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Antioxidant properties of individual and combined extracts of honey and mint

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INTRODUCTION & AIM

Plant-based substances have been used in traditional medicine systems around the world. Evidences from several studies have shown that polyphenols are useful as nutraceutical and display different health-promoting biological activities including antioxidant, antimicrobial, anti-inflammatory, cardioprotective, neuroprotective, anticancer, glucose regulation, modulation of gut microbiota, and several other biological and pharmacological properties [1].

Many formulations based on polyphenols have been prepared in specific ratios and used for multiple purposes [2].

The aim of the present study is to assess the level of bioactive compounds and the antioxidant activity of individual extracts of mint (*Mentha spicata*) and honey and their combination. The total phenolic contents (TPC) and the total flavonoid contents (TFC) were determined spectrophotometrically. The antioxidant activity was assessed using different in vitro assays, including DPPH, ABTS, reducing power, and iron-chelation tests.

METHOD

Honey and plant samples

Two multi-flora honey samples were used in this study. Green mint (Mentha spicata) was harvested, dried then ground into a fine powder. Plant material was extracted by agitation and maceration. Extracts (honey and mint) were studied separately or in combination in a specific ratio in distilled water.

Total phenol contents

Total phenol contents were estimated using the Folin-Ciocalteu method [3]. Aliquots (200 µl) were added to 500 µl of Folin-Ciocalteu (10%). After 5 min, 1500 µl of Na2CO3 (7.5%) were added then incubated for 30 min. Total phenolic contents were expressed as mg gallic acid equivalents (GAE)/g.

Total flavonoid contents

Total flavonoid contents were measured by colorimetric method [4]. One mL of of each extract and combination was reacted with 1 mL of aluminum chloride (2 %). After incubation for 1 h, the absorbance of the reaction mixture was measured at 420 nm. Total flavonoid contents were calculated as mg of quercetin equivalent (QE)/g.

Ferric reducing power

Reducing power was tested [5]. A volume of 2.5 mL of each extract and combination was mixed with 2.5 ml of phosphate buffer and 2.5 ml of K3[Fe(CN)6] (1 %). After incubation, 2.5 mL of TCA (10 %) were, followed by centrifugation at 3000 rpm for 10 min. The upper layer (1 mL) was mixed with 1 mL of distilled water and 0.5 mL of ferric chloride (0.1 %). The absorbance was measured at 700 nm.

DPPH radical-scavenging activity

Extracts were tested using DPPH technique [6]. 2 ml of each extract and combination were added to 0.4 ml solution of DPPH radical in methanol (0.5 mM). Absorbance was measured at 517 nm after 30 min. DPPH I% = 100 x (A blank A sample) / A blank.

ABTS radical-scavenging activity

Antioxidant activity was measured by using radical cation decolorization assay [7]. 2 ml of diluted ABTS^{o}+ to 20 µL of each extract and combination. The absorbance was taken 6 min at 734 nm after initial mixing.

Iron chelating activity

FIC activity was measured by the method of Dinis et al. [8]. One mL of 0.125 mM FeSO4 and 1 ml of 0.3125 mM ferrozine were mixed with 1 mL of of each extract and combination. The absorbance was measured at 562 nm.

RESULTS & DISCUSSION

Table 1 Total phenolic and flavonoid contents of honey and mint extracts and honey-mint combination

Samples	Phenolic content (mg GAE/g)	Flavonoid content (mg QE/g)
Honey 1	0.35 ± 0.01^{f}	0.23 ± 0.00^{g}
Honey 2	0.32 ± 0.01^{g}	0.12 ± 0.01^{h}
Mint 1	15.5 ± 0.06^{b}	27.49 ± 0.32^{b}
Mint 2	35.47 ± 0.08^{a}	32.03 ± 0.47^{a}
Honey 1 + Mint 1	0.71 ± 0.02^{c}	0.95 ± 0.00^{c}
Honey 1 + Mint 2	0.72 ± 0.02^{c}	0.73 ± 0.00^{e}
Honey 2 + Mint 1	0.65 ± 0.00^{d}	0.76 ± 0.00^{d}
Honey 2 + Mint 2	0.62 ± 0.01^{e}	0.55 ± 0.01^{f}

Results showed that the total phenolic and flavonoid contents of *Mentha spicata* were significantly higher than that of honey. All combined extracts exhibited various increases of total phenolic and flavonoid contents.

Table 2 Antioxidant activity of honey and mint extracts and honey-mint extracts combinations

Samples	Reducing power (700 nm)	DPPH (%)	ABTS (%)	Chelating power (%)
Honey 1	0,35 ± 0,02^{f}	42,01 ± 0,42^{c}	6,34 ± 3,55^{c}	7,46 ± 2,26^{c}
Honey 2	0,27 ± 0,04^{g}	35,28 ± 2,72^{d}	1,12 ± 0,77^{d}	7,63 ± 0,61^{c}
Mint 1	0,78 ± 0,02^{c}	51,20 ± 1,32^{a}	4,33 ± 0,95^{c}	77,77 ± 1,69^{a}
Mint 2	0,49 ± 0,01^{e}	45,56 ± 0,68^{b}	4,57 ± 0,54^{c}	0,66 ± 0,53^{d}
Honey 1 +Mint1	0,95 ± 0,01^{a}	49,04 ± 2,09^{a}	6,34 ± 2,94^{c}	14,55 ± 3,19^{b}
Honey 1 +Mint2	0,77 ± 0,01^{c}	40,74 ± 1,41^{c}	2,03 ± 1,19^{d}	2,11 ± 1,44^{d}
Honey 2 +Mint1	0,89 ± 0,02^{b}	47,88 ±1,61^{a}	22,96 ± 3,26^{a}	3,20 ± 1,07^{d}
Honey 2 +Mint2	0,70 ± 0,04^{d}	45,10 ± 0,23^{b}	18,35 ± 0,32^{b}	0,60 ± 0^{d}

Both individual mint and honey extracts exhibited antioxidant activity. When combined, the honey-mint extracts demonstrated enhanced antioxidant activity than the individual extracts. The synergistic effect was particularly evident in the reducing power, DPPH and ABTS assays. Whereas, honey-mint extracts showed low iron-chelating activity.

CONCLUSION

This study showed that individual extracts of honey and mint contained important quantity of phenolics and flavonoids and their combination was found to produce high antioxidant activity. These findings may afford useful basis for the alleged synergistic effects of natural food and facilitate their application in combination as functional foods and dietary supplements.

REFERENCES

[1]. Saleem et al. (2022). *Molecules.* 27(21), 7286.
[2]. Zhang et al. (2022). *Foods.* 11(15), 2189.
[3]. Chan E.W.C., Lim, Y.Y., Wong, L.F., et al. (2008). *Food Chem*. 109, 477–483.
[4]. Jain D.P., Pancholi S.S., Rakesh Patel R. (2011). *J. Adv. Pharm. Technol. Res.* 2, 177–183
[5]. Oyaizu M. (1986). *Jpn. J. Nutr.* 44, 307–315.
[6]. Lim T.T., Tee J.J. *Food Chem*. 103, 1003–1008 (2007)
[7]. Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C. (1999). *Free Radic. Biol. Med.* 26, 1231–1237.
[8]. Dinis T.C.P., Madeira V.M.C., Almeida M.L.M. (1994). *Arch. Biochem. Biophys.* 315,161–169.