Plant Archives Vol. 23, No. 1, 2023 pp. 81-89 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2023.v23.no1.014

SYNERGISTIC ANTIOXIDANT ACTIVITY OF HONEY BEE PRODUCTS AND THEIR MIXTURES

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(Date of Receiving : 13-10-2022; Date of Acceptance : 18-12-2022)

ABSTRACT

The goal of this study was to assess the impact of binary combination antioxidant synergistic effects of honeybee products (citrus honey, clover honey, sugar feeding honey, bee pollen, bee bread, bee wax, old wax comb, Egyptian propolis, Chinese propolis, royal jelly, Drone brood homogenate, worker brood homogenate, queen brood homogenate, bee venom) and The present study compared the antioxidant activity between ethanol and water extracts of bee products and evaluated the synergistic antioxidant activity effect of binary combination of bee products (water and ethanol extracts, separately), the antioxidant activity was analyzed via DPPH radical scavenging activity assay and found propolis as one of the most powerful antioxidant among all the honeybee products examined, and the ethanol (80%) extraction method recorded more antioxidant activity than the water extract, but in the royal jelly, drone brood homogenate, worker brood homogenate, queen brood homogenate and bee venom the water extract were the highest. The obtained results of honey bee product mixture activity affected by the interaction between chemical compositions of them, which had an impact on their antioxidant activity. And several of these binary combinations showed synergistic results; this might be because adding more antioxidant components from other products increased the antioxidant capacity.
Keywords : Antioxidant activity, binary combination, honeybee products, synergistic.

Introduction

Honey and related products have a lot of potential as dietary natural antioxidants (Silva *et al.,* 2011). Honey, pollen, propolis, bee venom and royal jelly are among the common popular natural products used in traditional medicines because of their effectiveness, therapeutic capabilities and bioactive chemicals contents (Brown *et al.,* 2016). Honey and related products have many effects as natural food antioxidants (Silva *et al.,* 2011). Honey, pollen, propolis, bee venom and royal jelly are popular as natural products used in traditional medicine because of their powerful medicinal properties and bioactive chemical content (Brown *et al.,* 2016). As a result, so many articles are currently reported on honey bee products chemical composition and their therapeutic benefits (Fratini *et al.,* 2016; Zhou *et al.,* 2015). Drone brood homogenate (DBH), which is almost unheard of in West of Europe. however, accessible in Romania and other Eastern European countries under the brand name Apilarnil, DBH-based bee product, first introduced in Romania by Nicolae Iliesiu (Iliesiu, 1981). It is made from honey bee combs which containing 7day old drone bee larvae that have been homogenized and lyophilized (Sawczuk *et al.,* 2019). Apilarnil is highly regarded in Romania for its biological activity and is used in cases of malnutrition, anorexia treatment, and depression treatment (Sawczuk *et al.,* 2019; Sidor and Dżugan, 2020).

Numerous traditionally utilized plants and honey bee products showed greatly improved pharmacological results when utilized in combination than when utilized independently (Boukraa 2008 and Xu *et al.,* 2014). In fact, synergistic interactions between the constituents of natural products impressively increase their biological efficacy. Yoirish (2001) reported that honey can be utilized in combination with many of medicinal plants.

Therefore, this study aims to investigate the antioxidant activities of honey bee products binary combination using different extract solutions (water and ethanol, separately).

Material and Methods

Pollen grains were collected in the spring and a common pollen trap was installed at the hive entrance and stored throughout the collection period. Every day, pollen was extracted from the traps, cleaned, and stored in airtight plastic bags (Barreto, 2004). Beebread was obtained directly from the combs and only beebread pieces were cleaned, and stored in airtight plastic bags. Drone, worker and queen brood homogenate samples were collected three times during the beekeeping season, shortly after being brought to the laboratory, the larvae were removed manually from comb cells and deep frozen, then crushed mechanically with a grinder in the ice bath (Schmidt and Buchmann, 1992). Collection of royal Jelly material was achieved in deprived colonies after 3 days of transferring the larvae. On the third day of transfer, the larvae were taken off and the royal jelly was collected from each cell, and transferred to plastic vials (Chen, 1993 and Zeng, 2013). Bee venom samples were collected from experimental apiary colonies by bee venom's

collection frame devices (Input Voltage: 11.5-13.5 VDC, Collector Frames: 40cm x 50 cm) and the collection time was 30 minutes. After 30 minutes, the collector frame was removed from the colony, and then the deposited bee venom on the glass plate was scrapped using a scraping knife (Rybak *et al.,* 1995). The wax sample was obtained by collecting the fresh formed wax comb pieces in the colonies during the spring season.

Pollen, bee bread, brood homogenate, royal jelly, and bee venom, wax samples were obtained from colonies of honey bee apiary located in the experiment apiary of honey bee research department, Plant Protection Research Institute (PPRI), Agriculture Research Center (ARC), Egypt, stored in a deep freezer at -18ºC after collection and preparation until use.

Samples of clover and citrus honey were collected by beekeepers between March and July 2020 in beehives found in various provinces of Egypt. The flower validity of the honey samples was confirmed by pollen analysis (Louveaux *et al.,* 1978). Feeding honey sample was collected after feeding the colonies (with empty combs from honey) by sugar solution 50% (sucrose solution (1: 1 w/v) which continuously provided for several days in the experiment apiary, and the sample stored in dark at room temperature (25ºC).

Egyptian, Chinese and old wax combs propolis samples were investigated; the Egyptian propolis was collected from experiment apiary through two years (2019-2020) (according to Breyer, 1995) and the Chinese propolis which was imported from China and the old wax combs propolis was collected from honeybee old wax combs (3-5 years old) from the experiment apiary.

Preparation of sample extracts was performed using distilled water and ethanol 80% as solvents. First, five grams of the sample material was ground up mechanically with a grinder in the ice bath with 100 ml extract solution (water or ethanol 80%), with continuous swirling for 3 days, then the extract was centrifuged (10 min at 4000 rpm) to obtain a clear supernatant liquid at a final concentration of 5g/100 ml(5%) as stock solution, but for bee venom sample the weight was 1g /20 ml (5%), the clear supernatant liquid stored in a deep freezer at -18 ^{0}C after preparation until antioxidant assay.

Water and ethanol extract samples were diluted with water or ethanol 80% at a ratio of 1:1 (2.5%) and mixed well by the vortex. In addition to providing the synergistic antioxidant activity of a binary combination of honey bee products, every two honey bee product samples (stock solution, 5%) were mixed in a 1:1 ratio for water and ethanol extracts individually.

Radical scavenging activity (Antioxidant activity): The samples radical scavenging activity for the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was analysed according to (Martins *et al.,* 2008), with slight modifications, 10 µl of sample solution was mixed with 3 mL of DPPH (Sigma-Aldrich) methanol solution (40 mg/liter) and the blank sample was methanol. The mixture was left for 30 min at room temperature and the absorbance (abs.) was measured at 517 nm. The antioxidant activity was determinate as follows:

Inhibition (%) = [(blank abs.- sample abs.) / blank absorbance] × 100. The mean values of concentration causing 50 % inhibition (IC_{50}) for each sample were calculated graphically (mg/ml). The antioxidant content was calculated using a standard curve of gallic acid, quercetin, and ascorbic acid (vit c) (Aldrich) treated in the same conditions as the samples. The mean of values was recorded, expressed as mg equivalent antioxidant / g of sample.

All values were expressed as mean ± SD. The significant differences were analyzed by using IBM SPSS Statistics 26. (to compare the antioxidant activity among honey bee products extracted with water and ethanol as an extraction solvent). Analysis of synergistic effects was performed following the method of Qiao *et al.* (2015) with slight modifications. The values from this study were analyzed by CompuSyn software to analysis the synergistic effect of a combination of binary samples and statistical analysis of results was performed and recorded as CI (combination index). CI value is a mathematical and quantitative representation of the pharmacological interplay of two drugs (CI>1: antagonism; CI = 1: additive; CI<1: synergism) (Chou, 2008).

Results

The present study deals principally with the results of an explorative investigation into the antioxidant activity of different honey bee products extracted by two different solutions (water and ethanol, 80%) and determines the synergistic effect of the samples binary combination (table, 1, 2 and 3).

The effects of DPPH (%), IC50, gallic acid, quercetin and vit. C equivalents (mg/g) of different honey bee products samples extracted by water and ethanol (80%) are summarized in Table 1. The antioxidant activity (IC50) varied from the highest value, which was observed in Chinese and Egyptian propolis, pollen, and old wax comb samples (66.533, 36.625, 80.012 and 55.238 mg/ml in water extract and 13.878, 19.740, 51.625 and 36.108 mg/ml in ethanolic extract, respectively), to the lowest value, which was recorded in sugar feeding honey and wax samples (1748.25, 942.78 mg/ml in water extract and 1644.7, 532.280 mg/ml in ethanolic extract, respectively). Propolis is the most potent antioxidant in all bee products tested. It is obvious that ethanolic extract had more antioxidant activity than the water extracts, but in bee venom, drone, worker, and queen brood homogenates samples, the water extracts were higher than the ethanolic extracts.

Significant differences were recorded among all honey bee products in water extract or in ethanolic extract. In addition, the antioxidant activity of honey bee product ethanolic extract had a significant difference when compared with water extract. but, there were no significant differences in antioxidant activity between the water extract and ethanolic extract in the sugar feeding honey and in the wax sample.

Table 1 : Antioxidant Activity of water and ethanolic extract of honey bee products (2.5%w/v)

product	Water extract					Ethanol extract					Prob.
	%	IC_{50}	Gallic acid	Quercetin	Vit C	%	IC_{50}	Gallic acid	Quercetin	Vit C	
			equivalent					equivalent			
		mg/ml	mg/g				mg/ml	mg/g			
CH	3.499±0.001	310.650	0.158	2.118	2.984	7.056±0.061	136.955	0.318	4.272	6.018	*
TH	3.903±0.176	255.630	0.176	2.363	3.329	5.307±0.001	240.135	0.239	3.213	4.527	*
FH	0.757±0.439	1748.250	0.034	0.458	0.646	0.758±0.201	1644.700	0.034	0.459	0.647	ns
P	15.560±0.001	80.012	0.702	9.420	13.271	27.623±0.001	51.625	1.246	16.723	23.560	*
B	9.469±0.181	126.930	0.427	5.732	8.076	15.621±0.543	77.900	0.705	9.457	13.323	*
W	1.539±0.620	942.780	0.069	0.932	1.313	1.807±0.799	532.280	0.082	1.094	1.542	ns
WOC	25.633±0.181	55.238	1.156	15.518	21.863	37.877±1.810	36.108	1.708	22.930	32.306	*
EPRO	35.000±0.001	36.025	1.579	21.189	29.852	58.200±0.001	19.740	2.625	35.234	49.640	*
CPRO	21.471±0.001	66.533	0.968	12.999	18.313	90.551±0.348	13.878	4.084	54.819	77.232	*
R	10.253±0.001	104.809	0.462	6.207	8.745	8.323±0.001	143.065	0.375	5.039	7.099	*
DH	8.986±0.543	131.035	0.405	5.440	7.664	5.789±0.121	298.570	0.261	3.505	4.938	*
WH	8.865±0.061	128.350	0.400	5.367	7.561	5.235±0.460	239.750	0.236	3.169	4.465	*
QH	8.624±0.302	143.655	0.389	5.221	7.356	5.728±0.001	273.985	0.258	3.468	4.886	*
V	14.822±0.107	84.290	0.668	8.973	12.642	7.288±0.001	177.855	0.329	4.412	6.216	*
		*						*			

Values (%I DPPH) are means ± standard deviations, IC50: 50% inhibitory concentration, CH: citrus honey, TH: clover honey, FH: sugar feeding honey, P: bee pollen, B: bee bread, W: bee wax, WOC: old wax comb, EPRO: Egyptian propolis, CPRO: Chinese propolis, R: Royal jelly, DH: Drone brood homogenate, WH: Worker brood homogenate, QH: queen brood homogenate, V:bee venom, *: significantly different, ns: no significantly different

The most elevated esteem of IC_{50} was reported in multi floral honey and the least in Persea Americana honey. Persea Americana honey recorded the heights activity of free radical scavenger (IC_{50}= 8.0 mg/mL), (Sánchez *et al.,* 2012).

Data were recorded from 15 samples of honey. The TPC concentrations (total phenolic compounds) values extended from 27.0 to 92.7 mg GAE (gallic acid eq.) /100g of honey, the highest values reported in polifloral honey, when compared to monofloral honey samples. Different in the TPC concentrations may be due to different botanical origins of honey. The radical scavenging ability of samples ranging from 7.3% to 25.9% I DPPH, in 30 min, as compared with gallic acid which expended 100% I DPPH (Almeida *et al.,* 2016).

Baltrušaitytė *et al*. (2007) found that bee bread exhibits a higher antioxidant activity than honey. The antioxidant activity of bee bread was demonstrated with IC_{50} of DPPH (0.05 ± 0.01 mg / ml), ABTS (0.08 ± 0.05 mg / ml), and the reducing power (0.05 ± 0. 04 mg / ml).

Sidor and his co researchers examined the antioxidant activity and total phenolic compounds of drone brood homogenate in several stages of brood development (Sidor *et al.,* 2021). A low-level of DPPH (%) appeared in white-eyed pupae (6.3 to 70% ethanol extract) while its highest level was recorded in the larval phase (20.5%).

Bee pollen ethanolic extract (from *Trifolium alexandrinum* L.) showed a higher radical scavenging activity than with other solvents (ethyl acetate, dichloromethane and petroleum ether). The highest DPPH scavenging activity was recorded in ethanolic extract (90%), followed by ethyl acetate (79%), and petroleum ether (75%), on the other hand, dichloromethane recorded moderate activities (63%) (Abd Elsalam *et al.* 2018).

Propolis is the leading displayer of antioxidant properties (Karadal *et al.,* 2018). (Nakajima *et al.,* 2009) illustrate that propolis (water and ethanolic extract) had the strongest antioxidant effects, among the tested bee products (bee pollen, royal jelly, and propolis). Honey bee pollen has strong antioxidant impacts, particularly against the H_2O_2 and O_2, however, its effects were only one-tenth stronger than that of propolis.

Bee venom antioxidant activity by using classical assays showed antioxidant properties, some data indicated that melittin alone has a much lower antioxidant activity compared to extracts of bee venom and this may well be due to the impact of other components of bee venom (Pavel *et al.,* 2014).

The propolis extract had very high antioxidant activity and honey samples had low antioxidant activity among bee products (Karadal *et al.,* 2018). Additionally, numerous investigators have recorded that propolis extracts have a strong antioxidant activity (Nagai *et al.,* 2001) and Nakajima *et al.* (2009) also reported that propolis extract is the most potent antioxidant among bee products (pollen, propolis, royal jelly and honey).

The antioxidant activity of the binary combined water or ethanolic honey bee products extracts were individually reported in Table 2 for water extract and Table 3 for ethanolic extract. Furthermore, in addition, to ensure interoperability between honey bee products, data were computerized in the CompuSyn software to determine the combination CI.

Data in Table 2, 3 and Fig. 1 reported that the Egyptian propolis, Chinese propolis and pollen water extract and clover honey, pollen and Egyptian propolis ethanolic extract combination showed more synergistic effects in the majority of the binary combinations, but the royal jelly water extract and worker, drone homogenate ethanolic extract samples showed the minority synergistic combinations effect.

Table 2 : Synergistic Antioxidant Activity of binary combinations of honey bee product water extract (2.5%w/v)

Product	CH	FH	P	B	W	WOC	EPRO	CPRO	R	DH	WH	QH	V
CH	7.20±0.56	4.40±0.67	18.52±3.99	16.67±0.65	4.87±0.64	29.45±1.56	36.08±1.23	24.49±2.07	11.64±0.92	10.79±0.57	11.96±0.72	10.05±0.91	15.58±1.10
CI	0.9421 sy	0.9324 sy	0.9462 Sy	0.6650 sy	0.9831 sy	0.8273 sy	1.0210	0.9440 sy	1.1111	1.0808	0.9441 sy	1.1354	1.1171
TH		4.08±1.02	17.01±3.28	12.25±1.77	4.76±0.59	26.87±2.95	38.10±5.89	23.13±0.59	12.25±0.00	11.57±0.59	10.20±1.77	10.54±1.18	16.49±3.13
CI		1.1227	1.0739	1.0032	1.1000	1.0405	0.9586 Sy	1.0303	1.0760	1.0279	1.1808	1.1095	1.0623
FH			16.40±0.00	11.69±0.00	2.00±0.10	27.76±0.28	36.15±0.94	23.49±1.46	9.31±0.50	9.470±0.496	9.470±0.57	8.225±0.57	15.46±2.36
CI			0.9698 Sy	0.8272 sy	1.1187	0.9185 sy	0.9706 Sy	0.9121 sy	1.1873	1.0040	0.9891 sy	1.1327	0.9849 sy
P				24.07±0.31	19.57±0.95	39.94±0.00	48.80±1.28	38.19±0.31	21.57±0.63	23.373±0.98	22.27±0.43	23.62±0.71	27.58±4.00
CI				0.8900 sy	0.8048 sy	0.8586 sy	0.8807 Sy	0.7890 sy	1.0607	0.8951 sy	0.9000 sy	0.8777 sy	0.9362 sy
B					11.42±0.50	30.83±2.89	42.78±1.92	23.61±4.81	15.00±2.89	16.67±0.22	15.28±2.41	14.5±1.828	22.8±0.53
CI					0.9069 sy	1.0147	0.9192 Sy	1.2081	1.2027	0.9747 sy	1.0781	1.1271	0.9176 sy
W						27.49±4.05	37.24±5.47	23.68±1.29	10.52±0.62	9.885±0.868	8.91±1.47	8.56±0.43	15.44±5.33
CI						0.9490 sy	0.9461 Sy	0.9251 sy	1.0881	1.0206	1.1384	1.1593	1.0250
WOC							56.36±1.44	40.77±3.90	29.47±2.79	29.30±0.74	29.26±4.01	28.53±1.02	34.36±1.19
CI							0.9059 Sy	0.9811 sy	1.1002	1.0700	1.0680	1.0952	1.0235
EPRO								54.62±0.18	44.06±0.54	47.60±2.31	44.06±1.49	47.39±2.82	49.00±0.00
CI								0.8648 sy	0.8992 sy	0.7981 sy	0.8740 sy	0.7923 sy	0.8626 sy
CPRO									29.28±1.65	28.90±2.04	28.62±0.95	28.69±5.81	31.52±0.53
CI									0.9384 sy	0.9151 sy	0.9229 sy	0.9126 sy	0.9928 sy
R										14.30±1.49	18.32±0.75	17.80±1.24	22.73±0.00
CI										1.2395	0.9033 sy	0.9232 sy	0.9559 sy
DH											14.45±3.85	12.34±0.00	21.96±1.60
CI											1.1195	1.3579	0.9428 sy
WH												14.33±0.58	22.20±1.39
CI												1.1213	0.9242 sy
WQ													20.66±0.00
CI													1.0018

Values (%I DPPH) are means ± standard deviations, CH: citrus honey, TH: clover honey, FH: sugar feeding honey, P: bee pollen, B: bee bread, W: bee wax, WOC: old wax comb, EPRO: Egyptian propolis, CPRO: Chinese propolis, R: Royall jelly, DH: Drone brood homogenate, WH: Worker brood homogenate, QH: queen brood homogenate, V:bee venom, CI : Combination Index, SY: synergistic.

Table 3 : Synergistic Antioxidant Activity of binary combinations of honey bee product ethanolic extract (2.5%w/v).

Product	CH	FH	P	B	W	WOC	EPRO	CPRO	R	DH	WH	QH	V
CH	11.00±0.72	7.25±0.33	33.28±2.66	23.76±5.41	7.94±0.00	44.23±0.87	62.96±3.30	95.40±0.27	13.49±2.18	9.76±0.87	8.94±0.64	9.10±0.92	12.79±1.39
CI	1.021	1.057	0.931 sy	0.815 sy	1.068	0.919 sy	0.976 Sy	1.012	1.023	1.234	1.299	1.329	1.007
TH		8.33±2.89	32.67±2.31	20.79±2.80	7.38±0.54	43.29±0.62	64.01±7.24	95.94±0.60	11.91±0.59	11.22±1.02	11.56±1.56	10.54±0.59	11.21±1.39
CI		0.671 sy	0.912 sy	0.893 sy	0.899 sy	0.916 sy	0.940 Sy	0.996 sy	1.043	0.880 sy	0.801 sy	0.943 sy	1.021
FH			29.09±1.82	15.88±2.70	3.12±0.19	45.52±1.38	56.97±5.84	93.21±1.05	9.68±0.00	5.38±0.93	5.65±0.81	5.46±0.00	8.00±1.64
CI			0.950 sy	1.014	0.783 sy	0.797 sy	1.033	0.985 sy	0.900 sy	1.210	1.035	1.132	0.976 sy
P				43.33±0.00	29.95±3.25	66.39±0.48	81.11±3.85	94.61±0.59	34.44±0.96	30.39±1.36	29.89±1.54	33.06±1.36	33.33±5.25
CI				0.825 sy	0.938 sy	0.826 sy	0.964 Sy	1.269	0.920 sy	1.014	1.022	0.908 sy	0.934 sy
B					17.72±0.92	45.56±0.48	72.22±4.81	96.56±0.315	23.28±5.30	18.63±0.50	22.38±1.10	22.76±1.96	23.79±3.87
CI					0.928 sy	1.040	0.927 Sy	1.075	0.886 sy	1.049	0.810 sy	0.811 sy	0.822 sy
W						40.35±0.00	60.98±2.16	93.51±0.40	9.25±0.50	6.67±0.72	5.975±0.865	5.63±0.55	8.55±0.79
CI						0.947 sy	0.961 Sy	0.989 sy	1.046	1.091	1.132	1.309	1.012
WOC							74.67±0.58	93.33±0.61	41.76±0.61	38.77±0.68	38.67±4.72	39.68±1.87	40.91±0.00
CI							1.193	1.341	1.013	1.066	1.059	1.033	1.021
EPRO								97.37±0.82	63.39±3.82	62.71±0.00	59.02±5.55	61.02±2.00	62.94±3.49
CI								1.566	0.982 sy	0.968 sy	1.034	0.996 sy	0.979 sy
CPRO									95.46±0.44	94.76±0.26	93.86±0.45	96.01±3.04	90.61±2.92
CI									1.021	1.006	1.008	0.999 sy	1.047
R										11.57±1.65	13.22±0.44	13.97±0.734	13.27±5.10
CI										1.121	0.913 sy	0.887 sy	1.063
DH											8.97±1.137	8.96±0.360	11.18±2.677
CI											1.142	1.201	1.068
WH												9.27±0.643	11.11±2.749
CI												1.092	1.541
WQ													13.33±0.001
CI													1.269

Values (%I DPPH) are means ± standard deviations, CH: citrus honey, TH: clover honey, FH: sugar feeding honey, P: bee pollen, B: bee bread, W: bee wax, WOC: old wax comb EPRO: Egyptian propolis, CPRO: Chinese propolis, R: Royall jelly, DH: Drone brood homogenate, WH: Worker brood homogenate, QH: queen brood homogenate, V:bee venom, CI Combination Index, SY: synergistic.

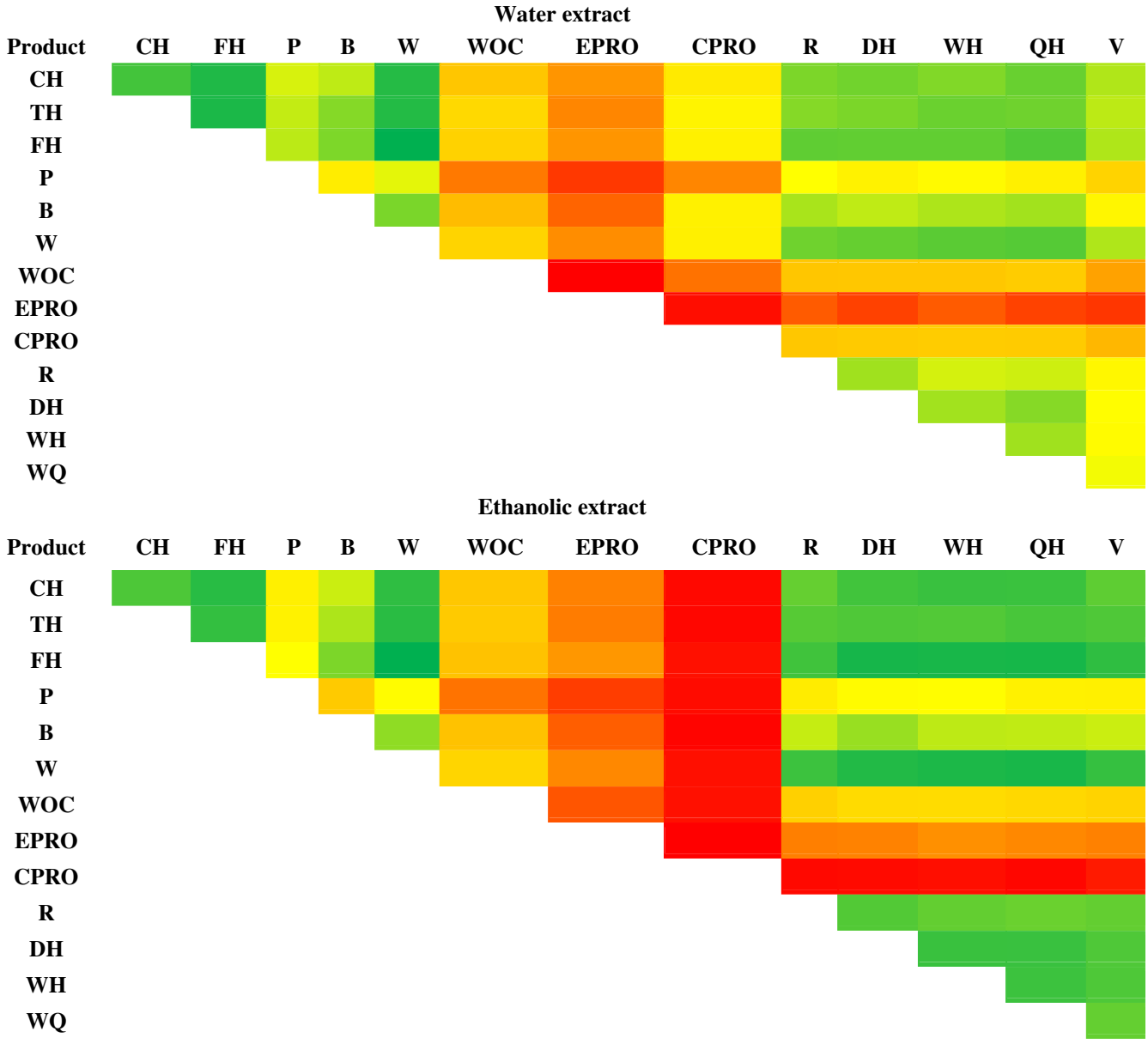

Fig. 1 : Heat mapping of synergistic Antioxidant Activity of binary combinations of honey bee product

Values (%I DPPH), CH: citrus honey, TH: clover honey, FH: sugar feeding honey, P: bee pollen, B: bee bread, W: bee wax, WOC: old wax comb, EPRO: Egyptian propolis, CPRO: Chinese propolis, R: Royall jelly, DH: Drone brood homogenate, WH: Worker brood homogenate, QH: queen brood homogenate, V:bee venom.

The average mean content of phenolic content and flavonoids content in poly flowers honey was 36.06 ± 10.18 mg gallic acid eq./100 g and 4.48 ± 1.69 mg quercetin aq./100 g, respectively. Mixing royal jelly did not significantly affect the phenolic compound content and antioxidant activity. Moreover, mixing honey with other bee products, (propolis, pollen and bee bread) recorded an insignificant increase in phenolic content, flavonoids content, antioxidant activity and reducing effects, but the highest effect recorded when mixing with bee bread. Significant linear correlations between phenolic content and flavonoid content and antioxidant activity and reducing effects have been reported (Juszczak *et al.,* 2016).

Beebread is arranged to apply with honey and some wax particles, also contains a higher antioxidant activity than natural pure honey. The wide range of distinctive components associated with the composition of honeybee products provides a synergistic impact on the other products (Čeksterytė *et al.,* 2016).

Compounds that are effective in extracting bee pollen can enhance (synergistic) or reduce (antagonistic) the helpful action of drug chemotherapy. There has been a surprising concern that some compounds that occur in the natural products may work antagonistically than synergistically with the drugs therapeutic activity (HemaIswarya and Doble 2006). Combination index values detailed in all interactions between cisplatin and bee pollen extract were less than 1. These Combination index numbers also prove the synergistic effect between bee pollen extract and cisplatin. The same data showed that bee pollen extract works synergistically with the drug chemotherapy, cisplatin and increases the effect of cisplatin drug even at lower concentrations (Wan Omar *et al.,* 2016).

Among the mixtures of bee products, the mixture of honey, royal jelly, propolis, and pollen had the most prominent antioxidant activity (72.98±3.08 mg AAE/g) and the triplet mixture of honey, royal jelly, propolis had little antioxidant activity. The antioxidant activity of propolis and royal jelly was reported as 267.37±0.33 mg and AAE/g 59.02±5.98 mg AAE/g. bee pollen, honey, and mixed samples reported a positive correlations with total phenolic content and antioxidant activity. In fact, honey and mixed samples have shown a positive relationship with total phenolic content and FRSA (Özkök and Silici, 2017).

Miguel *et al*. (2010) studied different extraction solvents (methanol, water and 70% ethanol), selectively for hydroalcoholic mixtures to extract phenolic compounds from propolis samples, given its excellent extraction efficiency and low toxicity if compared to methanol. Cavalaro *et al*. (2019) also considered the effect of ethanol/water concentration, solvent concentration, and extraction time relative to the amount of phenolic compound and the antioxidant activity of green Brazilian propolis, with assisted ultrasound. Prepare the process using a 99% ethanol solution and propolis 1:35: solvent (w / v), over 20 min.

Discussion

The results associated with the antioxidant activity of different bee products suggest that there are significant differences among the products under investigation. Propolis and pollen recorded the highest values of activity, but the lowest values were recorded in sugar feed honey and wax samples. That may be due to the fact that bee products are multicomponent natural substances and this component differs from honey bee products to another and therefore also contains other substances presenting antioxidant activity. That means that the difference in antioxidant activity is contributed to the different compounds in the honey bee products. The high content of phenolic compounds in propolis and pollen, which, reported by many researchers investigations, reflected the high antioxidant activity, the low antioxidant capacity in sugar feeding honey sample might be influenced by the absence of the honey floral source and its content of secondary plant metabolites

The natural antioxidant compounds present in honey provide antioxidant activity. These compounds include phenolic acids, flavonoids, ascorbic acid, organic acids, enzymes (catalase, glucose oxidase), amino acids, carotenoids, Maillard reaction products and proteins (Gheldof and Engeseth, 2002). Enzymes are naturally present in honey, like catalase, peroxidase and glucose oxidase (McKibben and Engeseth, 2002). These enzymes are known to have antioxidant activity. Different types of honey have different phenolic contents and as a result, have different antioxidant functions. In addition, handling, processing and honey storage may effect on its composition (Gheldof and Engeseth 2002; Turkmen *et al.,* 2005). A significant correlation was found between the phenolic content and antioxidant activity which was determinate by FRAP assay and DPPH assay, showed that phenolic compounds of acacia honey appeared to be responsible for the antioxidant activity (Krpan *et al.,* 2009).

Propolis ethanolic extract contains a high content of flavonoids, particularly quercetin, rutin, and kaempferol, and responsible for high total antioxidant capacity (Zhang *et al.,* 2016).

Phenolic compounds appear to be responsible for the biological activity of the three types of ethanolic extract propolis. Egyptian ethanolic extract propolis (EEP) content of phenolic compound were caffeic acid, salicylic acid, quercetin, pinostrobin, ferulic acid, genistein, pinocembrin, and daiazein higher than that in old wax combs ethanolic extract propolis (OEP) and Chinese ethanolic extract propolis (CEP), in addition, the phenolic compounds found in CEP were para hydroxy benzoic acid, phenol, trans-cinnamic acid, p. coumaric acid,, 3.5 dimethoxy benzyl alcohol, trans-cinnamic acid, galangin, chrysin, acacetin, and daidzin over EEP and OEP, on the other hand, OEP were protocatechuic acid, catechines, pyrogallic acid, more than that in EEP and CEP. It seems that the composition of phenolic compounds were different in the three types of ethanolic extract propolis and EEP contains phenolic compounds that are much higher than CEP and OEP. (Kamel *et al.,* 2013).

Some royal jelly compounds appeared to have an antioxidant impact; albumin in royal jelly have anti oxidative activity (Guo *et al.,* 2005). Protein and phenolic compounds of royal jelly have a high antiradical activity (determinate by FRSA) against reactive oxygen species (ROS) (Eraslan *et al.,* 2008). It's conceivable that the variety in antioxidant activity observed in this study is inferable to the solvent used. The ethanol (80%) extraction procedure antioxidant activity procedure was higher than that of the water extract. This may be because the antioxidant activity of the extracted extracts is influenced by the extraction solvents, their concentration, and polarity. Because the components of bee products have different structures, and although hydrophilic ones are better soluble in polar solvents such as alcohols, but, those with hydrophobic properties have a high affinity for non-polar solvents such as hydrocarbons, the composition of the obtained extracts is affected by the use of different polar solvents. It was found that the different types of extraction solvent had different effects on the concentration of bioactive compounds in the extracts.

It can be seen that the highest total antioxidant activity (TAA) is recorded in bee pollen extracted by ethanol, while water extract show lower TAA values. The higher elevated TAA values were detected in brown pollen and ochre samples extracted by ethanol. Similarly, the highest phenolic content values were recorded in ethanol bee pollen extract, followed by methanol extract and water extract (Sánchez *et al.,* 2012). Freire et al. (2012) reported the values of total phenolic and flavonoid contents compared with other studies which used ethanol, methanol and water extraction. They reported that the different types of solvent extraction had a different impact on bioactive compound concentration in the extracts. It is obvious that the total phenolic compounds of the propolis increased gradually with increasing the fractions volume of ethanol. The highest antioxidant activity was recorded in the ethanolic propolis extract, with the total phenolic compounds estimated at 317.65 mg AAE /g . The results confirmed the assumption that an increase in ethanol fraction in the extraction solvent should have a higher bility to dissolve different types of phenolic compounds due to the change in the solvent polarity, providing higher antioxidant activity in the extraction solution (Abdullah *et al.,* 2019). Moreover, both the biological activity and chemical composition of propolis extracts are highly dependent on the type of solvents used in extraction. (Sun *et al.,* 2015; Bittencourt *et al.,* 2015 & Narimane *et al.,* 2017).

Therefore, there may be significant changes in phenolic compounds and antioxidant activity phenolic compounds when comparing honey and its extract. Generally, according to the studies evaluated, honey dissolved in water yields higher phenolic content, on the other hand the extraction with methanol produced a higher flavonoid content (Mouhoubi-Tafinine *et al.,* 2016; Lianda *et al.,* 2012)

But in the present study the venom, royal jelly, worker, queen, and drone homogenates, the water extract revealed more antioxidant activity than the ethanol extract. This can be explained by several factors; the high protein content in these products makes them more soluble in water, and the ethanol causes aggregation, denature, reduce solubility, and precipitate the proteins, losing their biological activity.

Ethanol influences on the proteins in watery solution. It can denature the proteins (Gerlsma, 1968; Gerlsma and Stuur 1972; Herskovits and Mescanti, 1965; Mousavi *et al.,* 2008), often accompanied by transition in secondary structure of protein (Dufour and Haertlé 1990,1993) and decreased their solubilities (Yoshikawa *et al.,* 2012a,b). This study appeared that the high concentrations ethanol (above 50-60 %) alters the structure or the association state of bovine serum albumin and ribonuclease A, pH dependently (Yoshizawa *et al.,* 2014).

Conclusion

The present study has demonstrated for combined effects of antioxidant capacity of honey bee products water and ethanol extracts. The results obtained for the antioxidant activity of the honey bee products mixture were influenced by the interaction between their chemical compositions, which effected on their antioxidant activity. and some of these binary combinations showed synergistic results. this may be because the antioxidant capacity is expanded to include more antioxidants from other product. These results may help in the future design of functional foods based on bee hive products and it is a promising area to continue exploring in future studies.

Acknowledgement

None

Disclaimer

None

Conflict of Interest

None

Source of Funding

None

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