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# Evaluation of Antioxidant and Anti-mutagenic Activity of Naturally Fortified Honey with *Curcuma Longa* Extract

**SUNDUS HAMEED AHMED**

Al- Mustansiriyah University/ Science College, Baghdad, Iraq.

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**ABSTRACT**

The current research planned to explore the activity to anti-mutagenic and anti-oxidant to naturally fortified honey with *Curcuma longa* extract. The fortified honey was evaluated by GC MASS method. The activity of the anti-oxidant elevated due the activity of the free radicals in 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH), nitric and super oxide. Scavenge is achieved by alkaline DMSO and lipid per oxidation method, While anti mutagenic activity were assessed by mitotic index (MI), chromosomal aberration (CA) and Micronuclei (MN) tests. The outcomes data from the current work shows fortified honey had a highest antioxidant activity in compare with *Curcuma longa* extract .Results of genotoxic activity showed that furfural increased formations of MNs, CA and MI in blood culture associated with control, opposing, natural honey by itself didn't display any effect in geno-toxic while the effect in anti-mutagenic of honey could be related the capability of scavenge power to antioxidant for free radical.

**Keyword:** fortified honey, DPPH, GC MASS, DMSO, MI, MN, CA.



## INTRODUCTION

Honey composition depends on its flower sources, ecological influences[1], many Studies have shown that honey with dark color have the highest concentration of antioxidant than the light color[2]. Honey usually consider as a product come formed in nature and because of its high nutrition value in addition to the properties that influence the health of human being as antioxidant, antibacterial and anti-inflammation, all these properties and more rise its consumption[3]. Antioxidants existing in honey are so special because it contains both ascorbic acid which is not an enzyme compound with peroxidase and catalase which are enzymes[4], moreover honey contain tocopherol[5], organic acids like amino acids, carotenoids in addition to proteins, , Maillardreaction[6] *Curcuma longa* belong to the family: Zingiberaceae itœs extract have antioxidant properties[7]. Curcumin sulphate and glucuronide is The major constituents of the extracts. It causes apoptosis for cancer cell including colon, fore-stomach, duodenum and and etc[8]. Tha aim of our study detection the antioxidant activity of fortified honey with *Curcuma longa* active component, and studying the active component of fortified honey and its ability to prevention Cell mitosis.

## MATERIAL AND METHODS

Spectrophotometrical method used for determination total polyphenols content, we used by using Folin-Ciocalteau reagent. One gm of honey fortified with *Curcuma longa* mixed with 5 ml of meth-alcohol to determine it, after mixing the sample and make it homogeneous, 0.5 ml of it was

taken using pipette and placed it in volumetric flask (10 mL) and 0.5 mL of D.W. to dilute it, then 0.5 ml of Folin-Ciocalteau was placed in the solution then a solution of sodium carbonate (1.2 ml ) was also add to the solution. Then a 20 minutes was gave to the mixture complete the reaction, after that the spectroscopy measurement was achieved using 765 nm. The expression of TP is (mg GAE 100/g) which represent the weight of Gallic acid in milligrams (mg) per weight of honey (normally 100 g).

**Detection active component**

Active component of control honey and *Curcuma longa* extract and fortified honey with date palm fruit by:

**GC-MS**

GC/MS analyses were begun with injected 1 ìL of the sample in split mode 1/10; The Injection carried at 250 Celsius. Using DB-5MS column (30 m × 0.25 mm × 0.25 ìm) was applied in a continuous current of the carrier gas (1 mL/ minute). The start degree of the temperature was 5 °C, and kept for 3 minutes then rise 10 °C every minute until it reached 290 °C. Mass spectroscopy was achieved and the data base and the peaks was collected at 230 the mode of ionization was 70 eV and (m/z) was 35–450.

**The activity of antioxidants (*In-vitro*)**

Standard solutions were prepared using meth-alcohol (methanol) as a solvent to dissolve ascorbic acid to prepare five different concentrations (starting from 10 to 50 ìg/ml) rising 10 ìg/m to test the free radicals activity of DPPH.

**Preparation of DPPH solution**

Dissolved about 8.6 mg of DPPH in 6.6 ml meth-alcohol (methanol); test tubes incubate in dark to protect from light.

**Estimating the scavenge activities to DPPH (Protocol)**

Solution of DPPH around (150 µL) was added to 3 ml meth-alcohol (methanol), the absorptivity was immediately taken using 516 nm, the read was used as control for comparison. Changing the volume in the levels of test sample ( from 100 to 200) 20 ìl every time using methanol as solvent to increase the volume. The absorptivity was taken to each solution using (Shimadzu, UV-1700 spectrophotometry) at the same wavelength. The calculations of $IC_{50}$ and the decreasing percent were done as depicted in the below equation: The free radical scavenging activity (FRSA) (% antiradical activity) was calculated using the following equation:

$$\%FRSA = \frac{\text{Absorptivity of control - Absorptivity of sample}}{\text{Absorptivity of control}} \times 100$$

(Where FRSA represent the scavenging activities of the free radicals)

Each testing was repeated three times and outcomes actually are the average of them (mean % ± SD10).

**Reagents**

DMSO (dimethylsulphoxide), Nitro-blue tetrazolium.

**Preparing stock solutions**

Dissolve 1 gram of curcumin in 1 mL of distilled water (DW) then dilute this solution using DW to achieve different concentrations (10 to 50 ìg / Ml).

Using the equal quantities of methanoic extract powder and dissolve it same volume of DMSO to achieve a (1 mg/ml solution), dilute the stock solution by using DMSO to prepare different concentration (from 100 to 600 ìg / mL).

- To prepare Alkaline DMSO: take 2 ml alkaline DMSO containing, 10 mMol of sodium hydroxide in 0.2 ml of DW and 1.8 mL of DMSO
- Nitro-blue tetrazolium (NBT): Dissolve 50 mg of NBT was in 50 mL of DMSO to achieve (conc. 1 mg/mL).

Take 0.2 mL from nitro-blue tetrazolium (2 mg / mL stock prepared above) and 0.6 mL of the standard extracted in DMSO, 2 mL from alkaline DMSO (10 m M of sodium hydroxide dissolved in 0.2 mL DW) the two stock solutions were added to each other's to get 2.8 mL volume, the absorptivity was taken at the same wave number (560 nm), (1.2 mL, 80 mM) of hydrogen peroxide was added to the above stock solutions (100-600 ìg / mL), 600 ìL

of DMSO, 0.2 ml NBT solution and 2 ml alkaline DMSO were mixed and the absorptivity of this solution was used as control reading.· The percent of was determined as depicted below: :

**Culture of Lymphocyte**

Lymphocytes culture from human peripheral was used as the test.

This research was achieved due to donation of blood from three not smokers and healthy donators and there ages were (between 20 to 30 years ages) with no medication for at least 4 weeks. For the sister chromosomal exchange (SCE) chromosomal abnormalities. CA researchers, add 5.0 from chromosome to 0.4 of blood (contains heparin, phytohemagglutinin, fetal bovine serum and antibiotics) enhanced with 20 lg/mL bromo-deoxyuridine. All these cultures incubated at 37 Celsius for three days (72 hr ). with fortified honey, no changes in acidity of medium were noticed.

Anthracene $40x10^{-5}$ were used to treat the human lymphocytes for one to two days, the positive and negative controls (mitomycin-C = MMC, 0.40 lg/ mL) were also preserved in all experimental cells. The preparation of CA was achieved according to methods[8] in and SCE tests with minor modifications (9). 200 from each donor of well-spread metaphases (in total 400/dose) chromosomal deviations score. Mitotic Index (MI) were specified by counting 2000 cells from every giver. For the SCE assess, the samples were marked with Giemsa, according to Evans methods [10, 11]. The number of SCEs studied according[12].

**Micronuclei**

Preparation of micronuclei was achieved as the protocols in references[13,14]. Propagations of cells were assessed using CBPI (Cytokinesis Block Proliferation Index) which showed the middling quantity of cell series, specified cell had undertaken[15, 16, 17]. The main damage in DNA was due to the presence of Anthracene which was specified by means of comet evaluation [18], with few changes. The details of method that follows the comet assess in human lymphocytes was specified in the work[19].

To distinguish the impracticality of cells, we used exclusion assessment of trypan blue which were (>98%). Lymphocytes were isolated and incubated using stimulated honey with (1 mg / ml for 1 hour at room temperature). Positive and negative controls (Anthracene $20x10^{-5}$) involved as well. The percent of tail moment were around (100/slide) which represent 200 comets in total for each concentrations which were resolute through certain image. The investigation method was (Comet Assay IV, Observant Instruments Shimadzou). The data of the outcomes was studies carefully and the test was realistic to specify the percent of unusual cells with MI, CBPI, MN, CA, CA/cell, Dose–response, RI, SCEs. Relations and correlations were resolute from the abnormal cells percentage, MN, SCE, CA/cell.

$$\%\text{ scavenging activity} = \frac{\text{Treatment absorbance value}}{\text{Blank absorbance}} \times 100$$

## RESULT AND DISCUSSION

Table (1) showed that the total phenol and total flavanoid of the honey control, *Curcuma* extract and fortified honey. We found that fortified honey had the highest value of total phenol and total flavanoid as shown: 35.14± .0.017and 25± 0.0245 in compare with control honey and *Curcuma* water extract.

**Table 1: Total Phenol and total flavonoid of *Curcuma* water extract, Honey none fortified with *Curcuma* active component and Fortified with *Curcuma***

| Total Flavonoids | Total Phenols (mg of GAE/g) | Material |
|---|---|---|
| 17.03± .0.08 | 17.13± .0.024 | *Curcuma* water extract |
| 18.98± .0.022 | 22.65± .0.032 | Honey non fortified with *Curcuma* active component |
| 25.47± .0.024 | 35.14± .0.017 | Fortified with *Curcuma* |

Figure (1)and Table(2) showed the active component of all samples¡ we found there is many new anticancer component and anti clinical diseases were found in fortified honey as: 2propanone1,3dihydroxychromelin, Ethyl Ester, Dl-Glyceraldehyde dimer, Dl-Glyceraldehyde dimer,

**Table 2: Active component in fortified honey by GC-MS**

| No | Chemical Component | Fortified Honey | Curcuma | Control Honey |
|---|---|---|---|---|
| 1 | 2propanone1,3dihydroxychromelin | + | | |
| 2 | Ethyl Estar | | | |
| 3 | Dl-Glyceraldehyde dimer | | | |
| + | + | | | |
| 4 | phosphoric triamide | + | | + |
| 5 | n-Hexanal | + | | + |
| 6 | ? -phellanderene | | + | |
| 8 | ? -zingiberene | | | |
| 9 | Propranolol | + | | |
| 10 | 1-propanol,2- aminoalaninol | + | | |
| 11 | Octane | + | | + |
| 12 | p-cymene | | + | |
| 13 | phosphoric triamide | + | | |
| 14 | Heptanoic acid | + | + | |
| 15 | Cineol | | + | |
| 16 | Methylene asparagine | + | | + |
| 17 | Terpinolene | | + | |
| 18 | n-Nonane | | | + |
| 19 | Coumarin | + | | + |
| 20 | 2-Phenylethanol | | | + |
| 21 | Benzylalcohol | + | | + |
| 22 | 2-Furaldehyde | | | + |
| 23 | Botanic acid | + | | + |
| 24 | ?-Caryop[illegible] | | + | |
| 25 | L- prop[illegible]e | + | | + |
| 26 | Succi[illegible] acid | + | | |
| 27 | 1,3 P[illegible]ndi | + | | + |
| 28 | 3-Deoxyma[illegible]ic acid | + | + | |
| 29 | (+)Ascorpic acid | + | | + |
| 30 | Curcumin | | + | |
| 31 | ? -turmerone | | + | |
| 32 | 8,11-Octadecaoic acid | + | + | |
| 33 | Heptadecanoic acid | + | | + |
| 34 | 9,15-Octadeca-dien-1-ol | + | | |
| 35 | 9,12,15-Octadecatrienoic acid | + | | + |
| 36 | ? -bisabolene | | + | |
| 37 | sesquiphellanderene | | + | |
| 38 | r-turmerone | | + | |
| 39 | Tetradecane | + | | |
| 40 | Inositol | + | + | |

phosphoric triamide, n-Hexanal, á -phellanderene, Propranolol, 1-propanol,2- aminoalaninol , Octane, p-cymene, phosphoric triamide, Heptanoic acid, cineol, Methylene asparagines, Coumarin, 2-Phenylethanol, Benzylalcohol, Botanic acid, L-propline, Succinic acid, 1,3 Propandiol, 3-Deoxymannoic acid, (+)Ascorpic acid, 8,11-Octadecaoic acid, Tetradecane, Inositol.

**In-vitro antioxidant activity**

**DPPH free radical scavenging activity**

Table (3) Illustrates increases the dose of DPPH free radicals scavenging according to the high capability to scavenge to fortified honey extract. The value of $IC_{50}$ was 8.02ìg/ml in compare with other samples. The scavenge activity to the active oxygem of nitric oxides were affect the pathology action. Nitrogen species reactivity are in the following sequence $NO_2$, $N_2O_4$, $N_3O_4$ respectively, while the nitrate $NO_3^-$ and nitrite $NO_2^-$ shows high activity. We found that fortified honey had the lowest value $IC_{50}$ value 22.14 ìg / ml, the scavenging activity of radicals liberate from superoxide's using alkaline DMSO process are well-known in its harmfully to cells components.

Due to the reaction between (alkaline DMSO) and the (NBT) the free radicals were formed

**Table 3: $IC_{50}$ value of different antioxidant activity**

| Test | Fortified honey | *Phoenix dactylifera* | Control honey |
|---|---|---|---|
| DPPH scavenging | 8.02±0.012 | 25.7±0.0[illegible] | 18.68±0.022 |
| Nitric OxideScavenging | 22.14±0.015 | 1[illegible].005 | 120.94±0.033 |
| Super oxideScavenging | 53.04±0.02[illegible] | 108.31±0.022 | 155.13±0.044 |

**Table 4: Chromosomal aberration by Furfural**

| Treatment | Type of chromosomal aberration | | | | | | | Total CA |
|---|---|---|---|---|---|---|---|---|
| | M[illegible] | G' | G" | B' | B" | Deletion | SF | |
| Negative Control | [illegible] | | | | | | | |
| Fortified honey negative control | 9.39 | 0.10 | 0.02 | | | | | 0.12 |
| Positive control furfural (10x10$^{-5}$) | 5.[illegible]3 | 0.68 | 0.57 | 0.28 | 0.27 | 0.05 | 0.05 | 1.9 |
| Fortified honey after furfural | 11.[illegible] | 0.31 | 0.18 | 0.12 | 0.10 | - | - | 0.99 |
| Fortified with furfural | 12.26 | 0.47 | 0.33 | 0.20 | 0.0.12 | - | - | 1.12 |
| Furfural after fortified honey | 12.57 | 0.51 | 0.38 | 0.23 | 0.13 | 0.01 | 0.01 | 1.27 |

**Table [illegible] Number of Distribution of MN on the cell**

| Treatment | No of the testing cell | Distribution of MN on the cell | | | | No of the cells contain MN | No of MN | No MN /Total cells No |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 3 | 3 | 0.003 |
| Negative Control | 1000 | 998 | 3 | - | - | 5 | 5 | 0.005 |
| Fortified honey negative control | 1000 | 997 | 5 | - | - | 5 | 5 | 0.005 |
| Positive control furfural (20x10-5 | 1000 | 972 | 13 | 3 | 2 | 18 | 25 | 0.025 |
| Fortified honey after furfural | 1000 | 995 | 7 | - | - | 7 | 7 | 0.007 |
| Fortified with furfural | 1000 | 988 | 10 | 2 | - | 8 | 14 | 0.010 |
| Furfural after fortified honey | 100 | 988 | 9 | 2 | 1 | 12 | 16 | 0.016 |

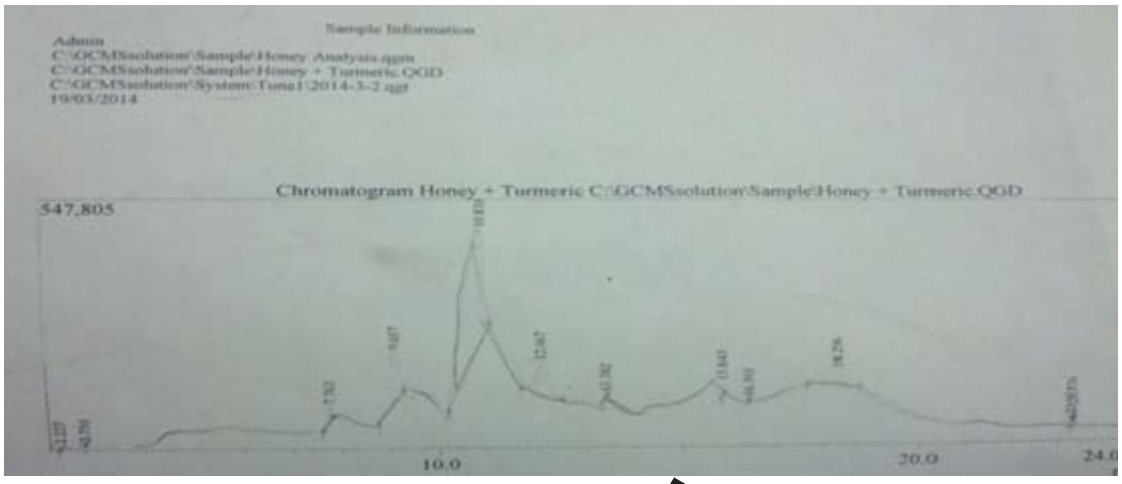


**Fig. 1: Active component in fortified honey by GC-MS**

to give the painted diformazen. The radical oxides in fortified honey cause the formation and the inhibitions of formazan as shown in Table 4. (The concentration of ascorbic acid used was 53.04ig/Ml).

In vitro genotoxicity exams discovered the complexes that cause the harms in genes, in both direct and indirect ways, in diverse routes. They were counted as a primary biologic signs that is of exposure to carcinogenic chemicals[20].

The data gathered from all tests shows toxicity of additives in all their concentration, while the treating time reduced the mitotic index.

Furthermore the frequencies of CA / Cell were increases in every treated group comparing to negative control. Six categories of chromosome deviation where caused by fortified showing its clastic effect. The experiments and the data that was gathered in this research indicate the breaks in chromosome as the main deviation which lead to rearrangement in the structure that increase the irregularities in chromosome isolation during mitosis[21]. The increases in the risks of cancers were related to increases in CA level[22]. We found that fortified honey decreased chromosomal aberrations in all treatments as shown in table (3). Anthracene cause increases in the frequencies of MN depending on doses. In addition to its reflection to genomic variability, MN analyzes both chromosomes clast (breakage of chromosomes) and chromosomes lag because of dysfunction[23].

Our experiments, fortified honey reduced the mitotic index in all actions. MI lessening may be because of G2 block which prevent cells to enter mitosis otherwise it could be due to lessening in the levels of ATP pressure resulted from the production of energy centers. Inhibition of certain cell cycle-specific enzymes, like polymerases of DNA, and its necessarily in generation of DNA, and other enzymes as well were involved directly with their orientations or the assemblies, this could clarify the effect of anti-mitosis and the frequency changes of diverse cell steps as well (24). Nuclear divisions and duplications indicate the of chemical additives effect, Table (5) Chromosomal aberration by Anthracene.

(B'= Chromatid break, B"=Chromatid break, G' Chromatid gap, G' Chromosomal gap, while SF=Simple fragment)

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