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# Protective activity of some antioxidant plant against hydrogen peroxidase genotoxic effects on human lymphocyte

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**Abstract**
The study aims to evaluation protective activity of different plants extracts, methanol extract of *Barley (Hordeum vulgare L.);* sage *(Salvia officinalis L)* and Pomegranate cortex (*Punica granutum*) and cold and hot water extract of Ginger ( *Zingiber officinale*) and soaking method for saffron *(Ccinerocus sativus)* against oxidative stress caused by $H_2O_2$ on human lymphocyte *in vitro* using DNA fragmentation test . Plant extracts were characterized using thin layer chromatography and detection phenol compounds using ferric chloride reagent. Results showed that some plant extract consist of different compounds and have phenolic compounds in its extracts, some of this extracts didn't have any effect on DNA when it use alone, but others have genotoxic effects, also its appeared protective effect against $H_2O_2$ which it caused fragment in DNA.
**Key words**: oxidative stress, DNA fragmentation, antioxidant activity.

## 1. Introduction

As a results of pollution in environment and increased our life problems which is caused elevate in oxidative stress and decreased in antioxidant system that conduce to harmful effects on cell compartment.
DNA is the most important particles in our life it is consider as secret of life which carrying all genetic information for cell structure and functions. DNA affected by oxidative stress that and this effects was become more affecting than others cell particles because it accumulated in lapse that causes genetic mutations and disease (Langseth, 1995).
Oxidative stress caused by increased reactive oxygen species (ROS) and reactive nitrogen species (RNS) where antioxidant system cannot balanced between free radicals production and its degradation thus it interact with cell partials such as proteins, lipids and nucleic acid (DNA , RNA) (Al-Saadi *et al*., 2012) .
Hydrogen peroxidase $H_2O_2$ have major roles in the cell activities , it is formed by superoxide anions transform or spontaneously in $O_2$ reduction by super oxide dismutase , $H_2O_2$ have free pair of electron in outer membrane its soluble in lipid thus it can cross threw membranes of cells also it generate in inflammation during immune system response (al-Saadi *et al*., 2012). Oxidative stress causes structural changes in DNA like point mutation, rearrangement, deletion, insertion and duplication in some sequences. Oxidative stress causes changes in DNA double helix such as breaks in strands, changes in major and minor grooves and changes in double helix . Also it causes cross link between DNA-DNA and DNA-protein , miss match between nucleotide paring(Aust and Eveleigh, 1999)
Esteve *et al*., (1999) Clarify that Mitochondrial genome more effect by oxidative stress than genomic DNA because mitochondria is a source of free radicals that produced in oxidative phosphorylation thus it may be causes dieses that relative with mutation in mitochondrial genome . To remove or reduced harmful effects of free radicals; cells use antioxidant systems that consist from different mechanisms which is responsible of remove excess of free radicals or interact with it to prevent its harmful effects (Shulaev *et al*., 2006)

Antioxidant system consist of enzymatic and non-enzymatic mechanisms, enzymatic mechanisms include antioxidant enzyme that prevent free radicals or oxidative materials from interact with cell compartment, like glutathione enzymes, catalase, thirodixin, superoxide dismutase, non-enzymatic mechanism include primary, secondary and natural antioxidant mechanisms, the last type of these is the axis study because it contain from natural compounds that present in nutrient or synthesis in the body, this types consist from upiquinol, uric acid, melatonin, lipoic Alfa, vitamins, carotenoid, flavonoids phenolic compounds, anthocyanin's and trace elements . (Langseth,. 1995; Halliwell and Chirico, 1993; Levonen,. 2000)
Antioxidant can be gotten from natural source, some plant have antioxidant activity in one part or whole plant. Literature review report plant extract roles as antioxidant agents in vivo and in vitro experiments.
Al-Terehi *et al.,* (2012) used licorice root extract as protective agent against oxidative stress caused by anticancer drug they found that extract reduced harmful effect of oxidative stress on DNA.

 

Al-Saadi *et al.,* (2013) use *Orignm vulgara* fractionation extract as antioxidant against UV irradiation that induced formation free radicals in human lymphocyte.
Also green tee extract was used as antioxidant agents against cyclophospomide drug and x-ray in white albino rats (al-Saadi 2013; AL-gebalee, 2007).

## 2. Materials and methods

2.1 Optimization $H_2O_2$ concentration.
In present study uses 50 µm of 10% $H_2O_2$ for 1 hours according to (Benhusein *et al*., 2010), then uses same concentration for 3 hours, last uses absolute concentration 37% for 1 hours and three hours.
2.2 plant extract preparation
A-Methanol extract of ginger, *Barley ,* Sage *and* Pomegranate cortex: it was prepared according to the method of Sato *et al*., (1990) with some modification. The modification procedure is as following, specific weight of the plant and it is mixed with the average of 1g to 3 ml of the dispert solution (20 %methanol: 80 % distilled water volumes), The mixture is uniformed by electric blender for 30 minutes in room temperature. The solution is filtered by using gauze fabric for getting transudate solution. It is put in the incubator at 50 C° for 24 hours for getting the dried dispert. The dispert is kept in a dry place until it is used.
B-Hot water extract: boiling water was added to specific weight of ginger with mix for 1 hours, then it filtered, transudate solution deride as mention above.
C-Cold water: DW was added to specific weight of ginger for 24 hours in 4 C then it filtered and deride as mention above.
D-Soaking methods : this extract prepared by added 1 gm. of suffran to 30 ml DW for 24 hours in 4 C . Then it filtered and kept in 4C.
E-Plant extract aliquot*:* ginger (hot, cold, and methanolic deried extract) sage, *Barley and* Pomegranate cortex was prepeaie by added 1 gm to 9 ml of DW. Then mixture was centrifugation 300rpm\10 min then supernant was filtered twice by Whitman filter paper. Suffran aliqot prepare by add 1 ml of disperd to 9 ml of DW. Then it pass the same procedure of mention above.
F-Thin layer chromatography Plant extract characterized by TLC, by use solvents (ethyl acetate: DW: methanol) (60:20:20 V/V/V) as mobile phase for all plant extract unless barley that characterized using (chlorophorm: hexane: ethanol) (1\1\1 v\v\v) then bands exam in visible and UV light in 312 wave length to detect retardation factors (Vekiari *et al.,* 1993 ).
G-Phenol reagent ; phenol reagent solution prepare according to ( Al-Shami , 1982) , 1 gm of ferric chloride dissolved in 99 ml of D.W, for detected phenol in plant extract dispread filter paper by plant extract solution then added some drops of reagent, appeared blue color is positive result. .
2.3 DNA fragmentation test performed according to DNA ladder stander curve as fallowing: DNA 50-1000 bp was electrophoresis in 1% agarose for 1 hours 70 V and 20mA in 10x15 Cm tray (clever scientific electrophoresis unit) then the distance between well and every bands was calculated in cm length, this distances was drawing as stander curve using (Microsoft excel 2010) , DNA stander curve as show in figure (3)and DNA molecular size calculated by Equation: **Y = -26.84X+833.59,** lyses level detection by equation **1000 – DNA molecular size** ,then result was classified as fallowing ≤200 semi lyses, 200-700 partial lyses, ≥ 700 complete lyses.

## Results

The results of present study show that Plant extract consist of variant phytochemicals compounds that have different polarities as show in figure (1) and table (1) that clarified retardation factors and color of TLC profile of plants extracts. Also table (2) show phenol detection in Sage, Pomegranate cortex and cold water extract of ginger have phenol in its extracts.
The results of DNA studying show that Using 50 µm of 10% $H_2O_2$ don't effect on DNA for 1houe , 3 hours and absolute concentration for 1 hours as show in figure (3) and table (3) , while absolute concentration for 3 hours causes completely lyses in DNA as show in figure (4) and table (4).
When lymphocyte treated with plant extract only, Some Plant extracts causes low lyses level or semi lyses in DNA like barley , hot extract of ginger and sage, where others causes partial lyses such as cold water extract of ginger and saffron, methanol extract of ginger don't effect on DNA as show in figure(4) and table (4).
When use plant extract as antioxidant activity it causes protect DNA from oxidative stress unless sage, cold water of ginger and barley it causes partial lyses as show in figure (3) and table (4).
Saffron , pomegrantate cortex, methanol extract of ginger and hot water extract of ginger show best protect of DNA against oxidative stress. As show in figure (5).

 

**Discussion**

A result of increased environmental pollution and increased stress of life, physician recommended us to return to natural products because it have low side effects, so increased industrials products that consist of chemicals compounds responsible of harmful side effects on human life and polluted environment; thus this study was suggested to evaluate protective effects of common plants which are used in different applications in our life.

This study used different types of extraction because solvent that used in it responsible of the types of phytochemicals that extracted from plant. Methanol and water is used to extracted high polar compounds because water and methanol is polar solvent, also this type of solvent mixture has low toxicity and no side effects when it used in animal labs (Al-turiahe *et al.,* 2012).

Many research use cold water for extracted because of this method protect some phytochemicals compounds that may be destruction by heat. In another hand boiled water use to extract some compound can don't extracted exempt previous methods, in spite of negatively attitude of these methods it using in large scale in researches and in different application as food additive and cosmetics, thus four types of extraction methods used in present study for studying side effects of this plant and its roles in body healthy ( AL –saadi *et al* 2012).

Also dispert methods was used to as common methods for used saffron in food preparation ,so this method protect some compound may be disrupting by deride, saffron extracted by this methods because it mimic natural uses of it.

Type of plant that extracted and uses in this study was chose according to previous study that improved anti-oxidant activity of this plant, Gismondi *et al*., (2012) improved antioxidant activity of saffron using DPPH method, its have free radicals scavenger activity, Omwamba ,(2010) also used DPPH test to detect antioxidant activity of barley grain he found that antioxidant activity of barley as result of barley contain phenol compound in methanol extraction of barley grains.

Sage have antioxidant activity according to Pizzale *et al*., (2002). Bua-in et al., improved antioxidant activity of ethanol extract of ginger that collected from different loci using DPPH assay. Pomegranate extract have Free radical scavenging activities were examined using an ESR technique with spin trapping; DMPO for hydroxyl (·OH) and superoxide ($O_2^{\cdot-}$) radicals; and [$(MGD)_2Fe^{2+}$] for nitric oxide (NO). ( Noda et al., 2002).

Thin layer chromatography of plant extract show that plants consist from different phytochemicals compounds that have different polarities this is useful in protective activity of extracts against $H_2O_2$ direct and indirect effects on human lymphocyte. This method is general characterization of plant extract thus needed to quantification and qualification technique to characterize these extract.

DNA fragmentation Results showed that some plant extract have low level or semi lyses of genotoxic effects, this is may be because this extract have genotoxic in high concentration or because in vivo using, other plant don't have toxic compounds or it don't have ability to interact with DNA particles in harmful interaction or resulted from its effects on epigenetic system that affected on enzyme, factors chemical structure of nucleotides (Sarkar *et al*., 2008) , this experiment consider short term thus these plant extract need to long term experiment to detect cytotoxic and genotoxic effect of it in vivo and in vitro.

For detection optimum concentration of $H_2O_2$ effect on DNA in human lymphosite cell , using 2 titers 10% and 37% for 1and 3 hours ,

Results show that used 10% of $H_2O_2$ for 1,3 hours and 50% for 1 hours these don't appear any effects by DNA fragmentation test this may be because $H_2O_2$ effects on one base level like point mutation, deletion, insertion or duplication or translocation short sequence of DNA. This effect cannot be appearing in DNA fragmentation test that deal with large harmful effects, or no effect of $H_2O_2$ in 10 % on human lymphocyte . Also this resistance of lymphocyte according to its antioxidant system in side cells such as SOD, catalase and other antioxidant partials.

When used 37%, for 3 hours genotoxic effects clearly papers in DNA it causes DNA completely lyses, this may be because free radicals effects, which interacted with DNA nucleotides to form 8-OXOdG , this compound destroy and break down the DNA double helix. (Cooke *et al*., 2003; Pizarro *et al*., 2009). Also this was causes breaking in DNA strands which lead to fragment DNA in to small parts. In the other hand it may cases cross link between DNA- DNA strands or DNA- protein. Benhusein *et al*., (2012) found that DNA was affected by exposure human hepatoma cell to 50mm of $H_2O_2$ FOR 60 min uses commet assay.

This results may be consequence of apoptosis in lymphocyte because large errors in this cells causes by un balance between free radicals and antioxidant system, Probably high concentration of $H_2O_2$ causes disrupted cell membrane and cell death that causes liberality DNA and direct effected by $H_2O_2$ .

As a results of genotoxic effect of oxidative stress and its effect on DNA this studying suggested use common plant which are known as anti-oxidant in different chemicals experiments to evaluated its protectively against $H_2O_2$ on DNA

 

Some plant extract appear variant protective effect on DNA that show in figure (5). Protective ability probably by many reasons which put under light in this study.

In review of literature some previous experiment show that plants have different phytochemicals compounds that responsible on antioxidant activity AL-Joubori (2012) used ginger extract to treated oxidative stress which induced in diabetic animal labs with other mixture of plant extract they found successful result in reduced oxidative stress effects on DNA, proteins, chromosomes and DNA repair system on insulin receptors genes.

Protective activity of plant extract against oxidative stress induced by $H_2O_2$ may be because plant extract contain of phenolic compound which have antioxidant activity due to its ability to donor proton to free radicals also it have protective activity to antioxidant enzyme from oxidative its protein by free radicals (Dimitrios , 2006) some of plants that used in present study have phenolic compound in its extracts.

Flavonoids consider as antioxidants this activity result from its interact with minerals that inducing free radicals or destructive free radicals partials or donor proton ion (Pratt and Miller, 1984; Al-saadi *et al* ., 2012).

Saffron, pomegranate cortex, methanol extract of ginger and hot water extract have best protective effect against $H_2O_2$ while barley, cold water of ginger and sage have low protective effect on DNA, we concluded from this study that don't used plants in high concentration in spite of its remedies , may be causes harmful side effects.

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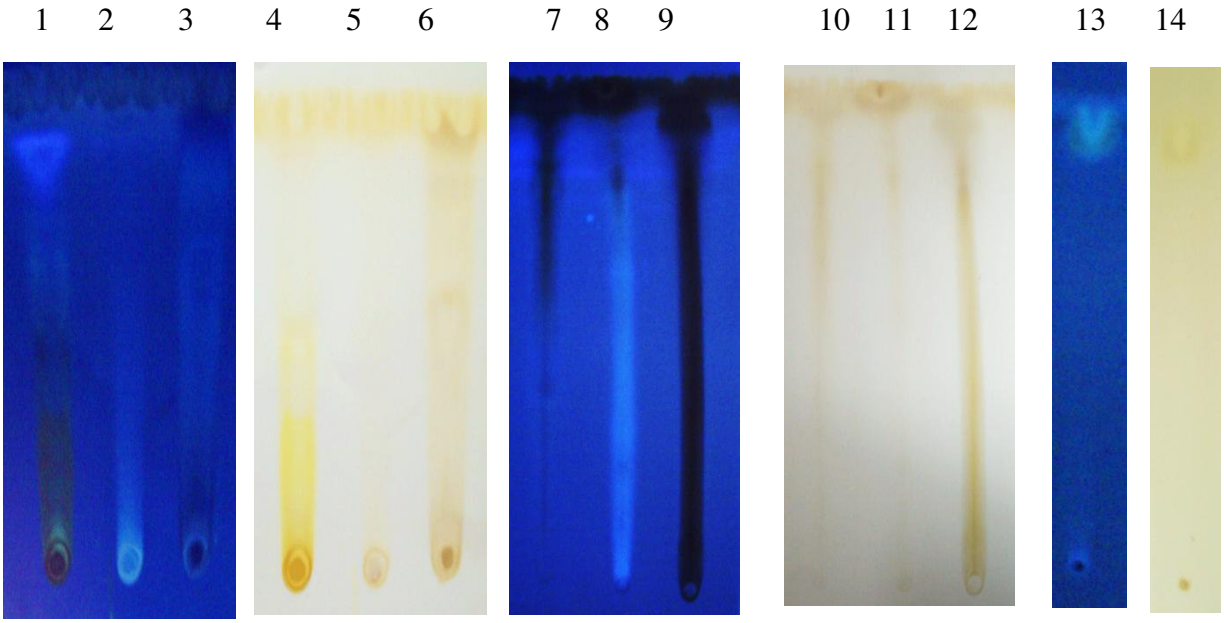


**Figure (1)** Thin layer chromatography profile of plant extract in visible light and UV light.
1,4; Saffron under UV and visible light.
2,5; Ginger hot water extract under UV and visible light.
3,6; Ginger cold water extract under UV and visible light.
7,10; Sage under UV and visible light.
8,11; Ginger methanol extract under UV and visible light.
9,12; Pomegranate cortex under UV and visible light.
13,14; barley under UV and visible light

 

**Table (1)** Characterization of plant extract (Retardation factors($R_f$ )and colors) of TLC profile

| Plant extract | Visible light | | UV | |
|---|---|---|---|---|
| | $R_f$ | color | $R_f$ | color |
| **Suffern** | 0.2 | Yellow | 0.05 | yellow |
| | 0.85 | Orang | 0.45 | brown |
| | | | 0.70 | Brown |
| | | | 0.85 | Violet |
| **Ginger hot water** | - | - | o.1 | Blue |
| **Ginger cold water** | 0.1 | Brown | 0.15 | Black |
| | 0.55 | Brawn | | |
| | 0.8 | Brawn | | |
| | 0.9 | Brawn | | |
| **Ginger methanol** | 0.82 | Yalow | 0.21 | black |
| | 0.93 | Brawn | 0.78 | black |
| | 0.97 | Yellow | 0.91 | Black |
| | 0.98 | Brawn | | |
| **Sage** | 0.69 | Yellow | 0.39 | Black |
| | 0.82 | Yellow | 0.82 | Black |
| | 0.95 | Brown | | |
| **Pomegranate cortex** | 0.78 | Brown | 0.95 | Black |
| | 0.91 | Brown | | |
| | 0.97 | brown | | |
| **Barley** | 0.93 | Yellow | 0.89 | Blue |
| | 0.96 | Green | 0.91 | dark blue |
| | | | 0.94 | Brown |

 

**Table (2)** Phenol compounds assay of plant extract using ferric chloride reagent.

| **Plant extract type** | **saffron** | **Ginger** | | | **Sage** | **Pomegranate cortex** | **Barley** |
|---|---|---|---|---|---|---|---|
| | | Hot W. | Cold W. | methanol | | | |
| **Phenol test** | - | - | + | - | + | + | - |

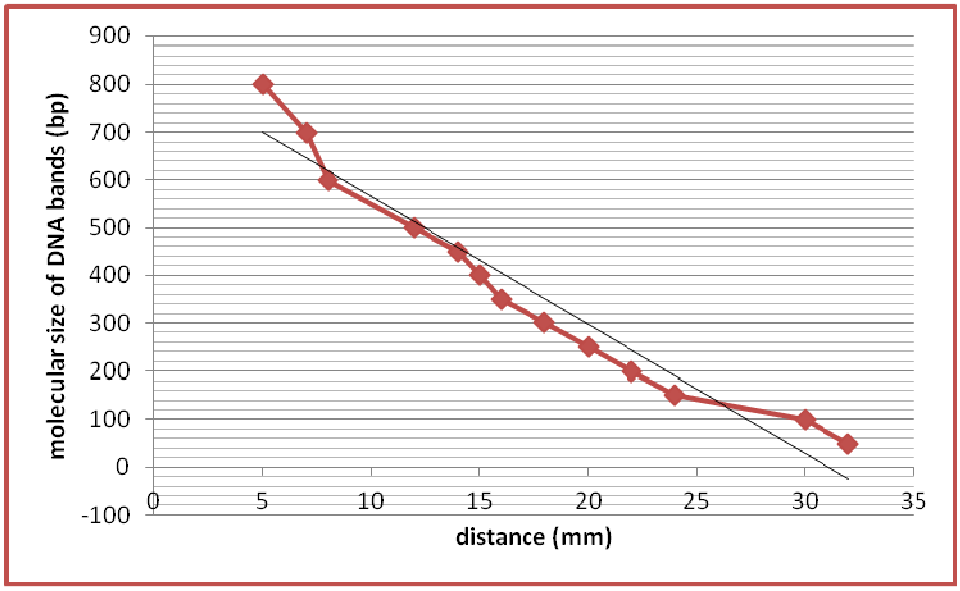


**Figure (2)** DNA stander curve (50-1000)bp in 1% agarose for 1 hours

 

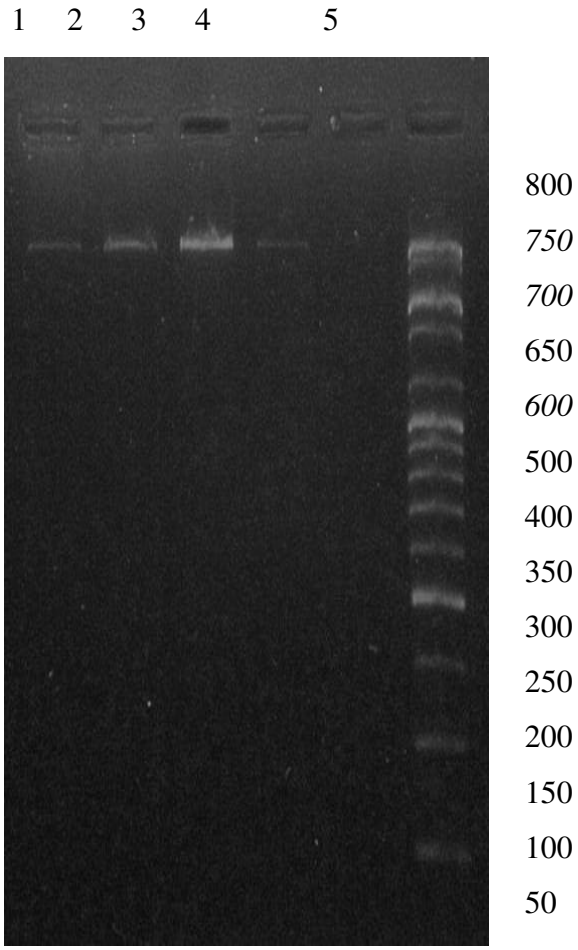


**Figure (3)** DNA lyses level of human lymphocyte exposure to different H2O2 concentration for different time.
Lane 1,Negative control.
Lane 2,50 µm 10% (1) hour.
Lane 3, 50µm 10% (3) hours.
Lane 4, Absolute concentration 37% (1) hour.
Lane 5,DNA ladder

 

**Table** (3) DNA lyses level for $H_2O_2$ , time and concentration optimize

| No. of sample in figure | H2O2 concentration | Type of lyses | Lyses level |
|---|---|---|---|
| **1** | Negative control | No lyses | - |
| **2** | 50 µm 50% (1) hour | No lyses | - |
| **3** | 50µm 50% (3) hours | No lyses | - |
| **4** | Absolute concentration 50% (1) hour | No lyses | - |
| **5** | DNA ladder | No lyses | - |

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

**Figure** (3) DNA lyses level of human lymphocyte exposure to $H_2O_2$ and different plant extract that clarified in table (2).

 

**Table** (4) lyses level of human lymphocyte DNA that exposureto H2O2 and different plant extract.

| Number of sample In figure (1) | Plant extract | Types of lyses | DNA M.S. | Lyses level bp |
|---|---|---|---|---|
| **1** | saffron | Partial lyses | 457.8 | 542.2 |
| **2** | Barley | Semi lyses | 820.17 | 179.83 |
| **3** | Pomegranate cortex | Semi lyses | 825.53 | 174.47 |
| **4** | Ginger methanol | No lyses | - | - |
| **5** | Ginger Cold water | Partial lyses | 790.6 | 209.4 |
| **6** | Ginger Hot water | Semi lyses | 820.17 | 179.83 |
| **7** | Sage | Semi lyses | 828.22 | 171.78 |
| **8** | $H_2O_2$50% | Complete lyses | Smear | 1000 |
| **9** | Suffrn+$H_2O_2$ | No lyses | - | - |
| **10** | Barly+$H_2O_2$ | semi lyses | 812 | 188 |
| **11** | Pomegranate cortex +$H_2O_2$ | No lyses | - | - |
| **12** | Ginger mthanol+$H_2O_2$ | No lyses | - | - |
| **13** | Ginger H.W.+$H_2O_2$ | No lyses | - | - |
| **14** | Ginger C.W. +$H_2O_2$ | Semi lyses | 825.5 | 174.5 |
| **15** | Sage +$H_2O_2$ | Semi lyses | 840 | 160 |

H.W hot water, C.W cold water

 

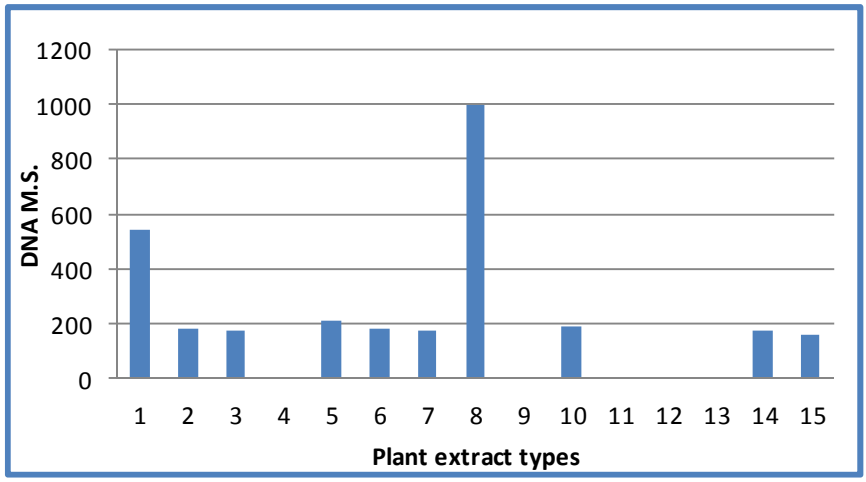


**Figure** (5) DNA lyses level of human lymphocyte exposure to H2O2 and plant extract
**1**,suffuran; **2**,Barley; **3**,Pomegranate cortex; **4**,Ginger methanol **5**,Ginger Cold water; **6**, Ginger Hot water**;** **7**,Sage; 8,$H_2O_2$50%; **9**,Suffrn+$H_2O_2$; **10**,Barly+$H_2O_2$; **11,** Pomegranate cortex +$H_2O_2$; **12**, Ginger mthanol+$H_2O_2$; **13**,Ginger H.W.+$H_2O_2$; **14,**Gingr C.W. +$H_2O_2$; **15**,Sage +$H_2O_2$, (DNA M.S. DNA molecular size).