

The Relationship Between Antioxidant and Anti-ulcer Activity in Saudi Honey Samples Harvested From Various Regions in Different Seasons

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Introduction

Honey, which is used in both domestic and medicinal applications, has been widely used as a sweetener since ancient times. The composition of honey varies depending on the geographical and nectar sources of a region. The quality of honey depends on its physio-chemical and sensory properties. Hence, knowledge about its constituents is essential for assessing its quality.

OBJECTIVES

The main goal of this project is, to study chemical components of Saudi honey (composed of winter and summer honeys), accordingly to **anti-ulcer** and **antioxidant** activity by using phytochemical and chromatographic analysis.

ABSTRACT

- The 13 Saudi honey samples (composed of winter and summer honeys) were studied according to **anti-ulcer** and **antioxidant** activity by using phytochemical and chromatographic analyses.
- **Phytochemical screening** of ethyl acetate and water extracts was used to detect the presence of carbohydrates, flavonoids, amino acids, and phenolic acids.

Table 1. Types of Saudi honey.

Sample no.	Honey/location	Floral sources <i>species</i>	Family	Type of honey season
1	Farming cells/Khamis Moshiet	Mixed different flowers		Winter
2	Samra ^a /Mhail Aseer	<i>Acacia Etbaica</i>	Leguminosae	Summer
3	Sidir Aseer/Aseer Mountains	<i>Ziziphus Spina</i> Christi L	Rhammnaceae	Winter
4	Magra ^a /Rijaal Alma 'a	<i>Hypericum Perforatum</i>	Gutteferae	Winter
5	Kina Kampher/Khamis Moshiet	<i>Eucalyptus camaldulensis</i>	Myrtaceae	Summer
6	Somra-Sihaiah ^a /Rijaal Alma 'a	<i>Acacia</i> App.	Leguminosae	Summer
7	Sidir Tehambh/Rijaal Alma 'a	<i>Ziziphus Spina</i> Christi L	Rhammnaceae	Winter
8	Mixed different mountains trees (sidir somiran, shihha, sihaiah)/Rijaal Alma 'a	<i>Acacia</i> Spp., <i>Ziziphus</i> Spp., <i>Artemisia juncus</i> Spp.,	Leguminosae Rhammnaceae, Composttae Juncaceae.	Summer & Winter
9	Somiran ^a /Rijaal Alma 'a	<i>juncus</i> Spp.,	Juncaceae.	Winter
10	Sihaiah/Khamis Moshiet	<i>Artemisia</i>	Composttae	Winter
11	Somiran ^a /Khamis Moshiet	<i>juncus</i> Spp.,	Juncaceae	Summer
12	Somiran/Aseer Mountains	<i>Ziziphus Spina</i> Christi L.	Rhammnaceae	Summer & Winter
13	Farming cells/Mhail Aseer	<i>Juniperusprocera</i>		Summer

ABSTRACT

- **The anti-ulcer activities** were evaluated, showing that oral administration of the honey samples reduced the intensity of ulcer scores when compared to the control group.
- Similarly, there was a highly significant reduction in the values of the ulcer indices and areas in rats that received the same sample ($P^* < 0.05$ and $P < 0.01$) in comparison with those of the ulcer control rats.
- The **antioxidant activity** of the honey samples was evaluated, revealing a proportional relationship between the **anti-oxidant** and **anti-ulcer** activities.

P* = percentage of protection

Phytochemical Analysis & Chemical Constituent HPLC Analysis

For Amino Acids:

Fifteen amino acids or related compounds were identified, **Proline is dominant in all of the honey samples**, in addition to aspartic acid and glutamic acid.

For Flavonoids:

- **More than eight flavonoids** were identified in honey samples:

Phytochemical Analysis & Chemical Constituent

honey sample	1	2	3	4	5	6	7	8	9	10	11	12	13
Proline,	116.9	20.7	17.1	34.5	98.1	10.3	76.5	175.6	-	106.4	52.9	203.3	51.4
threonine,	14.3	1.84	6.4	-	3.2	2.1	-	-	-	-	10.8	105.5	-
serine,	15.3	6.3	5.7	3.2	5.7	7.8	4.5	53.1	-	-	21.8	-	14.1
glutamine,	27.9	43.8	13.1	22.2	4.4	29.0	7.9	55.1	-	-	43.7	12.4	30.8
glycine,	13.3	4.3	-	16.2	4.2	-	-	85.9	-	-	9.3	88.2	4.6
alanine,	26.6	15.5	-	25.7	23.3	9.6	6.8	187.6	-	-	41.2	216.9	25.1
valine,	17.2	16.1	7.7	3.4	14.9	18.3	9.6	47.4	-	-	25.3	58	17.8
isoleucine,	15.4	7.4	21.9	-	11.6	9.1	4.5	24.1	-	-	16.4	26.4	11.2
leucine,	28.6	10.8	15.8	4.9	28.3	15.8	7.1	72.5	-	-	42.7	83.4	21.3
tyrosine,	10.6	-	-	-	-	-	-	-	-	-	-	-	-
phenylalanine,	20.7	-	-	-	3.1	15.9	-	34.7	-	-	19.4	76.4	2.7
lysine,	35.6	4.7	-	6.4	10.7	6.4	-	60.7	-	-	13.8	79.5	4.8
arginine	35.7	-	-	10.8	5.5	-	-	93.2	-	-	4.0	79.3	-
histidine	2.3	-	-	-	9.7	-	-	16.1	-	-	7.1	22.8	-
aspartic acid	31.1	83.7	9.0	18.4	26.7	56.33	21.4	110.9	-	-	52.5	105.5	38.4
glutamic acid	88.8	103.7	26.85	15.9	18.3	85.0	18.8	224.9	-	-	70.6	231.5	43.2

HPLC Analysis of amino acids in
13 samples of honey

Flavonoids

- More than eight flavonoids were identified in honey samples:

Apigenin	4',5,7-trihydroxyflavone
Luteolin	3',4',5,7-tetrahydroxyflavone
Acacetin	5,7-dihydroxy-2-(4-methoxyphenyl)chromen-4-one
Tricetin	3',4',5,5',7-pentahydroxyflavone.

Flavonols

Rutin	3,3',4',5,7-pentahydroxyflavone-3-rutinoside
Quercetin	3,3',4',5,7-pentahydroxyflavone
Myricetin	3,3',4',5,5',7-hexahydroxyflavone
Kaempferol	3,4',5,7-tetrahydroxyflavone

Phytochemical Analysis & Chemical Constituent

HPLC Analysis

For Carbohydrates

- In all of the honey samples, 5 carbohydrates were evaluated and quantified: monosaccharides (fructose, glucose, arabinose, and galactose) and disaccharides (maltose). Regarding the **galactose contents in all of the 13 honey samples**, three of the samples (Samples 3, 5, and 8) had a higher galactose content than the others.

The anti-ulcer activities

- The anti-ulcer activities, showing that **oral administration*** of the honey samples reduced the intensity of ulcer scores when compared to the **control group****.
 - Similarly, there was a highly significant reduction in the values of the ulcer indices and areas in rats that received the same sample ($P < 0.05$ and $P < 0.01$) in comparison with those of the ulcer control rats.
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- * Experimental dose of 100 mg of honey samples / kg of the rats' weight,
 - ** The reference drug; ranitidine was given orally at a certain dose (30 mg/ kg)

The anti-ulcer activities

A) Determination of LD₅₀

All rats treated with different doses of the honey samples (up to 4000 mg·kg⁻¹) were alive during the 24 h of observation. The animals did not show visible signs of acute toxicity, suggesting that the oral LD₅₀ of each of the honey samples was higher than 4 g·kg⁻¹. The tested honey samples are considered to be highly safe because substances possessing LD₅₀ higher than 50 mg·kg⁻¹ are nontoxic.

The anti-ulcer activities

B) Induction of Gastric Ulcers

Gastric ulcers were induced in all of the rats (except for the normal control) following oral administration of 50% ethanol at dose of 10 mL·kg⁻¹. In the normal control rats, an equivolume of distilled water was dosed instead of ethanol. One hour after ethanol administration, all rats were visualized to see the ulcer size which is measured using a medical endoscope through the pharynx.

The anti-ulcer activities

- The **percentage of protection** (P%) availed to the animals through various treatments was calculated using the following formula:

$$P\% = \left[\frac{(\text{UA control} - \text{UA treatment})}{\text{UA control}} \right] \times 100$$

The anti-ulcer activities

C) Results

- No pathological changes were observed in the normal control group, suggesting that the handling procedure did not interfere with the experimental results.
- It was noticed that oral medication with the Samples **2, 5, 6, 8, 11, and 13** ($100 \text{ mg}\cdot\text{kg}^{-1}$), prior to ulcer induction, significantly reduced the intensity of ulcer scores into 3.3, 3.6, 3.7, 3.5, and 3.4, respectively, as compared to 4.8 in the ulcer control group.
- Similarly, there is a high significant reduction in the values of the ulcer indices and ulcer incidence rate that

The anti-ulcer activities

C) Results

- Conversely, Samples **3, 10, and 12** exhibited a lower anti-ulcerogenic efficacy, reducing the ulcer scores, indices, and areas ($P < 0.05$).
- Moreover, Samples **2, 5, 6, 8, 11, and 13** induced a protection against ulcers ranging between 27.6% and 34.2%. To a lesser extent, oral administration of Samples 3, 10, and 12 produced protection at ratio between 18.4% and 21.0%.
- The other honey samples (Samples **1, 4, 7, and 9**) failed to improve the gastric damage.

Effects of Honey samples on the macroscopic parameters of gastric ulcers induced by 50% ethanol in rats (n = 5).

Groups	Ulcer Score (0-5)	Ulcer Index (mm)	Ulcer Area (mm ²)	Percentage of protection
Normal Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	-
Ulcer Control	4.8 ± 0.37	6.1 ± 0.22	7.6 ± 0.24	-
Ranitidine	2.7 ± 0.37 ^b	3.9 ± 0.18 ^b	4.5 ± 0.26 ^b	40.8
1	4.4 ± 0.24	5.6 ± 0.15	7.2 ± 0.55	5.3
2	3.3 ± 0.20 ^b	4.3 ± 0.29 ^b	5.0 ± 0.40 ^b	34.2
3	3.9 ± 0.18 ^a	5.2 ± 0.30 ^a	6.0 ± 0.54 ^a	21.0
4	4.5 ± 0.22	5.6 ± 0.21	7.3 ± 0.68	3.9
5	4.6 ± 0.40	5.8 ± 0.40	7.2 ± 0.68	28.4
6	3.6 ± 0.24 ^b	4.6 ± 0.29 ^b	5.4 ± 0.60 ^b	28.9
7	4.6 ± 0.24	5.7 ± 0.44	7.2 ± 0.24	5.3
8	3.7 ± 0.20 ^b	4.6 ± 0.29 ^b	5.5 ± 0.29 ^b	27.6
9	4.6 ± 0.24	5.8 ± 0.30	7.4 ± 0.56	2.6
10	3.8 ± 0.12 ^a	5.1 ± 0.18 ^a	6.2 ± 0.38 ^a	15.3
11	3.5 ± 0.22 ^b	4.5 ± 0.24 ^b	5.3 ± 0.38 ^b	30.3
12	3.9 ± 0.24 ^a	5.1 ± 0.13 ^a	6.1 ± 0.24 ^a	19.7
13	3.4 ± 0.40 ^b	4.5 ± 0.14 ^b	5.1 ± 0.24 ^b	32.9

Data are represented as mean ± SEM. **a** and **b** denote significant difference vs.

Antioxidant Activity

- The results of this experiment showed that the anti-oxidative activity in all honey samples was very high it has been shown that summer samples (2, 5, 6, 8, 11, and 13), has highly effective than the others , whereas the rest of the samples slightly lower than the summer honey samples, possibly because of the types of flavonoid compounds that have high oxygenation patterns.

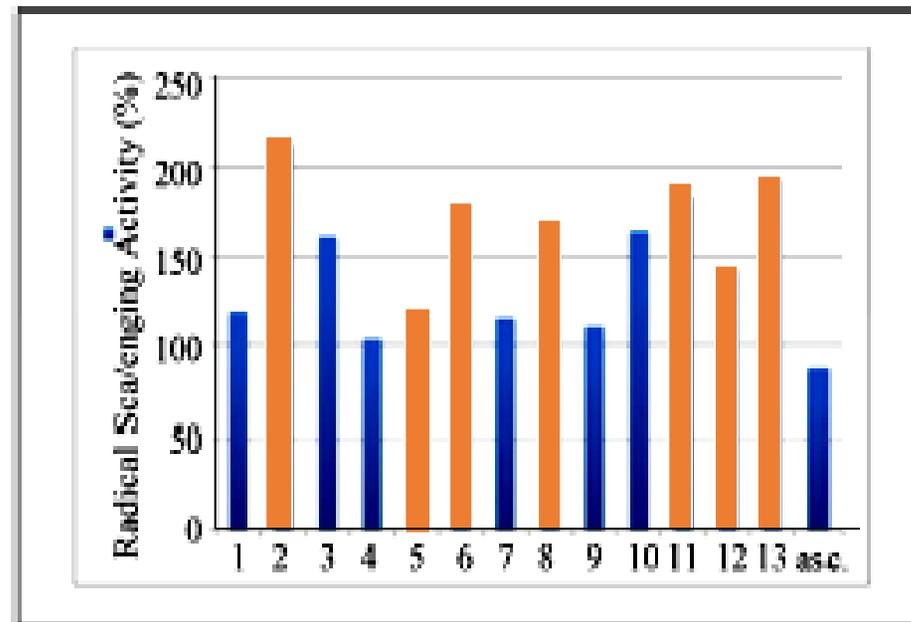
Antioxidative Activity

Summer honey

(2, 5 , 6, 8,
11, 12 and
13)

Winter

(1, 3, 4, 7,
9, 10)



Antioxidant activity (%) of summer and winter honey samples

Conclusion

- The results of this study could be reached that the effectiveness of honey as an anti-ulcer increase with the increasing its antioxidant activities.
- The findings, indicate that the geographical area of harvested honey plays a role in determining the traits of honey and its activity.
- Furthermore, honey that demonstrates high anti-oxidant activity and contains certain types of flavonoids can be used to treat stomach ulcers effectively.

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Thank You For Your Kind Attention

