table 2. Although *P. nimpha* and *P. dominula* were different species of the same genus, the nest fibers were very different. The oral secretions from *the P. nimpha* nest were seen as a membrane composed of both small and largely organic and inorganic fragments with thick fibers passing through the middle. Many inorganic particles were seen in the SEM micrographs (Figure 1C, 1D) The average fiber thickness of the envelope of *the P. dominula* nest was calculated as 6.03±0.053 µm (min. 5.12±0.543 mm - max. 6.98±0.042 mm) and that of *V. crabro* nest as 7.52±0.052 mm (min. 5.85±0.032 mm - max. 8.15±0.096 mm), (n = 30 for each nest) (Figure 1A, 1B). In contrast, the plant fibers of *P. dominula* were long, thick woody scrapings, that were glued regularly. The plant fibers of *V. crabro* were short, thin woody scrapings. The average fiber thickness of the envelope of *P. nimpha* nest was calculated as 4.10±0.067 µm (min. 3.88±0.345 µm - max. 4.78±8.534 µm) They were intertwined and disordered (Figure 1E-1H). The measurements of the edge length, diameter and depth of the combs' cells of *V. crabro, P. dominula* and *P. nimpha* were as min., max. and average was calculated (Table 3). The surface of the comb of *V. crabro, P. dominula* and *P. nimpha* were medium 254.340 cm^2, 153.860 and 113.040 cm^2 respectively.

Table 2. The thickness of the fibers of the nest (in µm).

Bee species	Big PD			Small PD			TR bee		
Thickness (µm)	outer surface	inner surface	honeycomb cover	outer surface	inner surface	honeycomb cover	outer surface	inner surface	honeycomb cover
Min.	8.3	13.0	4.1	4.0	7.3	1.4	3.6	4.5	3.2
Max.	14.9	15.3	11.1	20.0	21.0	4.6	15.1	11.9	8.7
Avarage	10.9±0.8	14.7±0.7	7.1±0.5	12.8±0.7	13.1±0.1	3.9±0.7	7.7±0.3	5.1±0.6	5.1±0.5

3.3. Antimicrobial Test

The antibacterial and antifungal activities of extracts of the three different nests were assayed in vitro agar disc diffusion method against 9 bacteria and one fungi species. Table 3 summarizes the microbial development inhibition of the ethanol extracts of the screened nests. The three ethanol nest extracts demonstrated antibacterial and antifungal activity. While the ethanol extracts of almost all the nests showed antibacterial and antifungal activity towards one or more bacterium against all the microorganisms used in this study. The alcohol PD-and VC-A extract of the nest demonstrated antibacterial activity (18.59±0.54 and 20.59±0.021 mm 25 μL^{-1} inhibition zone) against S. *aureus*. and B. *ceraus*, respectively, while the alcohol PN-A extract

of the nest demonstrated the highest antibacterial activity (19.13±0,53 -25.66±0.21, mm 25 μL^{-1} inhibition zone) against, S. *aureus* and B. *ceraus*, respectively, and the highest antifungal activity (19.45±0,00 mm 25 μL^{-1} inhibition zone) against C. *albicans*. The ethanol extracts of the investigated nest samples of 25 µL (from 1 mg mL^{-1}) showed maximum antibacterial and antifungal activity against gram-negative *P. aeruginosa*, gram-positive *B. cerausand C. albicans* (see Table 3).

Table 3. Results of antimicrobial screening of hornet and wasp nests' *V. crabro, P. dominula* and *P. nimpha* extracts determined by the agar diffusion method; VC-A, PD-A, PN- A V. *crabro*, P. *dominula* and P. *nimpha* extracts in ethanol respectively

Bacterias	*Vespa crabro*	*Polistes dominula*	*Polistes nimpha*	Ampicillin	Cephazolin	Nystatin
E. coli	14.20±0.54	17.78±0.46	16.80±0.64	19.00±0,00	19.00±0.00	NT
C.freundii	6.00±0.00	6.00±0.00	6.00±0.00	16.53±0.55	16.36±0.06	NT
P. aeruginosa	15.84±0.46	17.66±0.33	16.33±0.56	32.26±0.46	28.33±0.03	NT
K. pneumoniae	14.27±0.78	14.48±0.54	15.70±0.23	15.27±0.10	17.27±0.01	NT
S. aureus	18.59±0.54	18.32±0.72	19.13±0.53	10.76±0.45	6.00±0.03	NT
B. subtilis	16.60±0.73	15.61±0.75	17.81±0.33	32.60±0.32	34.26±0.11	NT
Y.enterocolitica	6.00±0.00	6.00±0.00	6.00±0.00	26.66 ±0.57	34.33 ±0.57	NT
B. ceraus	20.59±0.02	20.50±0.34	25.66±0.21	26.59±0.021	27.50±0.03	NT
C. albicans	19.45±0,00	18.53±0.00	15.50±0.12	NT	NT	17.00±0.00
E. fecalis	14.53±0.55	13.23±0.23	13.32±0.42	32.50±0.06	24.27±0.04	NT

-: no inhibition, NT: Not tested,.*Pseudomonas aeruginosa* ATCC®27853 Gram (-), *Escherichia coli* ATCC®25922 Gram (-), *Klebsiella pneumoniae* ATCC®13883 Gram (-),*Citrobacter freundii* ATCC® 43864 (-),*Bacillus subtilis* B209, Gram, *Staphylococcus aureus* ATCC 6538 Gram (+),*Yersinia enterocolitica* ATCC®27729 Gram (-), *Bacillus cereus* ATCC®10876 Gram (+), *Enterococcus feacalis* ATCC® 29121(+), *Candida albicans* ATCC®10231

3.4. EDX Analysis

The outcomes demonstrate that the main elements of the surfaces are carbon, oxygen and nitrogen followed by potassium and calcium atoms in descending order (see Table 4). Minor amounts of sodium, magnesium, iron, aluminum, chlorine and silicium were also detected. P. *dominula's* inner and outer membrane was not found in nitrogen. In contrast, high levels of nitrogen were found in comb materials of a nest of *P. nimpha* and *V. crabro*. However, nitrogen was found to be highest in the honeycomb cover of all three wasp nests. EDX spectra for the inner surface of *P. dominula* were shown in Figure 2 as a representative example.

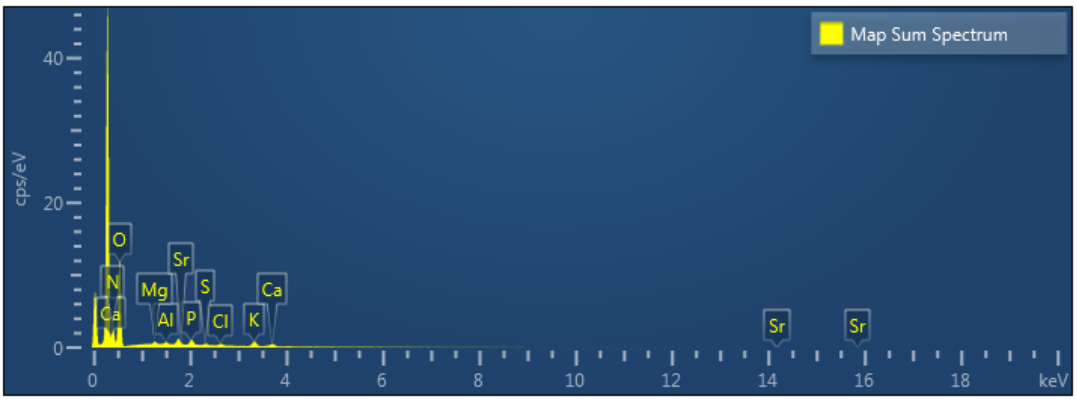

Figure 2. EDX spectrum of the elements embedded in the inner comb surface of *V. crabro (VC) P.nimpha (PN)* and *P. dominula (PD).*

In this essay, elemental analysis was carried out with extracted samples of nest materials. Concerning the outcomes of the elemental analysis (see Table 4), it is shown that the greatest nitrogen content was in VC- PD, honeycomb cover material, while the PN of honeycomb cover material is less than the other nest material. The highest carbon content in the PN-outer surface, the highest oxygen content in PD- inner surface and the highest potassium.

Table 4. EDX analyses of the inner and outer parts of the comb surfaces and honeycomb cover *V. crabro* (VC) *P. nimpha* (PN) and *P. dominula* (PD).

	Weigth %			Weigth %			Weigth %		
Element	*P. dominula* outer surface	*P. dominula* inner surface	*P. dominula honeycomb cover*	*P. nympha* outer surface	*P. nympha* inner surface	*P. nymphaof honeycomb cover*	*V. cobra* outer surface	*V. cobra* inner surface	*V. cobra of honeycomb cover*
C	58.75	54.17	52.03	55.87	52.98	52.71	55.12	52.79	43.10
O	40.16	45.32	27.05	26.85	33.43	29.94	30.89	32.48	30.07
Si	0.23	0.00	0.00	0.32	1.51	0.00	0.09	0.52	0.00
Na	0.11	0.00	0.00	0.00	0.00	0.00	0.03	0.05	0.00
Ca	0.21	0.16	0.00	0.33	0.05	0.42	0.06	0.06	0.08
K	0.22	0.29	0.30	0.54	0.24	0.34	0.04	0.22	0.00
P	0.07	0.06	0.16	0.36	0.10	0.34	0.04	0.14	0.01
S	0.07	0.00	0.18	0.12	0.07	0.09	0.04	0.09	0.06
Mg	0.04	0.00	0.00	0.13	0.00	0.29	0.03	0.07	0.09
Al	0.10	0.00	0.05	0.10	0.00	0.00		0.00	0.00
CI	0.03	0.00	0.16	0.15	0.07	0.41	0.08	0.08	0.38
N	0.00	0.00	20.06	15.11	11.55	15.47	13.47	13.50	26.21
Sr	0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.00	0.00
Total	100	100	100	100	100	100	100	100	100

3.5. Total Phenolic and Total Flavonoid Content

Electron transfer-based methods measure the ability of the antioxidant to reduce oxidants by color change. The antioxidant concentration of the samples and the degree of color change are related. ET-based methods include total Folin-Ciocalteu reagent and total phenolic method, iron ion reducing antioxidant power (FRAP), DPPH (2,2-diphenyl 1-picrylhydrazyl). In this study, nest materials belonging to *P. nimpha and P. dominula* species collected from Samsun *and V. crabro* species collected from Trabzon were used.

To study, nest materials belonging to *P. nimpha* and *P. dominula* species collected from Samsun *V. crabro* specie collected from Trabzon. The phenolic contents of 3.121 *P. nimpha*, 1.881 *P. dominula* and 0.635 mgGAE / g *V. crabro* were determined. For Total flavonoid content, *P. nimpha* had the highest amount of TFC (0.853), followed by *P. dominula* 0.425 mgQE/g while *V. crabro* could not be found (see Table 5).

Table 5. Total Polyphenol, Flavonoid Content, FRAP and DPPH tests results of samples.

Samples	TP (mgGAE/g sample)	TF (mgQE/g sample)	FRAP (µmol$FeSO_4 7H_2O$/g	DPPH SC_{50} (mg/mL)
P. *nympha*	3.121±0.017	0.853±0.002	24.645±0.104	3.185±0.061
P. *dominula*	1.881±0.054	0.425±0.006	21.639±1.356	4.325±0.027
V. *crapro*	0.635±0.067	-	11.978±0.075	6.338±0.029
Trolox				0.0004±0.000

3.6. FRAP and DPPH Assays

The FRAP test is a measure of the sample's Fe^{+3} Fe^{+2} reduction capacity. The samples from the *P. nimpha* and *P. dominula* showed a high ferric ion reducing capacity, 24.645 and 21.639 µmol$FeSO_4 7H_2O$/g whereas *V. crabro* the low 11.978 µmol$FeSO_4 7H_2O$/g (see Table 5).

The radical scavenging ability of samples was determined by DPPH decoloration assays in comparison with Trolox. The DPPH radical scavenging test measures the free radical scavenging activity of samples in solution, by granting one electron (Huang *et al.*, 2005). The radical scavenging activity of the samples was found to be 3.185 *P. nimpha*, 4.325 *P. dominula*, and 6.338 *V. crabro* mg / mL (see Table 5).

3.7. Phenolic Compounds

High-performance liquid chromatography (HPLC) is the most widely used for the characterization of polyphenolic compounds. In this study, the phenolic composition of the samples was determined by RP-HPLC-UV. Epicatechin, resveratrol, and rutin could not be detected in all three samples, while other components were detected in samples at different rates (see Table 6). As major components were protocatechuic acid, chrysin, myricetin, *P. nimpha* catechin, protocatechuic acid, chrysin, caffeic acid, *P. dominula* chrysin, myricetin was detected in *V. crabro*. In all three samples, chrysin was detected as the major component.

Table 6. Phenolic compounds of samples.

Standards	*P. nympha* (µg phenolic/g sample)	*P. dominula* (µg phenolic/g sample)	*V. crapro* (µg phenolic/g sample)
Gallic Acid	2.043	1.302	n.d.
Protocatechuic Acid	35.304	35.256	2.651
p-OH Benzoic Acid	13.208	13.284	2.461
Catechin	28.644	20.418	6.413
Caffeic Acid	10.144	42.022	7.045
Syringic Acid	15.382	21.931	4.778
Epicatechin	n.d.	n.d.	n.d.
p-Coumaric Acid	28.002	15.106	5.123

Table 6. Continues.

Ferulic Acid	11.606	8.284	16.849
Rutin	n.d.	n.d.	n.d.
Myricetin	30.627	15.456	26.400
Resveratrol	n.d.	n.d.	n.d.
Daidzein	1.821	n.d.	2.391
Luteolin	17.282	10.573	9.476
t-Cinnamic Acid	n.d.	0.423	0.751
Hesperetin	1.791	2.001	3.026
Chrysin	30.068	22.326	29.652
Pinocembrin	18.054	4.617	3.541
CAPE	20.615	1.685	4.301

*n.d.:not detected.

4. DISCUSSION and CONCLUSION

The topic materials of the work are *P. nimpha*, *P. dominula* and *V. crabro* nests. This work primarily consists of several parts. In the first step, the physicochemical feature of the nest materials was investigated in terms of water absorption capacities, oral secretion, plant lift material ratios and oil substance percentage. Then, SEM and stereomicroscope were used to establish the surface of nest properties, and the surface chemical content was established by EDX technique and elemental decomposition techniques were used. At the last stage, antioxidant and antimicrobial activity works were accomplished with the extracts of the materials in distinct polarities. Bagriacik (2011), determined the water absorption capacity of the nest materials of Turkish origin as 91% and 100% for *V. orientalis* and *V. crabro*, respectively. Ertürk & Bağdatlı (2019), the water absorption capacities of the nests were 100% and 53.19% for *P. dominula* and *V. crabro* nest materials, respectively. In this work, the water absorption capacities of the nests were 283.01%, 242.71% and 154.354% for *V. crabro*, *P. dominula* and *P. nimpha* nest materials, respectively. The material from which bee nests are built generally depends on the ecological and vegetative vegetation it depends on. The results we found in our study may be different from other studies and the use of different plant species in the nest. The water absorption capacity of nest material of *V. crabro* and *P. dominula* is very similar to each other. According to the oil content, plant material, oral secretion, and oil content (%) determination, plant material-oral secretion ratios of the samples nest material of *V. crabro* and *P. nimpha* were similar, *V. crabro* 28.846%, %30.322 % whereas *P. dominula* sample of nest material was different 19.205. However, the oil content was similar between the two paper wasps, but the oil rate of *V. crabro* nest material was very different detected. These results indicated that the plant material, oral secretion and oil content of nest materials of *P. nympha*, *P. dominula* and *V. crabro* are not related to the species, but the fact that the nest depends on the environment and depends on the plant. It is expected that these two species have different nest characteristics. The fact that species belong to the same genus does not mean that the nest structures will have the same structure. This situation could be emphasized if changes in nest characteristics could be observed in different populations of the same species.

Paper wasps structure umbrella-figurated nests hanging underneath fringe and protrusion. Bald-faced hornets construct large, football-shaped nests. Construction begins with finding suitable support for the nest – a window shutter, a tree branch, or a root in the case of subterranean nests. regardless of where a wasp constructed its nest or what figured the nest is, the process wasps handling to establish their nests is usually similar.

Paper wasps and hornets all build paper nests, even though the dimensions, figures, and positions of their nests differ. Most social wasps construct combs from herbal plant fiber, mud and oral secretions (Wenzel, 1991). The comb consists of a particular unit, the cell, which is generally hexagonal and contains only one offspring. This modular planning and organized

structure are influential amongst wild social wasps and some bees. Despite these modular structures, the combs of wasp image large variety, both inter-and intra-particularly. At present, the problem of microbial strength is rising. Therefore, some precautions should be taken, such as controlling antibiotic use, conducting modern work to better understand the genetic mechanisms of resistance, and continuing research for new synthetic or inherent drugs. The aim is to propose antimicrobial drugs that are suitable and influential for the patient. In this research, all three nest extracts have potential against microorganisms as antimicrobial compounds. So, they can be used in the cure of contagious diseases reasoned by resistant microorganisms. Therefore, our outcomes once again highlight the significance of effective native extracts against antibiotic-resistant bacteria that pose a threat to human health. To the best of our knowledge, this is the first comprehensive study on the nesting materials of all three species originating from the Black Sea region: *Vespa crabro*, *Polistes dominula,* and *Polistes nimpha*.*V. crabro, P. dominula* and *P. nimpha*. The nest materials of the three species with the same origin have distinct specifications like physical appearance, water absorption capacity, the proportion of plant material to oral secretion, and their bioactive and spectral features. Found some choice of foundresses in correlation to these three parameters. Nests are most frequently started on *Hypericum* spp., *Tanacetum* spp., *Daucus spp.,* and *Achillea* spp., plants. These plants have antimicrobial properties. The highest antimicrobial activity of the essential oil of *Tanacetum walteri* was observed against *Staphylococcus aureus, Enterococcus faecalis* and *Klebsiella pneumoniae* with MIC value of 0.63 mg/mL. In some studies, bees start building nests with more Hypericum spp., Tanacetum spp., Daucus spp., and Achillea spp., plants. Therefore, these plants are found in bee nests. These herbs have antimicrobial properties. The highest antimicrobial activity of *Tanacetum walteri* essential oil was observed against *Staphylococcus aureus, Enterococcus faecalis* and *Klebsiella pneumoniae* with a MIC value of 0.63 mg / mL (Ghaderi & Sonboli, 2019). Finally, aqueous solutions of *Hypericum perforatum* teas were found to be antimicrobial against gram-positive bacteria with specific activity against methicillin-resistant strains of Staphylococcus aureus (Reichling *et al.,* 2001). (Ghaderi & Sonboli, 2019). Latterly, hydrous solutions of *Hypericum perforatum* teas were found to be antimicrobial effective against gram-positive bacteria with special activity towards methicillin-resistant strains of *Staphylococcus aureus* (Reichling *et al.,* 2001). In conclusion, the three nest ethanol extracts have an extensive spectrum of efficiency against a panel of bacteria accountable for the most common bacterial diseases. These promising extracts open the possibility of evidence of new clinically influential antibacterial and antifungal compounds.

Determination of the activities of products with antioxidant properties is important in the use of many areas. In this study, especially the previously unstudied *P. nimpha, P. dominula* and *V. crabro* were determined of biological activities.

The total phenolic content of samples as gallic acid equivalent, *P. nimpha* was found 3.121 mg/g, *P. dominula* for the sample to 1.881 mg/g and *V. crabro* for the sample 0.635 mg/g. In another study total phenolic content was found of *V. crabro* and *P. dominula* nests collected from the Black Sea region ranged from 6.41 to 11.93 mg GAE/g (Ertürk & Bagdatli, 2019). The total phenolic content of the samples in our present study was found to be lower in the literature. Total flavonoid content was determined in the study was 0.853, 0.425 mgQE/g *P. nimpha* and *P. dominula* respectively, while the amount of flavonoid substance in *V. crabro* was not detected.

One of the antioxidant methods is the most widely used FRAP method. This study measured Fe^{+3} Fe^{+2} reduction capacity in the samples. FRAP results were determined linearly proportional to total phenolic and flavonoid content. Again, the highest activity was determined 24.645 µmol$FeSO_4 7H_2O$/g in P. *nympha* bee nests.

The DPPH has been widely used to determine the free radical scavenging ability of the samples. The free radical scavenging activity of the samples depends on their hydrogen-donating ability (Silva *et al.,* 2006). The highest effective sample extract was *P. nimpha*, *P. dominula* and the

lowest was *V. crabro* with SC_{50} 3.185, 4.325 and 5.87 mg/mL, respectively. In the study conducted by Ertürk & Bagdatli (2019), DPPH results of *Vespa crabro* and *Polistes dominula* nests were found to be an experiment and IC_{50} values for BHA and ascorbic acid are 175.87 ± 1.34 and 156.87 ± 1.62 µg ml^{-1}, respectively.

In this study, phenolic contents were determined using an RP-HPLC-UV device. HPLC analysis of phenolic compounds in wasp nests from the Black Sea region in Türkiye showed that many phenolic compounds were present in all wasp nests analyzed.

The concentrations of individual phenolic compounds in wasp nests are presented in Table 6. As major components were found chrysin, myricetin in V. *crabro*. Chrysin was determined as a major component in three samples. Chrysin and Myricetin were found as major components in *V. crabro*. On the other hand, Chrysin was found to be the main component in all three samples. It has been found in studies conducted with chrysin flavonoids that it has strong anti-inflammatory and antioxidant properties and promotes cell death by inhibiting cell cycle progression (Samarghandian *et al.*, 2011). Protocatechuic acid was found 35.304 and 35.256 µg phenolic/g sample in a nest of *P. nimpha* and *P. dominula* nest, respectively. Chrysin was found in 29.652 µg phenolic/g sample in *V. crapro* bee nest. Chrysin is found in excess amounts in plants which were reported to have many biological activities including antibacterial, antioxidant, antiinflammatory, antiallergic, anticancer, antiestrogenic activities (Babu *et al.*, 2006). Chrysin has an inhibitory effect on the tyrosinase enzyme (Kubo *et al.*, 2000). Accordingly, it can be thought that wasp nests in the study may be a potential tyrosinase inhibitor.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Zehra Can: Investigation, Resources, Visualization, Software, Formal Analysis, and Writing - original draft. Supervision, and Validation. **Ömer Erturk**: Methodology, Formal Analysis, and Writing -original draft. **Mustafa Yaman**: Methodology, Formal Analysis, and Writing -original draft.

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