Introduction to Nutrition: Innovation Technologies on Nutritional Quality and Health
The “nutrition” word first appeared in 1551 and comes from the Latin word nutrire, meaning “to nourish.”

Nutrition is the sum of all processes involved in how organisms obtain nutrients, metabolize them, and use them to support all of life’s processing.
Nutritional science covers a wide spectrum of disciplines

- personal health,
- population health,
- planetary health

Nutritional science concern the research and investigation of how an organism is nourished.

New trends in food processing and technology affects the nutritional quality and quantity and the product manufacturing quality and thereby HEALTH...
The describing and explaining of innovative technologies impact on
- Food production and processing
- Nutritional value and foods and drinks
- Food safety and preservation
- New foods
- Fortified foods and drinks
- Nutraceutical foods and drinks
- Nanotechnological foods
- Edible anticancer foods
New technologies in food production and processing are driven by:

- knowledge and new techniques gained from research investigations;
- attempts to increase efficiency, reduce environmental effect of production;
- competition between food companies;
- consumer demand.
Innovation in food production, processing and new product development can offer benefits for consumers and the environment.

1. Environmental Sustainability
2. Dietary and Health Needs and Consumer Demand
3. Farming and Agriculture Capacity for Biotechnical Considerations, New Crops and Nanotechnological Products
4. The Usability of New Techniques and Technologies for Food Improving and Developing, for Food Safety, and for Nutraceutical Foods and Edible Anticancer Agents
Environmental Sustainability

Challenges include:

- sustainable, affordable food supply and demand,
- stability in food supplies,
- achieving global access to food and ending hunger;
- reducing the impact of food production on the world’s environmental systems.

Controlled Innovative Technologies are Necessary
Dietary Needs and Consumer Demands

Through medical and nutritional research there is more knowledge available on nutrition and dietary needs. This includes information about preventative nutrition and nutrition through life. This creates a demand for new products in the marketplace.

Controlled Innovative Technologies are Necessary
3

Farming and Agricultural Capacity

The availability of new techniques from biotechnology and genetic research provides an opportunity to control cell metabolism and breeding.

This makes it possible for developers to meet more specific requirements e.g. to increase a specific nutrient in a food.

Innovative Technologies are Necessary
**Consumer Demands**

- With less additives
- With high nutritional value
- High quality
- Less thermal damage
- Good sensory properties
- Safe products

Thereby, food manufacturing designed for better food safety and quality.
Strategies for Food Processors

- Premium food products
- Long lasting Foods
- Convenience foods
- Minimally processed foods
- Ready-to-cook meals
- Ready-to-eat foods
- Low-fat foods
- Low-carbohydrate foods
- Specialities in foods
  (For Health Treatments-
   For Anticancer Support
   For Kids
   For Military
   For Pregnants
   For Sportmans)
**Nonthermal**
- High Hydrostatic Pressure
- Pulsed electric fields
- Ultrasound
- Ultraviolet
- Irradiation
- Cold Plasma
- Dense Phase Carbon Dioxide
- Ozone
- Chemicals

**Thermal**
- Microwave
- Radiofrequency
- Ohmic Heating
- Induction Heating
EMERGING TECHNOLOGIES

- Shelf Life Extension
- Innovative Fresh Products
- Unwanted OR Reduced Constituent
- Unwanted Enzyme Inactivation
- Pathogen Inactivation
- Clean-label Products
In Nutrition Concept

Innovative Technologies are effective on all concepts.
Polyphenols occur as plant secondary metabolites and widely distributed in the plant kingdom and represent an abundant antioxidant components of the human diet. Interest in the possible health benefits of polyphenols has increased due to the corresponding antioxidant activities...
Polyphenols divide into several subgroups including majorly flavonoids...and especially anticarcinogen.
Flavonoids Family in Food Plants

Flavonoids

- Flavons
  - Apigenin
  - Luteolin
  - Baikalein
  - Krysin
  - Diosmin
  - Genkvin
  - Ikarohofolin
  - Rhofolin
  - Tektokirisin

- Isoflavons
  - Daidzein
  - Genistein
  - Biokenin A
  - Formononetin
  - Glisitein
  - Daidzin
  - Genistin
  - Glisitin
  - 6'-O-Asetildaidzin
  - 6'-O-Asetilgenistin
  - 6'-O-Asetilglisitin
  - 6'-OMalonildaidzin
  - 6'-OMalonilgenistin
  - 6'-OMalonilglisitin

- Flavonols
  - Quercetin
  - Kaempferol
  - Miricetin
  - Quercitrin
  - Isoquercitrin
  - Rhamnetin
  - Isorhamnetin
  - kaempferid
  - Rutin
  - Astragalin
  - Hiperosid

- Flavan-3-ols
  - (+)-Catechin
  - (-)-Epicatechin
  - (-)-Epicatechingallate
  - (-)-Epigallocatechin
  - (-)-Epigallocatechingallate

- Flavanons
  - Hesperetin
  - Hesperitin
  - Naringenin
  - Naringin
  - Narirutin
  - Didimin
  - Eriositrin
  - Eriodiktiol
  - Neoriositrin
  - Neohesperitin
  - Izosakuranetin
  - Pinosembrin
  - Ponsinbrin
  - Prunin

- Anthocyanidins
  - Cyanidin
  - Malvinidin
  - Delfinidin
  - Pelargonidin
  - Petunidin
  - Peonidin

- Chalcones

- Flavononols (Dihydroflavonols)

- Anthocyanins
  - Grape extract

Tokusoglu, 2001; Tokusoglu & Hall, 2011
Chemoprevention & Phytochemicals.....

Chemoprevention is an active cancer (CA) preventive strategy to inhibit, delay or reverse human carcinogenesis using naturally occurring or synthetic chemical agents.

Studies have resulted that several new phytochemicals possess cancer preventive effect such as polyphenols. Several cellular mechanisms including contribute to the overall cancer preventive effects of dietary phytochemicals...
Signal transduction pathways as POTENTIAL MOLECULAR TARGETS for chemoprevention by dietary phytochemicals

- Increasing expression of detoxifying enzymes and/or antioxidant enzymes,
- Inhibiting cell cycle progression and cell proliferation
- Inducing differentiation and apoptosis
- Inhibiting expression and functional activation of oncogenes
- Increasing expression of tumor-suppressor genes,
- Inhibiting angiogenesis and metastasis by modulating cellular signaling pathways
Case Study

Antioxidant and Cytotoxic Properties of *Salvia fruticosa* and Its Effects of Some CYP450 and Antioxidant Enzymes in Colon Cancer HT-29 Cell Line
OUTLINE

1-Introduction

Phytochemicals

*Salvia fruticosa* *M.*

*Colorectal Cancer*

Cytochrome P450 system (CYP450)

Antioxidant enzymes

Aim of The Study

2-Material and Methods

3-Results and Discussion

4-Conclusion
**Taxonomy**

Kingdom: Plantae

- Subkingdom: Tracheobionta
- Division: Magnoliophyta
- Class: Magnoliopsida
- Subclass: Asteridae
- Order: Lamiales
- Family: Lamiaceae
- Genus: Salvia
- Species: S. fruticosa Miller
Salvia Genus-----

Antioxidant
Anticancer
Antimicrobial
Antiinflammatory
Antimutagenic

Coloretic
Anticolinesterase

Improving cognitive performance and mood
Reducing work relating stress
Phenolic Contents of *Salvia fruticosa* (SF)

Caffeic acid

Rosmarinic acid
Dihydroxy caffeic acid

Caffeic acid

Rosmarinic acid

Salvianolic acid derivatives

Sagerinic acid

Sage Phenolics
Colorectal Cancer

Most common cancer type

1 million colon cancer is diagnosed every year and 500 000 patients die due to the disease
Phase I enzymes

- **Oxidation**
  - Cytochrome P450 monooxygenase system
  - Flavin-containing monooxygenase system
  - Alcohol dehydrogenase and aldehyde dehydrogenase
  - Monoamine oxidase
- **Reduction**
  - NADPH-cytochrome P450 reductase
- **Hydrolysis**
  - Esterases and Amidase
  - Epoxide hydrolase
Xenobiotic Metabolism

- Occurs in two phases of reactions:
  - Phase I reactions
  - Phase II reactions

- The overall purpose of two phases is to increase polarity and enhance excretion
Cytochrome P450s (CYPs)

- Cytochrome P450 enzymes are responsible for most phase I reactions
- Heme containing enzymes
- P450 absorbs light at 450nm (Omura and Sato 1964)
- Located mainly on the endoplasmic reticulum and the inner membrane of the mitochondria

\[ \text{O}_2 + \text{NADPH} + \text{H}^+ + \text{RH} \rightarrow \text{NADP}^+ + \text{H}_2\text{O} + \text{ROH} \]
<table>
<thead>
<tr>
<th>Human CYP Families</th>
<th>Names</th>
<th>Function</th>
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<tr>
<td>CYP1 (3 genes)</td>
<td>1A1, 1A2, 1B1</td>
<td>Xenobiotic metabolism</td>
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<tr>
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<td>2A6, 2A7, 2A13, 2B6,</td>
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<tr>
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<td>2C8, 2C9, 2C18, 2C19,</td>
<td>Xenobiotic and steroid metabolism</td>
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<td>2D6, 2E1, 2F1, 2J2,</td>
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<td>2R1, 2S1, 2U1, 2W1</td>
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<tr>
<td>CYP2 (16 genes)</td>
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<td>CYP3 (4 genes)</td>
<td>3A4, 3A5, 3A7, 3A43</td>
<td>Xenobiotic and steroid metabolism</td>
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<td>CYP4 (12 genes)</td>
<td>4A11, 4A22, 4B1, 4F2,</td>
<td>Fatty acid metabolism</td>
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<tr>
<td></td>
<td>4F3, 4F8, 4F11, 4F12,</td>
<td></td>
</tr>
</tbody>
</table>
CYP1A1

- Takes role in the conversion of pro-carcinogenic compounds to highly carcinogenic compounds

- PAHs are metabolized to carcinogens by CYP1A1
CYP1A2

- Expression is induced by some PAHs
- Caffeine, aflatoxin B1, acetaminophen and cigarette smoke are the substrates
- Vegetables such as cabbages, cauliflower and broccoli are known to increase levels of CYP1A2
CYP2E1

- Highly liver-enriched enzyme (Seliskar & Rozman, 2007)
- Expressed in a wide range of extrahepatic tissues (Botto et al., 1994).
- Metabolizes both endogenous substrates, such as ethanol, acetone, and acetal.
- Exogenous substrates including benzene, carbon tetrachloride and etc.
- Activates many pre-carcinogens and pre-toxins.
Phase II enzymes (GSTs)

- They catalyze detoxification or elimination of xenobiotics by conjugating with Glutathione.

- These enzymes are categorized as mitochondrial, microsomal and cytosolic (Sheenan et al., 2001).

- Cytosolic GSTs; alpha (GSTA), mu (GSTM), pi (GSTP), theta (GSTT), kappa (GSTK), zeta (GSTZ) and omega (GSTO).
Mainly located in the liver and in the intestine.

Play important roles on deactivation of many drugs and also activation of drugs or procarcinogens to carcinogen agents.

Involved in the metabolism of acetaminophen, codeine, ciclosporin (cyclosporin) and diazepam (Hashimoto et al., 1993).
GST Mu & GST Pi

- They detoxify several anticancer drugs
- The known substrates for GST Mu includes reactive epoxide intermediates
- Mutations of GST Mu gene have been linked with an increase in a number of cancers
- GST Pi have cellular protection against free radical and carcinogenic compounds due to its detoxifying function
- Some of the drugs including cisplatin and carmustine are substrates of GSTP1 and GSTM1 enzymes, respectively and they are excreted by conjugation with glutathione (Ban et al., 1996; Smith et al., 1989).
Antioxidant Enzymes

- Glutathione S-transferase (GSTs)
- Catalase
- Glutathione Peroxidase (GPx)

Glutathione S-transferase (GSTs):

\[ \text{Glutathione} + \text{Xenobiotic (X)} \rightarrow \text{Glutathione-S-Conjugate} \]

Catalase:

\[ 2\text{H}_2\text{O}_2 \xrightarrow{\text{Catalase}} 2\text{H}_2\text{O} + \text{O}_2 \]

Glutathione Peroxidase (GPx):

\[ 2\text{H}_2\text{O}_2 \xrightarrow{\text{GPx}} 2\text{H}_2\text{O} + \text{O}_2 \]
Antioxidant Enzymes

Catalase

- found in most of all living organisms.
- catalyzes the decomposition of hydrogen peroxide to water and oxygen molecule.
- have peroxidative function converting peroxides (ROOH) into alcohol (ROH) and water.

\[ 2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2 \]

Hydrogen peroxide Water Oxygen
Antioxidant Enzymes

Glutathione Peroxidase (GPx)

- Catalyzes the reduction of hydrogen peroxides to water and alcohols at the expense of GSH (Flohe, 1989 and Ursini et al., 1995).
- Protect the organism from oxidative damage.
The aim of this study was:

- Phenolic contents SF by RP-HPLC
- Antioxidant properties of SF
- Cytotoxic effects of SF on HT-29 colon cancer cell line
- mRNA expressions of some CYP 450 and antioxidant enzymes in HT-29 cell line

RESEARCH ON Salvia fruticosa (SF)
S. fruticosa M. was collected from Germiyan Village, Çeşme, İzmir, Turkey.
MATERIALS & METHODS

**Salvia fruticosa M.**

- Phenolic Profiles of the plant by RP-HPLC
- Extraction of Plants
- Determination of TPC & TFC Antioxidant Activities
- Cell Culture Studies
  - Cytotoxicity on HT-29 cells by XTT Assay
  - Enzyme Activities
  - Isolation of Protein
  - Determination of Protein Concentration
  - Screening the Antioxidant Enzymes
- Gene expressions
  - Total RNA Isolation from HT-29 cells
  - RNA Concentration Determination
  - cDNA Synthesis
  - Quantitative Real Time PCR
MATERIALS & METHODS

*Salvia fruticosa* M.

Phenolic Profiles of the plant by RP-HPLC

**Extraction of Plants**

Determination of TPC and TFC & Antioxidant Activities

Boiling for 30 min.

*S. fruticosa* extract
Extraction

- The extraction procedure was based on the work of Adamson et al.
  - The aerial parts of the plant was dried at room temperature, protected from direct sun light
  - 20 g of the dried plant was mixed with tap water (400 mL) and left to boiling for 20 min.
  - After filtration, the extract was concentrated under vacuum and lyophilized
  - The extracted powder was weighed and stored at -20°C in brown bottle until use
  - Extraction yield was determined using the following equation
  - Percentage extraction (w/w) = \([\frac{\text{Mass of SF (in extracted solution)}}{\text{mass of total SF}}] \times 100\)
Determination of TPC

TPC was determined by using the method of Singleton and Rossi (1965)

20 µL of the extracts + 100 µL of 1:4 diluted % Folin reagent + 80 µL of 10% Na2CO3

After 30 minutes of incubation at RT, read the absorbances at 750 nm

Gallic acid (GA) was used as a standard

Results were expressed by using the following equation

\[
\text{mg GA/g dry extract mass} = \frac{[(\text{Abs(sample)} - \text{Abs(sample blank)}) - \text{Abs(blank)}]}{\text{slope} \times \text{DF}}
\]
Determination of TFC

- Total flavonoid content of the plant extract was determined according to **Aluminum Chloride Colorimetric Assay** (Zhishen et al., 1999)
  - 20 µL of each extract + 80 µL of distilled water + 6 µL of 5% sodium nitrite
  - 5 min later add 6 µL of 10 % Aluminium chloride (AlCl₃)
  - 6 min later add 40 µL of 1 M sodium hydroxide (NaOH)
  - Complete up to 200mL
  - Measured the abs. at 510nm
  - Catechin was used as standard

Results were expressed by using the following equation

\[
\text{mg CE /g dry extract mass} = \frac{[(\text{Abs}(\text{sample}) - \text{Abs}(\text{sample blank})) - \text{Abs} (\text{blank})]}{\text{slope} \times \text{DF}}
\]
Determination of Antioxidant Activity

Free Radical Scavenging Capacity by DPPH Method

- DPPH• scavenging activity was determined according to Blois et al. (1958)
- 10 µL of each extracts + 140 µL DPPH solution
- After 20 minute absorbance changes was determined at 517 nm
- α- tocopherol was employed as the reference.
- IC$_{50}$ (50% effective concentration) value was calculated using the following equation

\[
\text{RSA (Radical scavenging activity)} \% = \left(\frac{A_0 - A_1}{A_0}\right) \times 100
\]
Free Radical Scavenging Capacity by ABTS Method

ABTS method was performed according to Re et al. (1999)

- 2.5 µL of each extracts + 250 µL of ABTS• solution
- After 6 min, absorbance was monitored at 734 nm
- Trolox was used as standart to calculate the trolox equivalent antioxidant capacity (TEAC) values.
- Percent inhibition was calculated using the following equation

\[
\text{Inhibition \%} = \left(\frac{A_0 - A_1}{A_0}\right) \times 100
\]
Determination of Metal Chelating Activity

- The chelating activity (ChA) of the extract was investigated according to methods of Dinis et al., (1994)
- 40 µL of extracts + 8 µL ferrozine (5 mM) + 4 µl iron (II) chloride (2 mM) + 148µl absolute methanol
- After 10 min incubation, read absorbance at 562nm
- EDTA was used as the chelating standard
- Chelating activity of the extract was expressed as IC50
- ChA % was calculated using the following equation

\[
\text{ChA} \% = \left(\frac{A_0 - A_1}{A_0}\right) \times 100
\]
HPLC-ANALYSIS

➢ First
For rosmarinic acid and caffeic acid; (Liu et al., 2013).
(A) water:tetrahydrofuran: trifluoroacetic acid (98:2:0.1)
(B) 0.1% trifluoroacetic acid in acetonitrile
The column was phenomenex Luna C18 column (250 x 4.6 mm, 5 μm) at 37 °C.

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>A%</th>
<th>B%</th>
<th>Flow</th>
</tr>
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<tbody>
<tr>
<td>30</td>
<td>88</td>
<td>12</td>
<td>1 mL/min.</td>
</tr>
<tr>
<td>45</td>
<td>80</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>70</td>
<td>30</td>
<td></td>
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</table>
RP-HPLC-ANALYSIS

Second

The other phenolic compounds; (Soraia I. et al., 2010)
A) 0.1% (v/v) formic acid in water
B) Acetonitrile

The column was a Inertsil ODS-3 (250 mm x 4,6 mm, 5 μm) at 30 °C

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>A%</th>
<th>B%</th>
<th>Flow</th>
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<td>80</td>
<td>20</td>
<td>1 mL/min.</td>
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<td>70</td>
<td>30</td>
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<tr>
<td>40</td>
<td>60</td>
<td>40</td>
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<tr>
<td>60</td>
<td>40</td>
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</tr>
<tr>
<td>80</td>
<td>20</td>
<td>80</td>
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Antiproliferative effects of *Salvia fruticosa*

-The cytotoxic effects of water extract of *S. fruticosa* and its phenolic contents (rosmarinic acid and caffeic acid) on HT-29 cells were investigated by using **Cell Proliferation XTT Kit.**

Reduction of XTT tetrazolium to XTT formazan by mitochondrial dehydrogenase in the presence of phenazine metho-sulphate.
Gene Expression Analysis

- **Total RNA Isolation**
  - Isolation of total RNA from HT-29 cells were performed according to *Thermo Scientific GeneJet RNA Prufication Kit*
  
  - Concentration of the isolated RNA was quantified by measuring the absorbance at 260 nm. Purity was assessed by $260/280$ nm ratio with NanoDrop.

- cDNA synthesis was performed according to *RevertAid™ First Strand cDNA Synthesis Kit*
Determination of mRNA expression

- mRNA expressions of CYP1A1, CYP1A2, CYP2E1, CYP3A4, GSTM1, GSTP1, GPx and Catalase genes in HT-29 cell line were analyzed by quantitative Real Time PCR (qRT-PCR)

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<th>Components</th>
<th>Reaction Volume (µl)</th>
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<td>SYBR Green PCR master mix</td>
<td>5 µL</td>
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<tr>
<td>Forward Primer (10µM)</td>
<td>0.2 µL</td>
</tr>
<tr>
<td>Reverse Primer (10 µM)</td>
<td>0.2 µL</td>
</tr>
<tr>
<td>cDNA</td>
<td>1.6 µL</td>
</tr>
<tr>
<td>RNAse free water</td>
<td>3 µL</td>
</tr>
<tr>
<td>Total volume</td>
<td>10 µL</td>
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<td>Total volume</td>
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<th>Cycluses</th>
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<td>Denaturing</td>
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<td>Amplification</td>
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<tr>
<td>Annealing</td>
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<tr>
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<td>GSTP1</td>
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<td>Catalase</td>
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<tr>
<td>Elongation</td>
<td>72</td>
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</table>
Determination of mRNA expression

- Relative mRNA expressions were calculated using GAPDH as an internal standard.

- **The Livak Method** \( (2^{-\Delta\Delta C_t}) \) used for relative gene expression analysis.

  In order to evaluate the results, \( \Delta\Delta C_t \) method was used.

  \[
  \Delta C_t = C_t \text{ (target)} - C_t \text{ (normalizer)}
  \]

  \( \Delta C_t \) is the difference between the CT values of the target and the normalizer that is GAPDH.

  \[
  \Delta\Delta C = \Delta C_t \text{ (sample)} - \Delta C_t \text{ (reference)}
  \]

  Comparative expression level = \( 2^{-\Delta\Delta C_t} \)
RESULTS

- Phenolic contents of S. fruticosa

RP-HPLC chromatogram of caffeic acid and rosmarinic acid standards at 322 and 330 nm with their retention times
RESULTS

Phenolic contents of *S. fruticosa*

HPLC chromatogram of phenolic standards mixture at 280 nm with retention times; (a; gallic acid, b; (+)catechin, c; (-) epicatechin, d; epigallocatechin gallate, e; syringic acid, f; p-coumaric acid, g; rosmarinic acid, h; t-resveratrol, k; quercetin).
RESULTS

- Phenolic contents of *S. fruticosa*

RP-HPLC chromatogram of the *S. futicosa* extract monitored at 330 nm. Peaks are indicated as follows: 1) caffeic acid 2) rosmarinic acid, respectively.
MATERIALS & METHODS

*Salvia fruticosa* M.

- Phenolic Profiles of the plant by RP-HPLC
- Extraction of Plants
- Determination of TPC & Antioxidant Activities

![Graph showing phenolic compounds](image)
(a; gallic acid, b; (+)catechin, c; (-) epicatechin, d; epigallocatechin gallate, e; syringic acid, f; p-coumaric acid, g; rosmarinic acid, h; t-resveratrol, k; quercetin).
Phenolic Profiles of the plant by RP-HPLC

Şiringik Asit & derivatives

Quercetin & derivatives

Gallik Acid

Resveratrol & derivatives
Salvia fruticosa M.

Phenolic Profiles of the plant by RP-HPLC

Extraction of Plants

Determination of TPC & Antioxidant Activities

- Total Phenolic Content (Folin Ciocalteu Method)
- Total Flavonoid Content (Aluminum Chloride Colorimetric Assay)

- Antioxidant Activity
  - DPPH Method
  - ABTS Method
  - Metal Chelating Activity
RESULTS on Antioxidant activity and phenolic content

*Salvia fruticosa extraction*

<table>
<thead>
<tr>
<th>Extract</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water extract of <em>S. fruticosa</em></td>
<td>16.5 ± 1.5</td>
</tr>
</tbody>
</table>
RESULTS

TPC of *Salvia fruticosa*

<table>
<thead>
<tr>
<th>Concentration (mg/mL)</th>
<th>Total Phenol Content μg GAE / mg dry extract + SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>178.84 ± 2.15</td>
</tr>
<tr>
<td>0.5</td>
<td>171.66 ± 2.61</td>
</tr>
<tr>
<td>Average</td>
<td>175.2 ± 5.07</td>
</tr>
</tbody>
</table>

Gallic acid standard curve

\[
y = 0.107x + 0.0036 \\
R^2 = 0.9982
\]
RESULTS

➢ TFC of *Salvia fruticosa*

<table>
<thead>
<tr>
<th>Concentration (mg/mL)</th>
<th>Total Flavonoid Content (µg CE / mg dry extract + SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>105.4 ± 3.12</td>
</tr>
<tr>
<td>0.5</td>
<td>108.9 ± 2.61</td>
</tr>
<tr>
<td>Average</td>
<td>107.14 ± 2.47</td>
</tr>
</tbody>
</table>

\[ y = 0.0022x + 0.0432 \]

\[ R^2 = 0.997 \]
Free radical scavenging capacity of S. fruticosa by DPPH Assays

<table>
<thead>
<tr>
<th>Sample</th>
<th>Antioxidant Activity</th>
<th>Maximum RSA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>IC50 µg/mL ± SD</td>
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<td>S. fruticosa extract</td>
<td>34.9 ± 0.009</td>
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<td>17.1 ± 0.014</td>
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RESULTS

• Free radical scavenging capacity by ABTS Assays

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<tr>
<th>Concentration (µg/mL)</th>
<th>TEAC value (mmol TE/g extract ± SD)</th>
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<tr>
<td>2,5</td>
<td>1.23 ± 0.0043</td>
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<tr>
<td>Average</td>
<td>1.23 ± 0.1900</td>
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</table>

\[ y = 2.857x - 4.3231 \]

\[ R^2 = 0.9785 \]
RESULTS

Metal chelating activity of *S.*

<table>
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<tr>
<th>Sample</th>
<th>Fe$^{+2}$ Chelating Activity</th>
<th>Maximum RSA (%)</th>
</tr>
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<tr>
<td>S. fruticosa extract</td>
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<td>75.8 ± 0.92</td>
</tr>
<tr>
<td>EDTA</td>
<td>0.107 ± 0.002</td>
<td>94.3 ± 1.08</td>
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</tbody>
</table>
## Phenolic Profiles

Quantitative RP-HPLC Analysis of *S. fruticosa M.*

<table>
<thead>
<tr>
<th>Phenolic compounds</th>
<th>mg/g dry extract ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rosmarinic acid</td>
<td>59.3 ± 3.82</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>15.16 ± 0.4</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>0.56 ± 0.024</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>0.74 ± 0.0104</td>
</tr>
<tr>
<td>Trans resveratrol</td>
<td>0.17 ± 0.06</td>
</tr>
<tr>
<td>Quercetin</td>
<td>0.73 ± 0.04</td>
</tr>
</tbody>
</table>
# Total Phenolics & Flavonoids Profile

**MATERIALS & METHODS**

Salvia fruticosa M.

**Determination of TPC & TFC Antioxidant Activities**

<table>
<thead>
<tr>
<th>Concentration (mg/mL)</th>
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</table>
RESULTS

**Salvia fruticosa M.**

Phenolic Profiles of the plant by RP-HPLC

Extraction of Plants

Determination of TPC and TFC & Antioxidant Activities

---

Antioxidant Activity by DPPH Method

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<th>Sample</th>
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<th>Maximum RSA (%)</th>
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<td>IC50 µg/mL ± SD</td>
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<td>17.1 ± 0.014</td>
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Antioxidant Activity by ABTS Method

\[ y = 2.857x - 4.3231 \]
\[ R^2 = 0.9785 \]

<table>
<thead>
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<th>Concentration (µg/mL)</th>
<th>TEAC value (mmol TE/g extract ± SD)</th>
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<td>2.5</td>
<td>1.23 ± 0.0043</td>
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<td>5</td>
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<td>10</td>
<td>1.15 ± 0.0046</td>
</tr>
<tr>
<td>Average</td>
<td>1.23 ± 0.1900</td>
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</table>
### RESULTS

**Salvia fruticosa M.**

**Phenolic Profiles of the plant by RP-HPLC** → **Extraction of Plants** → **Determination of TPC and TFC & Antioxidant Activities**

### Phenolic Profiles

- Sample: S. futicosa extract
- Chelating Activity: 0.43 ± 0.0035 mg/mL
- Maximum RSA (%): 75.8 ± 0.92

- Sample: EDTA
- Chelating Activity: 0.107 ± 0.002 mg/mL
- Maximum RSA (%): 94.3 ± 1.08

### Antioxidant Activity by Metal Chelating Activity

<table>
<thead>
<tr>
<th>Sample</th>
<th>Fe$^{+2}$ Chelating Activity</th>
<th>Maximum RSA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. futicosa extract</td>
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<tr>
<td>EDTA</td>
<td>0.002 mg/mL ± SD</td>
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</tr>
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</table>

---

This table shows the concentration (mg/mL) of different samples and their respective chelating activity and maximum RSA (%) values.
**Materials & Methods**

*Salvia fruticosa M.*

- Phenolic Profiles of the plant by RP-HPLC
- Extraction of Plants
- Determination of TPC and TFC & Antioxidant Activities
- **Cell Culture Studies**

**Cell Culture Studies**

- **HT-29 Cell line**: Colorectal adenocarcinoma
- **Type**: Adherent, epithelial like
- **Origin**: Female, 44 years

**Medium in cell culture**: McCoy's 5A; L-Glutamine; 10% fetal bovine serum; 1% penicillin-streptomycin and 1% Na-pyruvate.

**The cells were incubated at 37°C in a humidified atmosphere with 5% CO₂.**
**MATERIALS & METHODS**

*Salvia fruticosa M.*

- Phenolic Profiles of the plant by RP-HPLC
- Extraction of Plants
- Determination of TPC and TFC & Antioxidant Activities
- **Cell Culture Studies**

- **Viable Cell Counting**

![Viable Cell Counting Image](image-url)
Cytotoxicity determination on HT-29 colon cancer cells
RESULTS

HT-29 Growth curve

Number of cells (x10^4/well)

Time (days)

0  5  10  15  20
RESULTS

Cytotoxicity of *S. fruticosa* on HT-29 cells. Left: 48 hours 0.1 % DMSO treated control. Right: 48 hours IC\textsubscript{50} concentration (0.174 mg/mL) *S. fruticosa* treated.
RESULTS

- Cytotoxicity of *S. fruticosa* on HT-29 Cells

HT-29 cells. Left: 72 hours 0.1% DMSO treated control. Right: 72 hours IC\(_{50}\) concentration (0.228 mg/mL) *S. fruticosa* treated.
Cytotoxicity of *S. fruticosa* on HT-29 Cells

Viabilities of HT-29 cells in response to dose and time dependent treatment of *S. fruticosa*
Cytotoxicity of rosmarinic acid on HT-29 Cells

**HT-29 Cells 48 hr**

**HT-29 Cells 72 hr**
Cytotoxicity of caffeic acid on HT-29 Cells

Graph 1: HT-29 Cell 48 hr

Graph 2: HT-29 Cells 72 hr

Cell viability (%) vs. Concentration (mg/mL)
Cytotoxicity of *S. fruticosa*, rosmarinic acid and caffeic acid on HT-29 Cells

<table>
<thead>
<tr>
<th>Agents</th>
<th>Incubation</th>
<th>48 hr</th>
<th>72 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td></td>
<td>0.185 ± 0.0025</td>
<td>0.229 ± 0.0148</td>
</tr>
<tr>
<td>Rosmarinic acid (R.A)</td>
<td></td>
<td>0.148 ± 0.0036</td>
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<td>R.A + C.A</td>
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<td>0.169 ± 0.005</td>
<td>0.184 ± 0.0076</td>
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RESULTS

EFFECTS OF *Salvia fruticosa* on CYP1A1, CYP1A2, CYP2E1 AND CYP3A4 mRNA EXPRESSION
EFFECTS OF *Salvia fruticosa* on CYP1A1, CYP1A2, CYP2E1 AND CYP3A4 mRNA EXPRESSION

**CYP2E1**

**CYP3A4**
FOLD CHANGES OF CYP1A1, CYP1A2, CYP2E1 AND CYP3A4 mRNA EXPRESSION

<table>
<thead>
<tr>
<th>Genes</th>
<th>48 hr incubation</th>
<th>72 hr incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A1</td>
<td>8.14 ↑</td>
<td>4.12 ↑</td>
</tr>
<tr>
<td>CYP1A2</td>
<td>2.57 ↑</td>
<td>3.89 ↓</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>1.72 ↓</td>
<td>NS</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>1.75 ↓</td>
<td>4.02 ↑</td>
</tr>
</tbody>
</table>
RESULTS

EFFECTS OF *Salvia fruticosa* on GST M1, GST P1, GPx4 AND CATALASE mRNA EXPRESSION

**GST M1**

**GSTP1**
EFFECTS OF \textit{Salvia fruticosa} on GST M1, GST P1, GPx4 AND CATALASE mRNA EXPRESSION

\textbf{GPx4}

\textbf{CATALASE}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{result.png}
\end{figure}
## RESULT

### FOLD CHANGES OF GST M1, GST P1, GPx4 AND CATALASE mRNA EXPRESSION

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<th>Genes</th>
<th>48 hr incubation</th>
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<tbody>
<tr>
<td>GST M1</td>
<td>2.17 ↑</td>
<td>1.62 ↑</td>
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<td>GST P1</td>
<td>1.68 ↓</td>
<td>1.3 ↑</td>
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<td>GPx4</td>
<td>3.5 ↑</td>
<td>2.17 ↓</td>
</tr>
<tr>
<td>CATALASE</td>
<td>1.37 ↓</td>
<td>1.28 ↑</td>
</tr>
</tbody>
</table>
RESULTS

*Salvia fruticosa M.*

- Phenolic Profiles of the plant by RP-HPLC
- Extraction of Plants
- Determination of TPC and TFC & Antioxidant Activities
- Cell Culture Studies
  - Enzyme Activities
    - Isolation of protein
      - Determination of Protein Concentration
      - Screening the antioxidant enzymes
    - Gene expressions
      - Total RNA Isolation from HT-29 cells
        - RNA Concentration Determination
          - cDNA Synthesis
            - Quantitative Real Time PCR
  - Cytotoxicity on HT-29 cells by XTT Assay
EFFECTS OF *Salvia fruticosa* on Total GSTs, GPx4 AND CATALASE ACTIVITIES

(Statistically significant, p < 0.05)
RESULT

CHANGES OF Total GSTs, GPx4 AND CATALASE ACTIVITIES

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>48 hr incubation</th>
<th>72 hr incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTs</td>
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<td>↓</td>
</tr>
<tr>
<td>GPx4</td>
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<td>↓</td>
</tr>
<tr>
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## DISCUSSION

### Antioxidant Activity

<table>
<thead>
<tr>
<th>Sample</th>
<th>Antioxidant Activity</th>
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### Concentration (µg/mL) & TEAC value (mmol TE/g extract ± SD)

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### Sample Fe⁺² Chelating Activity

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### Ratio of TFC/TPC

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<tr>
<th>Concentration (mg/mL)</th>
<th>Ratio of TFC/TPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>0.59</td>
</tr>
<tr>
<td>0.5</td>
<td>0.63</td>
</tr>
<tr>
<td>Average</td>
<td>0.61</td>
</tr>
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</table>
Cytotoxicity of *Salvia fruticosa*

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## DISCUSSION

**Analysis of *Salvia fruticosa* on mRNA Expression and Antioxidant Enzyme Results**

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DISCUSSION

AHR Pathway

PXR-CYP CASCADE

Ligands enter cell

Receptor binds ligand

DREs CYP1A1

ARNT

Other factors?

mRNA

DREs Other genes

Proteosome

Degradation

Increased cytochrome P-4501A1

New proteins

Translation

Other gene products

Drug A

PXR RXR

CYP3A

CYP3A enzyme

Liver, intestine

Examples

Ethinyestradiol

Erythromycin

Atorvastatin

Inclavir

Elavirenz

Cyclosporin

Carbamazepine

Warfarin

Tamoxifen

Doxorubicin

Drug B

Drug B
CONCLUSION

➢ Our results underline the importance of studying flavonoids at the intestinal level to assess potential health risks linked to flavonoid enriched supplements or functional food ingestion.
This study also emphasizes the need for a careful investigation of the potential benefits and adverse effects before making recommendations regarding dietary supplements and the importance of educating the consumer on the potential drug and xenobiotic interactions.

These results provide an evidence that phenolic compounds (flavonoids), depending on their structures, are either beneficial antioxidants or acting as harmful pro-oxidant compounds.
THANK YOU FOR YOUR INTERESTS

GREETINGS FROM TURKEY

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TURKEY – İZMİR
İZMİR- KARŞIYAKA
WISHING SUCCESSFUL OMICS CONFERENCES
SEE YOU NEXT MEETING

Associate Professor Dr. Özlem TOKUŞOĞLU
ozlem.tokusoglu@cbu.edu.tr
tokusogluozlem@yahoo.com