Research Article

Variation in Total Polyphenolic Contents, DNA Protective Potential and Antioxidant Capacity from Aqueous and Ethanol Extracts in Different Plant Parts of *Hypericum perforatum* L.

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ABSTRACT

Background and Purpose: *Hypericum perforatum* belonging to the family Hypericaceae is a reputed medicinal plant including a wide ranges of important phytochemical components. Chlorogenic acid, rutin, hyperoside, quercitrin, quercetin, pseudohypericin, hypericin and hyperforin are of the major components. Crude extract and individual compounds of *H. perforatum* have been reported to exert antidepressant, antibiotic, and antitumoral activities. It is worthy to note that the quantity and efficacies of the crude extracts or individual compound are not constant, which are strongly influenced by different climatic conditions, harvesting times, harvested plant organs and post-harvest practices. Hence, numerous studies on *H. perforatum* collected from different parts of the World are carried out for their desired quality and biological efficacy. **Methods:** Wild collected plant materials were dried and preserved with a voucher specimen number and were extracted using maceration at room temperature for 24 h in dark. Subsequently, extracts were screened for their phenolic and flavonoid contents, plausible antioxidant activities using two methods namely DPPH radical scavenging and ferric-reducing antioxidant power (FRAP) assays and DNA protective activities. **Results**: The highlights of the study were are listed as 1) the highest total phenolic content in ethanol extracts of leaf, ii) the highest total flavonoid content in flower, iii) DPPH scavenging activity in leaf (80.51 %), flower (63.42 %) and stem (48.20 %), iv) highest ferric reduction capacity in ethanol extracts of stem were determined. Also, potent DNA protection activity was observed even at the lowest concentration value (25μg/ml) of the extracts. **Conclusion:** The phenolic content and strong antioxidant activities of ethanol extracts of different parts of the plant are reported. All the extracts exhibited strong DNA protective activities in response to the UV radiation in the presence of hydrogen peroxide.

Key words: Antioxidant, DNA protective activity, Flavonoid, *Hypericum perforatum* L., Phenolic.

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INTRODUCTION

Wide range of curative properties made *Hypericum* species reported as medicinal plants all over the world throughout the human history. Because of its distinguished phytochemical composition, especially rich in naphthodianthrones (pseudohypericin, hypericin, hyperforine etc.) of which pharmaceutical efficiency for a dozen of illnesses scientifically approved, HP has a special importance in pharmaceutical market.[1,2] Thus, scientific studies have recently been focused on this particular species. Wild collection and field cultivation of some cultivars are the main resources of HP in the market. Although field cultivation for standardized drug supply,

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wild collection (especially in Turkey) has continued now. Wild collected and dried raw plant materials in Turkey have totally been exported to Europe, processed on there and marketed. Thus, wild grown HP plants throughout the Turkey maintain their importance and scientific works related to these materials have scientifically important.[1-3]

Free radical and reactive oxygen species are highly unstable molecules that are either synthesized endogenously e.g. a product of energy metabolism as a part of oxidation chemical process or produced as a consequences of exogenous factors such as stress, cigarette smoking, imbalanced diet, alcohol, pollutants or sunlight.[3] Subsequently, production or an overload of free radicals modify the structure of lipid, carbohydrate, protein and complex macromolecules and are subsequently linked to the certain diseases including cancer, diabetes, cardiovascular, age related and neurological disorders.[4-9] The degradation or removal of the free radicals may not be completely controlled by endogenous enzymes such as superoxide dismutase, glutathione, but herbal products rich in phenolic contents are reported to possess important roles in the prevention of oxidation deterioration and decreasing the levels of free radicals and for this reason, many traditional plants have been screened for their phenolic content and free radical scavenging activities.[10,11] Hydrogen peroxide, superoxide anion, hydroxyl and nitric oxide radical are free radicals, that producing during cellular oxidation naturally, are unstable and highly reactive molecules.[12] Hydrogen peroxide (H_2O_2) in the presence of UV, a known reactive oxygen species (ROS) generating system, is capable of producing molecules containing unpaired electrons named as free radicals that can damage biomolecules including proteins, lipids, carbohydrates, enzymes, RNA and DNA. When reactive oxygen species interact with DNA molecules, it affects the formation of DNA strand and causes single and double strand breaks in the DNA.[13] This change of the DNA formation may result in development some disorders in genetically and also lead to develop diseases such as cancer, diabetes, aging, coronary artery, myocardial infection, rheumatoid arthritis, Alzheimer's disease and Parkinson's disease, etc. Adverse and toxic effects of the H_2O_2 can be prevented by antioxidants, which have important roles in scavenging all oxidant molecules by their hydroxyl group, thus providing protective effects on the DNA formation.[14-16] HP contains potential antioxidants that are effective against the DNA damage by directly reacting with the radicals, reducing peroxides and stimulating the antioxidative system. Therefore, in the current research, flower, stem and leaf of HP were extracted using water and ethanol, and aimed to investigate free radical scavenging activity by DPPH and ferric reducing assays, total phenolic and flavonoid contents of the extracts and thereafter DNA protective potentials in the presence of ultraviolet and hydrogen peroxide.

MATERIALS AND METHODS

Plant material

All the above said parts of HP were collected in the flowering season from Kilis, Turkey in 2014 and after authentication; voucher specimens have submitted in the Biology Department at University of Kilis 7 Aralik, Turkey.

Preparation of Extracts

The air-dried and finely powdered stem, leaves and flower of HP (5 g) were stirred with 100 ml of pure methanol for 30 min, respectively. Extraction was carried out using maceration at room temperature for 24 h followed by filtration through Whatman No.4 filter paper. The extracts were then concentrated in vacuum at 40°C using a Rotary Evaporator. Then the extracts were preserved in sealed vials at 4°C until further analysis.

Determination of Total Phenolic Content

Total phenolic content was determined according to the Folin-Ciocalteu reagent method.[17] The amount of total phenol was calculated as mg/g (Gallic Acid Equivalents) from calibration curve of Gallic acid standard solution (R^2=0. 9993). An aliquot of each sample (0.1 ml) was diluted to 1 ml with distilled water. Briefly, 0.5 ml of Folin-Ciocalteu reagent (1:1 v/v) and 1.5 ml of 20 % (w/v) sodium carbonate was added to the diluted sample solution, and the mixture was then vortexed and allowed to stand for 2 hours at room temperature for color development. The volume was completed to 10 ml with distilled water and their absorbance was measured at 765 nm (Evolution 201 UV-Visible Spectrophotometer). The total phenolic content was expressed as mg/g Gallic acid equivalents (GAE). All samples were analyzed in triplicate.

Determination of Total Flavonoid Content

The flavonoids content was determined by an aluminum chloride method using quercetine as a reference compound.[18] This method based on the formation of a complex flavonoid-aluminum. The amount of total flavonoid was calculated from calibration curve of quercetine standard solution (R^2 =0. 9815). 1ml of extracts or standard quercetine solution (500 µg/ml) was added to 4 ml distilled water and 0.3 ml of 5% $NaNO_2$ was added. After 5 minutes, 0.3 ml of 10 % $AlCl_3$ was added. After 6 min, 2mL of 1 mol L- NaOH was added and the

final total volume was completed to 10 ml with distilled water. The solution was thoroughly mixed. Afterwards the absorbance of the mixture was measured at 510 nm against prepared water as a blank. The total flavonoid content of extracts was expressed as mg quercetine equivalents (QE) /g of dried leaf material.

Scavenging Effect on 2, 2-diphenyl-1picrylhydrazyl (DPPH)

The antioxidant activity of the extracts was evaluated by DPPH radical scavenging assay described by Blois.[19] The reaction mixture including 1 ml of DPPH solution (0.1 mM in methanol) and leaf extracts (1 mg/ml-0.500 mg/ml-0.250 mg/ml-0.125 mg/ml) adjusted to 3 ml by adding methanol were left at room temperature in dark for 30 min and initial absorbance and absorbance after 30 min-incubation was measured at 517 nm. The assays were carried out in triplicate. The DPPH radical scavenging percentage was calculated from the following equation;

% DPPH radical scavenging = [(control absorbance – extract absorbance)/control absorbance] × 100

Reducing Power Assay

The reducing power was determined according to the method proposed byOyaizu.[20] An aliquot of 1 ml plant extracts (1 mg/ml-0.500 mg/ml-0.250 mg/ml-0.125 mg/ml) was mixed with 2,5 ml of phosphate buffer (0.2M, pH =6.6) and 2,5 ml of $K_3Fe(CN)_6$ (1%), shaken well and left for incubation at 50°C for 20 min. After incubation, 2,5 ml of TCA (10%) was added in order to stop the reaction and the mixture was centrifuged at 1000 rpm for 10 min. 2,5 ml of supernatant, 2,5 ml of distilled water and 0,5 ml of $FeCl_3$ (1%) was mixed and incubated for 10 min and absorbance was read at 700 nm on spectrophotometer. The assays were carried out in triplicate.

DNA Protective Potential

The other aim of this study was to investigate DNA damage protection potentials of HP against to the DNA damage generated by hydrogen peroxide (H_2O_2) and ultraviolet (UV)-known as reactive oxygen species (ROS) generating system. The DNA protective activity of the water and ethanol extracts from stem, leaves and flowers of HP was analyzed using pBR322 plasmid DNA (Vivantis) in the presence of UV/H_2O_2described previously[21,22] with some modifications. For analysis, different experimental groups were used, including control (untreated pBR322 plasmid DNA, treated with H_2O_2 and UV pBR322 plasmid DNA, treated with only H_2O_2 pBR322 plasmid DNA and treated with only UV pBR322 plasmid DNA) and treated groups with different concentration of extracts (ranging from 25µg/ml to 200 µg/ml) and H_2O_2 and UV. Briefly, the experiments were held a volume of 10 µl in a micro centrifuge tube, firstly pBR322 super coiled plasmid DNA (200ng) was added to each tube, after that plant extracts were added (except the control samples), and then H_2O_2 was added to a final concentration of 2.5 mmol/L (except one of the control sample), finally all of the samples exposed to UV light for 5-8 min on a UV trans illuminator at 300 nm at room temperature. At the end of the reaction, loading buffer (10 mMTris-HCl, 0.15% orange G, 0.3% xylene cyanol, 60% glycerol, 60mM EDTA) was added in each reaction tube, analyzed in 1.5% agarose gel for electrophoresis at 100 V for approximately 1 h, in Tris-borate-EDTA gel buffer (TBE-buffer) (45 Mm Tris-Borate, 1 mM EDTA, pH 8.2), the gels were stained with ethidium bromide (EtBr) (0.5 µg/ml), and photographed under UV trans illuminator gel documentation system (VilberLourmat), finally DNA fragmentation patterns were separated by agarose gel electrophoresis.

Statistical Analysis

SPSS statistical program was used to determine statistical significance levels and the differences between individual averages were considered to be statistically important at $p<0.05$ probability level.

RESULTS AND DISCUSSION

Polyphenolic contents of HP

A reagent that Folin–Ciocalteu was used to determine total polyphenol in plant extracts. Folin–Ciocalteu reagent consists of a yellow acidic solution containing complex polymeric ions formed from phosphomolybdic and phosphotungsticheteropoly acids.[23] The total phenolic contents in the different extracts of the flower, stem and leaves of HP were determined, and expressed as milligrams of gallic acid equivalent (GAE) per gram of dry plant. Polarity of different solvents was significantly influenced the total phenolic content ($P<0.05$). The studies were performed with water extracts that are usually consumed as herbal tea, and ethanol extraction was also used for comparison. The amount of total polyphenolic content in three ethanol extracts ranged 146. 35 to 182. 93 mg GAE/g and 88.93 to 175.41 mg GAE/g for water extracts, as shown in Table 1.

Flavonoids, which are most commonly found and widely distributed in plant polyphenol compounds, were in the range of 7.95 to 20.50 mg QE/g and 12.59 to 14.84 mg QE/g for ethanol and water extracts, respectively in this study. The highest value was determined in ethanol extracts of flowers and the lowest content was

ascertained in ethanol extracts of stem. Extractability of phenolic varied depending on the used solvents and plant parts, but worthy of mention is that the biological activity does not always depend on the amount of the polyphenol contents rather but depend on possible interaction of the bioactive components in the extracts.

Antioxidant activities of HP

Extracts of stem, leaf and flower of HP were subjected to a screening for antioxidant activity by two complementary tests, namely the reduction of 2,2-diphenyl-1picrylhydrazyl (DPPH) and ferric reducing. The results of antioxidant activity in different extracts and parts of the species compared with the ascorbic acid and gallic acid as positive controls. The DPPH scavenging activities of HP were summarized in Table 2.

DPPH assay was used as a rapid screening method for evaluation of antioxidant capacity. 2,2-diphenyl-1picrylhydrazyl, purple-colored stable free radical is reduced into the yellow colored diphenylpicryl hydrazine.[24] In this context, different plant extracts obtained using ethanol and water solvents were subjected to the DPPH free radical scavenging assay for comparison of their possible antioxidant activities and the results are expressed as an inhibition percentage of free radicals. The ability of the extracts for removal of DPPH was determined on the basis of their concentration, inhibition percentage and the highest inhibition percentage exhibits the highest effects of the extracts. Herein, four experimental doses (0.125 to 1 mg/ml) of extracts were used and the scavenging effects were determined to be dose-dependent for each extract (R^2=0.99). Accordingly, ethanol extracts of leaf demonstrated the highest inhibition (84%) and were followed by flower (72.37%) and stem (54.9%) whereas the inhibition percentages for water extracts of the samples were 60.33 % (flower), 53.07 % (leaf) and 33.47 % (stem) (Table 2).

Electron donation capacity with respect to the reducing power of compounds were reported to be associated with the antioxidant activity of the compound.[25] Ferric reducing capacity is measured by the reduction of Fe $[(CN)\ 6]^{3+}$ to Fe $[(CN)\ 6]^{2+}$. In this context, increase in absorbance due to the formation of the complex is the indicator of the increased reduction capacity.[26] Accordingly, similar to the DPPH assay results, ethanol extracts of the samples exhibited a highest ferric reduction capacity. The highest increased reduction capacity was determined in ethanol extracts of stem samples and the lowest activity was observed in water extracts of the stem samples (Table 3).

Table 1: Polyphenolic contents of *Hypericum perforatum* L.

Solvents/ parts	TPC (mg GAE/g DW)			TFC (mg QE /g DW)		
	Ethanol			Water		
	Flower	Stem	Leaf	Flower	Stem	Leaf
Ethanol extract	146,35 **d**	159,49 **c**	182,93 **a**	20,50 **a**	7,95 **f**	15,20 **b**
Water extract	175,41 **b**	88,93 **f**	125,99 **e**	14,68 **d**	12,59 **e**	14,84 **c**

Results are expressed as mean of three replicates. Data were analyzed by ANOVA and within each column different letters indicate statistically different values according to LSD-test at P< 0.05; **mg GAE/g DW,** milligram Gallic acid equivalent per gram dry extract; **mg QE /g DW,** milligram quercetine equivalent per gram dry extract; **TPC:** Total phenolic content; **TFC:** Total flavonoid content.

Table 2: DPPH inhibition percentage (%) of *Hypericum perforatum* L.

	Ethanol Extracts			Water Extracts			Standards	
	Flower	Stem	Leaf	Flower	Stem	Leaf	GA	AA
1 mg/ml	72,37 **b**	54,90 **cd**	84,00 **a**	60,33 **c**	33,47 **e**	53,07 **d**	95,88	96,93
0,5 mg/ml	40,00 **b**	29,53 **c**	50,37 **a**	32,70 **c**	17,97 **d**	28,23 **c**	95,67	66,04
0,25 mg/ml	24,00 **b**	18,17 **c**	27,50 **a**	18,80 **c**	11,17 **e**	16,30 **d**	84,10	32,74
0,125 mg/ml	11,13 **b**	10,93 **b**	14,60 **a**	11,10 **b**	6,60 **d**	9,40 **c**	47,76	13,31

Data are expressed as means of three replicates. The mean is significant at the 0.05 level and the results are expressed as inhibition percentage (%). Data were analyzed by ANOVA and within each column different letters indicate statistically different values according to LSD-test at P< 0.05; **GA:** Gallic acid; **AA:** Ascorbic acid; **Notes-2:** Standard antioxidant compounds, GA and AA, were excluded from statistical analysis. Mean of three replicates were presented in the Table 2.

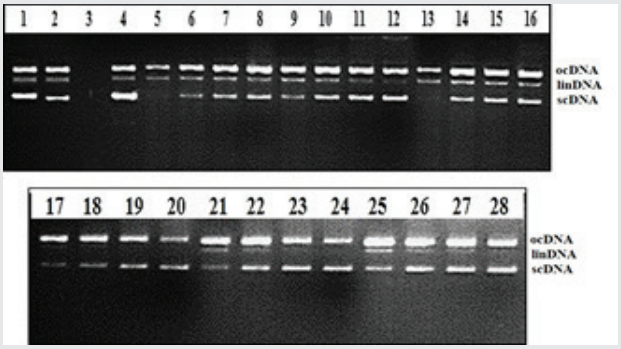

Figure 1: DNA protective activity of *Hypericum perforatum* L. extracts

Control Samples: Line 1, DNA + UV; Line 2, DNA; Line 3, DNA + H_2O_2 + UV; Line 4, DNA + H_2O_2. Line 5, DNA + UV+ H_2O_2 + the water extracts from leaves of *H. perforatum* at 25 µg/ml concentration; Line 6, DNA + UV + H_2O_2 + the water extracts from leaves of H. perforatum at 50 µg/ml concentration; Line 7, DNA + UV + H_2O_2 + the water extracts from leaves of *H. perforatum* at 100 µg/ml concentration; Line 8, DNA + UV + H_2O_2 + the water extracts from leaves of *H. perforatum* at 200 µg/ml concentration; Line 9, DNA + UV + H_2O_2 + the water extracts from flowers of *H. perforatum* at 25 µg/ml concentration; Line 10, DNA + UV + H_2O_2 + the water extracts from flowers of H. perforatum at 50 µg/ml concentration; Line 11, DNA + UV + H_2O_2 + the water extracts from flowers of H. perforatum at 100 µg/ml concentration; Line 12, DNA + UV + H_2O_2 + the water extracts from flowers of H. perforatum at 200 µg/ml concentration; Line 13, DNA + UV + H_2O_2 + the water extracts from stem of *H. perforatum* at 25 µg/ml concentration; Line 14, DNA + UV + H_2O_2 + the water extracts from stem of *H. perforatum* at 50 µg/ml concentration; Line 15, DNA + UV + H_2O_2 + the water extracts from stem of H. perforatum at 100 µg/ml concentration; Line 16, DNA + UV + H_2O_2 + the water extracts from stem of *H. perforatum* at 200 µg/ml concentration; Line 17, DNA + UV + H_2O_2 + the ethanol extracts from leaves of *H. perforatum* at 25 µg/ml concentration; Line 18, DNA + UV + H_2O_2 + the ethanol extracts from leaves of *H. perforatum* at 50 µg/ml concentration; Line 19, DNA + UV + H_2O_2 + the ethanol extracts from leaves of *H. perforatum* at 100 µg/ml concentration; Line 20, DNA + UV + H_2O_2 + the ethanol extracts from leaves of *H. perforatum* at 200 µg/ml concentration; Line 21, DNA + UV + H_2O_2 + the ethanol extracts from flowers of *H. perforatum* at 25 µg/ml concentration; Line 22, DNA + UV + H_2O_2 + the ethanol extracts from flowers of *H. perforatum* at 50 µg/ml concentration; Line 23, DNA + UV + H_2O_2 + the ethanol extracts from flowers of *H. perforatum* at 100 µg/ml concentration; Line 24, DNA + UV + H_2O_2 + the ethanol extracts from flowers of *H. perforatum* at 200 µg/ml concentration; Line 25, DNA + UV + H_2O_2 + the ethanol extracts from stem of *H. perforatum* at 25 µg/ml concentration; Line 26, DNA + UV + H_2O_2 + the ethanol extracts from stem of *H. perforatum* at 50 µg/ml concentration; Line 27, DNA + UV + H_2O_2 + the ethanol extracts from stem of *H. perforatum* at 100 µg/ml concentration; Line 28, DNA + UV + H_2O_2 + the ethanol extracts from stem of *H. perforatum* at 200 µg/ml concentration.

Table 3: Ferric reducing activities of *Hypericum perforatum* L.

	Ethanol Extracts			Water Extracts		
	Flower	Stem	Leaf	Flower	Stem	Leaf
1 mg/ml	3,25 b	3,27 a	3,20 c	1,21 d	0,59 f	1,01 e
0,5 mg/ml	3,18 a	3,14 b	3,18 a	0,71 c	0,31 e	0,52 d
0,25 mg/ml	2,86 a	2,38 c	2,75 b	0,40 d	0,16 f	0,28 e
0,125 mg/ml	2,01 a	1,51 c	1,92 b	0,20 d	0,09 f	0,14 d

Results are expressed as mean of three replicates. Data were analyzed by ANOVA and within each column different letters indicate statistically different values according to LSD-test at P< 0.05.

DNA protective activity of HP extracts

DNA protection effect of HP extracts was investigated with plasmid DNA derived from pBR322 in the presence of ultraviolet and hydrogen peroxide. pBR322 plasmid DNA, isolated from *Escherichia coli*, has 4361 base pairs (bp) and on agarose gel electrophoresis shows two bands including scDNA (supercoiled circular DNA) and ocDNA (open circular DNA). scDNA is the native form of DNA derived from pBR322 and moves faster than ocDNA on gels. When DNA is exposed to UV light, in the presence of H_2O_2, this situation is leading to produce free hydroxyl radicals, and change native formation of DNA (scDNA), produced ocDNA and linear DNA (linDNA).[22-27] The addition of the water and ethanol extracts of HP at 25µg/ml-200µg/ml concentration to the reaction mixture prevent to change the formation of linDNA, and help to protect the native formation of DNA (Figure.1).

Figure 1 shows the electrophoretic band pattern of pBR322 plasmid DNA after exposed to UV/H_2O_2 in the absence and presence of the extracts of HP in a dose dependent manner (concentration of the extracts ranging from 25µg/ml to 200µg/ml): first four lines are control samples, UV irritation of DNA in the absence of H_2O_2 (line 1), absence of UV and H_2O_2 (line 2), presence of UV and H_2O_2 (line 3), and presence of H_2O_2, without UV irritation of DNA (line 4), and the other lines are treated lines with the water and ethanol extracts from stem, leaves and flowers of HP.: lines 5 to 8 are the water extracts from leaves of HP, lines 9 to 12 are the water extracts from flowers of HP, lines 13 to 16 are the water extracts from the stem of HP at 25µg/ml to 200µg/ml concentration, lines 17 to 20 are the ethanol extracts from leaves of HP, lines 21 to 24 are the ethanol extracts from flowers of HP, lines 25 to 28 are the ethanol extracts from the stem of HP at 25µg/ml to 200µg/ml concentration. It was clearly demonstrated that all of the extracts from HP are able to provide DNA protection potentials against to UV radiation in the presence of hydrogen peroxide.

CONCLUSION

In conclusion, this study clearly evaluated that all of the extracts from different parts of HP are able to provide DNA protection potentials against to UV radiation in the presence of hydrogen peroxide. In addition, this plant has high total polyphenolic contents and strong antioxidant capacity was demonstrated in this research. Experimental evidence of the present study would help to understand that this plant has potential health benefits

as a natural antioxidant. Moreover, the extracts and/or compounds obtained from different parts of HP might be used as natural anticancer agents, as well as antiaging products in daily life for the future with validation by further studies.

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CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

ABBREVIATION USED

AA: Ascorbic acid; **DNA:** Deoksiribo nükleic acid; **DPPH:** 2,2-diphenyl-1picrylhydrazyl; **EtBr:** Ethidium bromide; **FRAP:** ferric-reducing antioxidant power; **GA:** Gallic acid; **GAE:** Gallic acid equivalent; **H_2O_2:** Hydrogen peroxide; **HP:** Hypericum perforatum; **linDNA:** lineer DNA; **ocDNA:** Open circular DNA; **QE:** Quercetine equivalents; **RNA:** Ribo nucleic acid; **ROS:** Reactive oxygen species; **scDNA:** supercoiled circular DNA; **TFC:** Total flavonoid content; **TPC:** Total phenolic content; **UV:** Ultraviolet.

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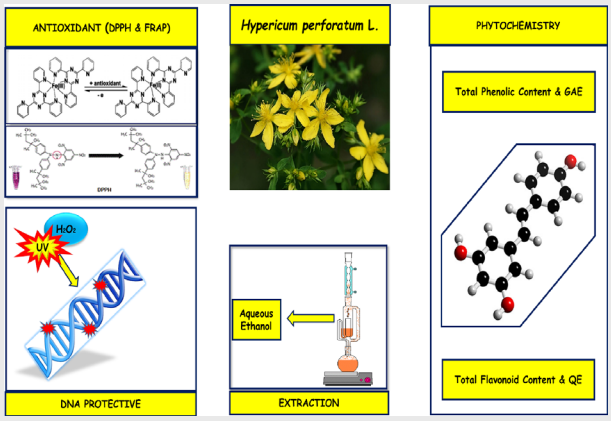
PICTORIAL ABSTRACT



SUMMARY

- *Hypericum perforatum* (St. John's Wort), a historical herbal remedy, is important potential medicinal plants due to its a wide range of important phytochemical components.
- The purpose of this paper is to determine total polyphenolic contents, DNA protective potential and antioxidant activity of HP.
- Results given in this paper are distinguished for this plant and DNA protective potential study for this plant is novel.
- As a result; phenolic content and strong antioxidant activities of ethanol extracts of different parts of the plant are reported here. All the extracts exhibited strong DNA protective activities in response to the UV radiation in the presence of H2O2.
- To determine phytochemical composition and biological activities are important for **pharmaceutical industry** in order to develop novel drugs and human health.

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Prof. Dr. Nazim Sekeroglu: He earned his B.Sc in Agricultural Engineering, Prof. Dr. Sekeroglu earned his PhD degree on *Medicinal and Aromatic Plants* Cukurova University, Turkey. He is interested in medicinal and aromatic plants since his undergraduate education. He is currently a full-time professor at Kilis 7 Aralık University, Department of Medicinal and Aromatic Plants. He published over 100 peer-reviewed articles and still active on his research area. Additionally, he has organized many international scientific meetings, including congress, symposiums and workshops as chairperson. Furthermore, he is the founder of the Association of medicinal and Aromatic Plants of Mediterranean (AMAPMED), also he is general coordinator of Global Federation of Medicinal and Aromatic Plants (GOFMAP), a worldwide non-profit organization gathering associations related 'Medicinal and Aromatic Plants'. Moreover, he is a member of scientific commissions about *Medicinal and Aromatic* Plants at Food, Agriculture, Forestry and Health Ministries in Turkey. He has visited more than 50 different countries for scientific purposes.

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