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Qualitative Analysis for Free Radical Scavenging and Acid Value of Honey Including GC-MS Spectra.

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ABSTRACT

Essentially it is a sweet flavouring natural product, which is consumed for its high nutritive value and effects on human health, with antioxidant, anti-inflammatory, antimicrobial and bacteriostatic properties as well as wound and sunburn healing effects**.** Honey is used as indigenous medicine in infants where as its antioxidant property of honey make its popular in the higher aged group peoples. Natural honey is a sticky and viscous solution with contents of 78-80% carbohydrate (mainly glucose and fructose), 15-17% water, 0.1-0.4% protein, 0.2% ash and minor quantities of amino acids, enzymes and vitamins as well as other substances. GCMS analysis of honey showed the higher concentration of6-Oxa-bicyclo [3.1.0]hexan-3-one, 1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl)ester, 1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl)ester, Cyclopropane, nonyl, and DL- Arabinose and the minimum concentration of 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3á,5Z,7E), d-Glycero-d-galacto-heptose, Paromomycin and d-Gala-l-ido-octonic amide. Many other substances also occurs in honey, but sugars are the major components. The composition of honey is variable and depends mainly on its floral source.

Keywords: Honey, antioxidant activity acid value and GC-MS analysis.

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INTRODUCTION

In the Pre-Ancient Egyptian times, honey was used topically to treat wounds.[1] The ancient Greeks believed that consuming honey could help you live longer. Even the Prophet Mohammed glorified the healing powers of honey. Over four thousand years ago, honey was used as a traditional ayurvedic medicine, and it was thought to be effective for treating material imbalances in the body.

The beneficial properties of honey have been explored by various scientists in modern times, and there is evidence to suggest that these historical claims may hold some truth. According to the US Department of Agriculture (USDA) National Nutrient Database, one tablespoon of honey (approximately 21 grams) contains 64 calories, 17.3 grams of carbohydrate (17.3 grams of sugar no fiber), 0 grams of fat and 0 grams of protein.[2]Honey is made up of glucose, fructose, and minerals such as iron, calcium, phosphate, sodium chlorine, potassium, magnesium, with the approximate percentage Fructose: 38.2%, Glucose: 31.3%, Maltose: 7.1%, Sucrose: 1.3%, Water: 17.2%, Higher sugars: 1.5%, Ash: 0.2%, Other/undetermined: 3.2%[3-5].The dark colored honeys have been reported to have higher total phenolic contents and, consequently, higher antioxidant capacities [4].

Honey has been utilized by civilisation for its medicinal properties[6-8] for over 2,000 years and continues its legacy as a multipurpose health aid few are listed below:-

ALLERGIES SUPPRESSOR

The anti-inflammatory, anti allergic effects and ability to soothe coughs has led to the belief that honey can be helpful in reducing seasonal allergic symptoms. Although there are no medical studies providing its this efficiency,

"A common theory is that honey acts like a natural vaccine." It contains small amounts of pollen, when if the body is exposed to small amounts of it, it can trigger an immune response that produces antibodies to the pollen. "After repeated exposure, person could build up these antibodies and the body should become accustomed to their presence so that less histamine is released, resulting in a lesser allergic response."

NATURAL ENERGY ELEVATOR

Honey is considered to be an excellent source of all-natural energy at just 17 grams of carbohydrates per tablespoon. The natural unprocessed sugar - fructose and glucose - directly enter in to the bloodstream and can deliver a quick boost of energy. This rise in blood sugar level acts as a short-term energy source for your workout, especially in longer endurance exercises.

MEMORY-BOOSTER

The ability of honey to help brain's health is indeed unbeatable. The brain needs calcium in order to process thought and make decisions. As we grow older did not care about good intake of vitamins and minerals, honey used to maintain it up to some extent. A 2011 survey published in Menopause found a daily spoonful of Malaysian honey may boost women's memory after menopausal period, which can provide an alternative therapy for the hormone-related intellectual decline. After four months of taking 20 grams of honey a day, the women were more likely to have better short-term memory than their counterparts who took hormone pills. The sweet nectar is loaded with antioxidants that may help in preventing cellular damage and loss within the brain.

COUGH RELIEVER

Honey is believed to be effective in common cough due to its ingredient dextromethorphan. It can be also used in treating upper respiratory tract infections. It has believed that persistent cough that won't go away can easily be remedied with two teaspoons of honey, according to a 2012 study published in the journal Paediatrics. It act as a good tonic for the children between the age of 1 to 5. The thick consistency helps of this magic golden liquid's coat the throat where as sweet taste is believed to trigger nerve endings that protect the throat from incessant coughing.

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SLEEP AID

Honey is a good medicine for the people suffering from sleepless nights. Honey helps in steady rise in insulin, according to **Brennecke**, causes the tryptophan in honey to enter the brain, where it is then converted into serotonin and then into melatonin, which is a sleep aid. This hormone is responsible for regulating sleep and wake cycles.

TREATS DANDRUFF

Honey is also helpful in bringing temporary relief to the scalp by curing dandruff. A 2001 study published in the European Journal of Medical Research is that "application of 10 percent diluted honey to problem areas and leaving it on for three hours before rinsing led to itch relief and no scaling within a week. The patients did not relapse even after six months of use.

ANTIBACTERIAL AND ANTIFUNGAL PROPERTIES

Many researcher have reported the antibacterial activity of honey and found that natural unheated honey has some broad-spectrum antibacterial activity when tested against pathogenic bacteria, oral bacteria as well as food spoilage bacterialt can also treat seborrheic dermatitis and dandruff, which are often caused by an overgrowth of fungus. Moreover, "honey also has anti-inflammatory properties, which address the redness and itching on the scalp,"[9-10].

WOUNDS AND BURNS HEALING AGENT

Since many years it can be used as a conventional treatment for wounds and burns by disinfecting wounds and sores from major species of bacterial. Honey is used as natural antibiotic that can act both internally and externally [11]. Such as methicillin resistant Staphylococcus aureus (MRSA). A 2005 study published in the British Journal of Surgery found all but one of patients who suffered from wounds and leg ulcers showed remarkable improvement after applying a topical application of honey. Antibacterial agent methylglyoxal (MGO) comes from the nectar of mānuka trees is having very good antibacterial and wound healing property.

MATERIALS AND METHODS

(i) TEST FOR THE ANTIOXIDANT ACTIVITY OF HONEY:

Take the samples of Honey and tested it for antioxidant capabilities. **DPPH** (2,2diphenylpicrylhydrazyl).This test was carried out by mixing 1.5 ml ethanolic solution of each sample with 2 ml of a 0.2mM ethanolic solution. Take three different concentrations of honey 2,4, 6 µg of sample is mixed with 1.5 ml of 0.2mm ethanolic DPPH solution. This mixture then put in dark for 30 minutes at 25^0C and the absorbance at 514 nm was recorded (A sample). Same experiment was also carried out without using the test material that is honey only having ethanolic DPPH solution. And the absorbance was recorded as (A blank).The free radical scavenging activity of each solution was then calculated as % inhibition according to the following equation [12].

$$\% \text{ Inhibition} = 100 - (\text{Abs sample} - \text{Abs blank}) \times 100 / \text{A control}$$

If % inhibition becomes equal to or more than 90% then it will show antioxidant properties

DPPH Control: - 0.473

(ii) TEST FOR ACID VALUE OF HONEY

Take 0.5 g of substance in conical flask; add 50-100 ml of 95% ethyl alcohol to it and 2-3 drops of phenolphthalein indicator. Then boil the mixture for 5-10 minutes on water bath and titrate it with 0.1N KOH solution.

$$\text{Acid value} = 56.1VN/W,$$

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Where V = Volume of KOH used,N = Normality of KOH,W = Weight in gram of sample.Titrate it with 0.1N KOH solution, during titration the volume of KOH used is 0.1 ml

Acid value of Honey = 56.1×0.1×0.5 = 2.805

(iii)GAS CHROMATOGRAPHY–MASS SPECTROMETRY ANALYSIS:

The GC–MS analyses were performed on a Thermo Scientific TSQ 8000 Gas Chromatograph - Mass Spectrometer. This mass spectrometer comes paired with the TRACE 1300 GC along with auto-sampler for automated sample handling. The analysts were separated in RT: 3.00 30.10 SM: 15G. The split/ splitless injector temperature was set at 400°C and volume was 1.0 µl.[13]

IDENTIFICATION OF COMPONENTS

The components of the essential oil were identified on the basis of comparison of their relative indices and mass spectra by computer matching with WILEY8 and National Institute of Standards and Technology (NIST08) libraries provided with the computer controlling GC-MS system [14].

RESULTS AND DISCUSSION

Antioxidant activity observed at different concentration of honey is mentioned in table no.1 given below, is showing its good free radical scavenging nature.

In total 40 components were identified. In which the higher concentration of 6-Oxa-bicyclo [3.1.0] hexan-3-one, 1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl)ester, 1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl)ester, Cyclopropane, nonyl, and DL- Arabinose are noticed and 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3á,5Z,7E), d-Glycero-d-galacto-heptose, Paromomycin and d-Gala-l-ido-octonic amide are observed with minimum concentration.

Table 1: Antioxidant activity of Honey at different concentration

S.No.	Concentration	Blank	Sample+ DPPH	% Anti oxidant Activity
1	10	0.057	0.128	84.98
2	20	0.065	0.117	89.00
3	30	0.071	0.115	90.69
4	40	0.076	0.111	92.60
5	50	0.082	0.105	95.13

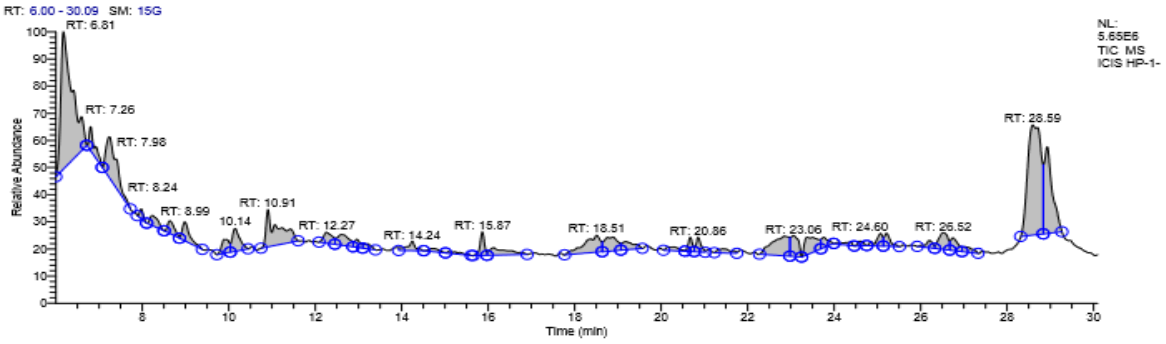

Fig 1: The GC-MS spectra showing presence of number of compounds in honey.

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Table 2: Components of Honey identified by GC-MS analysis

Sr. no	RT (min)	Area%	Peak Area	Compound name	Mol. Formula
1	6.18	19.04	52246262.21	6-Oxa-bicyclo[3.1.0]hexan-3-one	$C_5H_6O_2$
2	6.81	1.62	4456523.41	N-(1-Methoxycarbonyl-1- methylethyl)-4- methyl-2-aza-1,3-dioxane	$C_9H_{17}NO_4$
3	7.26	5.30	14549156.43	DL- Arabinose	$C_5H_{10}O_5$
4	7.98	0.42	1156263.16	d- Glycero-d-ido-heptose	$C_7H_{14}O_7$
5	8.24	1.02	2805391.91	d- Glycero-d-galacto-heptose	$C_7H_{14}O_7$
6	8.64	1.08	2973640.66	Methyl 6-oxoheptanoate	$C_8H_{14}O_3$
7	8.99	1.67	4588072.32	Butanoic acid, 4-(1,1-dimethylethoxy)-3-hydroxy-, methyl ester, (R)-	$C_9H_{18}O_4$
8	9.90	1.23	3378308.72	L-Glucose	$C_6H_{12}O_6$
9	10.14	2.10	5766910.87	N-(1-Methoxycarbonyl-1-methylethyl)-4-methyl-2-a za-1,3-dioxane	$C_9H_{17}O_4$
10	10.91	5.49	15069237.21	Cyclopropane, nonyl	$C_{12}H_{24}$
11	12.27	1.16	3171208.44	Desulphosinigrin	$C_{10}H_{17}NO_6S$
12	1.62	1.61	4413576.40	d-Gala-l-ido-octonic amide	$C_8H_{17}NO_8$
13	12.97	0.60	1656968.52	d-Glycero-d-galacto-heptose	$C_7H_{14}O_7$
14	13.22	0.45	1245611.79	d-Gala-l-ido-octonic amide	$C_8H_{17}NO_8$
15	14.24	0.94	2583381.26	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	$C_{30}H_{40}O_5Si_6$
16	14.77	0.39	1060482.41	d-Glycero-d-galacto-heptose	$C_7H_{14}O_7$
17	15.22	0.34	933992.64	d-Gala-l-ido-octonic amide	$C_8H_{17}NO_8$
18	15.87	1.12	3317296.89	Hexadecen-1-ol, trans-9-	$C_{16}H_{32}O$
19	16.12	1.62	4449753.69	Furan-2-carbohydrazide, N2-(3-indolylmethylene)-	$C_{14}H_{11}N_3O_2$
20	18.51	3.44	9436138.14	3-Butoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	$C_{19}H_{54}O_7Si_7$
21	18.76	2.26	6208855.28	á-D-Glucopyranose, 4-O-á-D-galactopyranosyl-	$C_{12}H_{22}O_{11}$
22	19.18	1.20	3291678.70	d-Glycero-d-galacto-heptose	$C_7H_{14}O_7$
23	20.31	0.57	1564731.86	Paromomycin	$C_{23}H_{45}N_5O_{14}$
24	20.67	0.72	1981831.06	Hexadecen-1-ol, trans-9	$C_{16}H_{32}O$
25	20.86	0.88	2419583.52	Diethyl Phthalate	$C_{12}H_{14}O_4$
26	21.43	0.63	1741278.20	Paromomycin	$C_{23}H_{45}N_5O_{14}$
27	22.85	4.15	11385707.07	Desulphosinigrin	$C_{10}H_{17}NO_6S$
28	23.06	2.00	5489508.83	Desulphosinigrin	$C_{10}H_{17}NO_6S$
29	23.37	2.58	7072549.24	Paromomycin	$C_{23}H_{45}N_5O_{14}$
30	23.76	0.82	2243774.25	Paromomycin	$C_{23}H_{45}N_5O_{14}$
31	24.38	0.37	1008528.46	Paromomycin	$C_{23}H_{45}N_5O_{14}$
32	24.60	0.34	928816.05	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3á,5Z,7E)-	$C_{27}H_{44}O_3$
33	25.07	0.99	2722744.15	2-Heptadecanol, acetate	$C_{19}H_{38}O_2$
34	25.21	0.95	2595983.90	2-Cyclohexen-1-one, 4-hydroxy-3,5,6-trimethyl-4-(3-oxo-1-butenyl)-	$C_{13}H_{18}O_3$

35	26.21	0.64	1764074.73	Isoxazolo[2,3-b]benz[1,2]oxazine, perhydro-2-methoxycarbonyl-10-methyl-5a-trimethyl silyloxy	$C_{16}H_{29}NO_5Si$
36	26.52	1.89	5196319.85	2-Phenyl-2,3,4,5-tetrahydro-1H-benzo[d]azepine	$C_{16}H_{17}N$
37	26.75	1.13	3110224.38	2-Phenyl-2,3,4,5-tetrahydro-1H-benzo[d]azepine	$C_{16}H_{17}N$
38	27.03	0.64	1750012.13	1-Naphthol, 1,2,3,4-tetrahydro-2-methyl-	$C_{11}H_{14}O$
39	28.59	17.77	48766752.22	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	$C_{24}H_{38}O_4$
40	28.93	8.73	23958370.05	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	$C_{24}H_{38}O_4$

CONCLUSION

The slightly acidic pH level of honey (between 2.9 and 4.5) helps in preventing the growth of bacteria,Low acid value of honey favour its role in various medicinal ailments and it is even safe for infants. It has always been recommended as multipurpose medicine. While its antioxidant constituents cleans up free radicals even as ant-agingcompound. The physical properties of honey vary depending on the specific flora that was used to produce it, as well as its water content.

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