OMICS Group
Accelerating Scientific Discovery

4th International Conference and Exhibition on
Nutrition
October 26-28, 2015 Chicago, Illinois, USA

KEY NOT PRESENTATION

Özlem Tokuşoğlu
Associate Professor Dr
CONFERENCE CHAIR

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.”

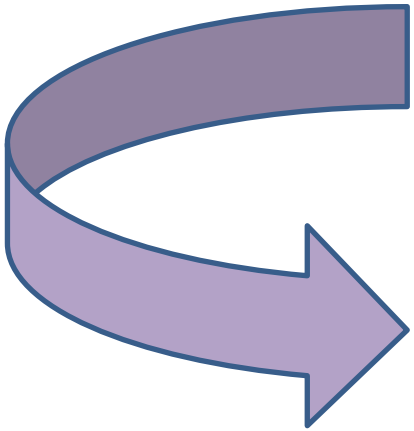
Nutrition is the sum of all processes involved in how organisms obtain nutrients, metabolize them, and use them to support all of life’s processing.



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**
- **Fortificated 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 forBiotechnical Considerations, New Crops and Nanotechnological Products

4. The Usability of New Technigues and Technologies for Food Improving and Deveoloping, for Food Safety, and for Nutraceutical Foods and Edible Anticancer Agents





1

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



2

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.

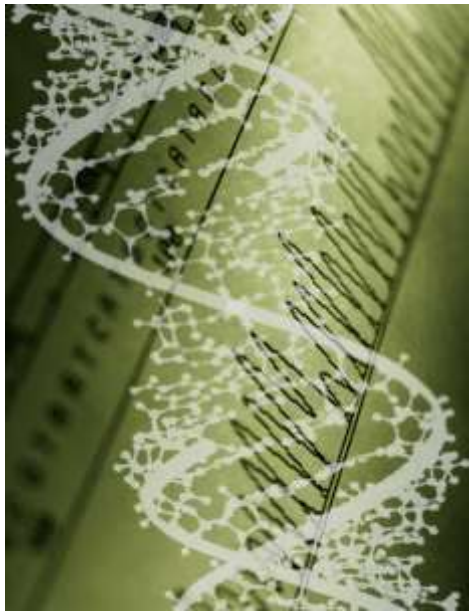
Controlled Innovative Technologies are Necessary

3

Farming and Agricultural Capacity

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

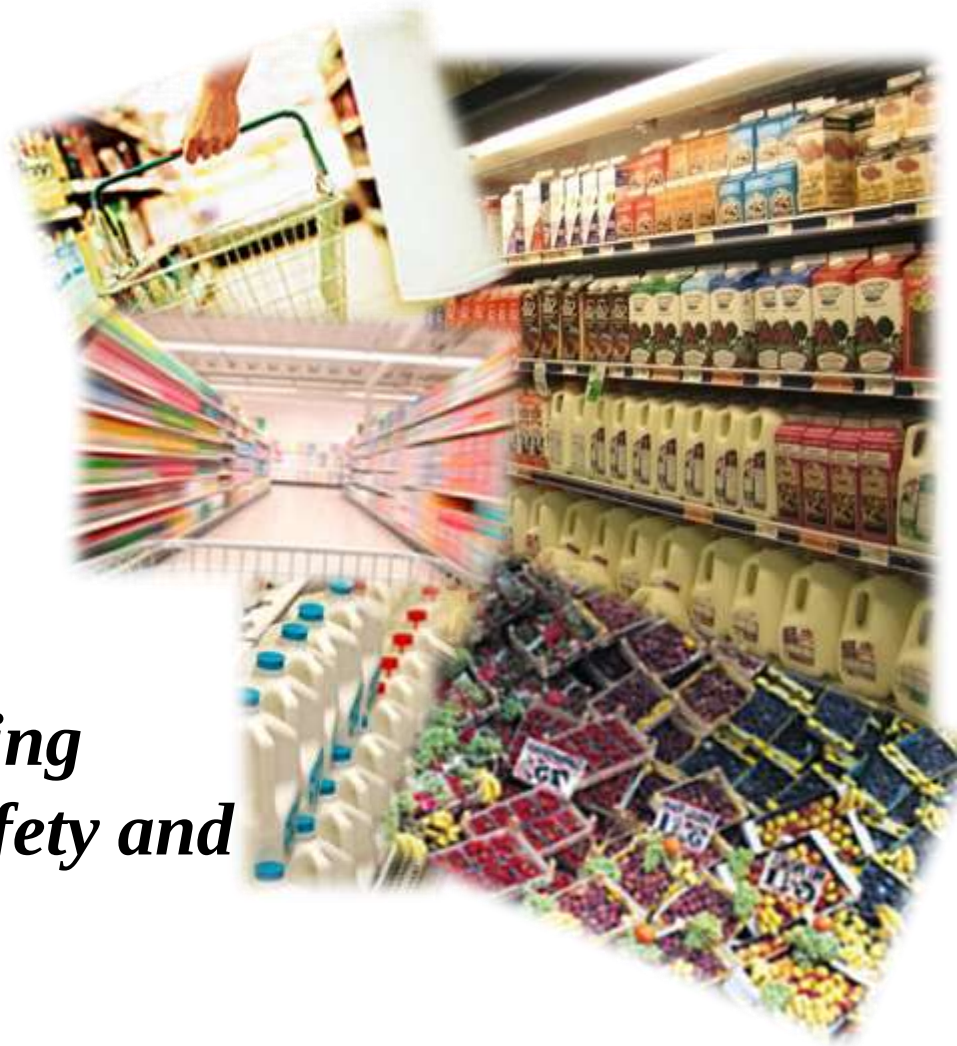
This makes it possible for developers to meet more specific requirements e.g. to increase a specific nutrient in a food.



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)



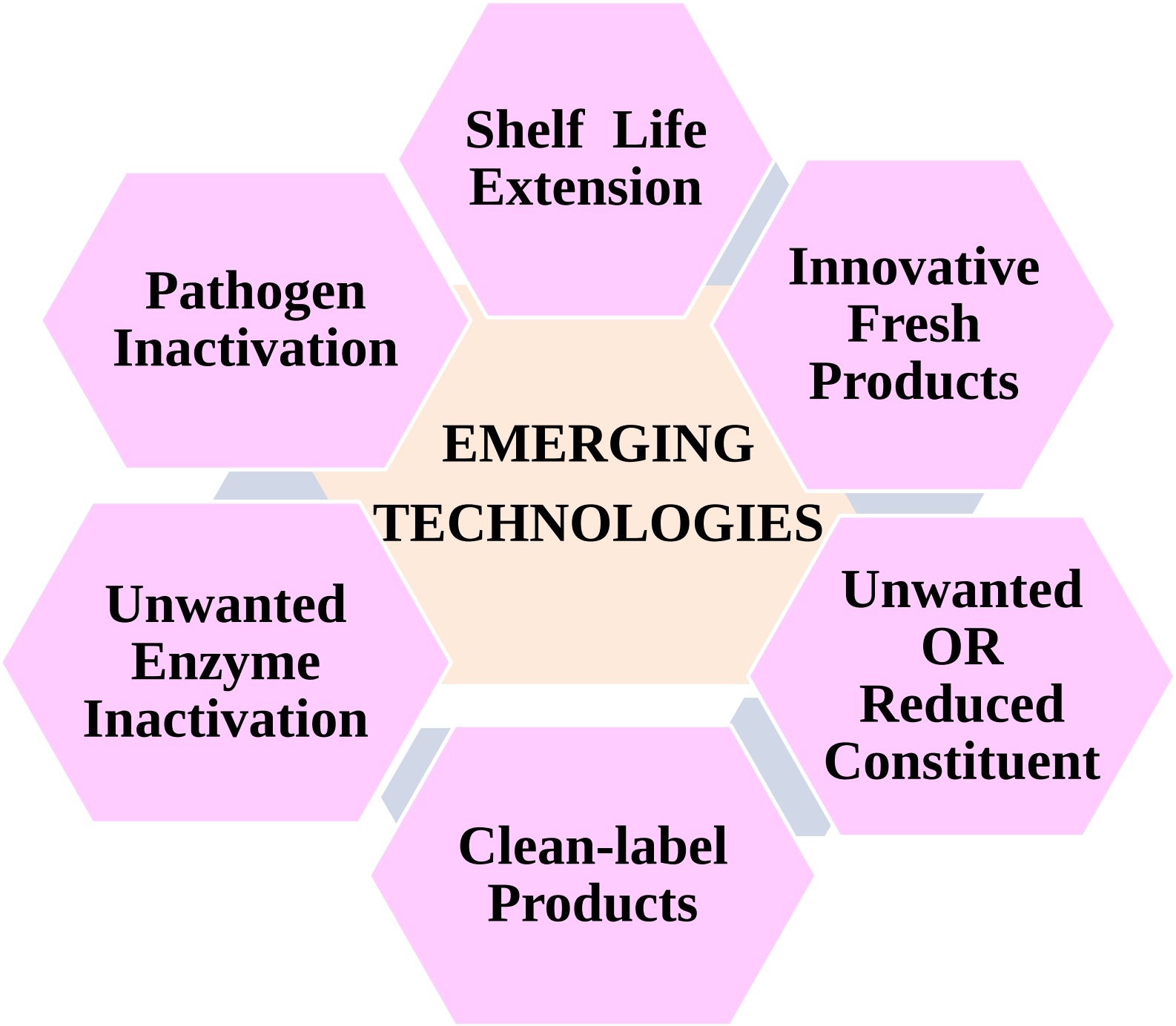
NONTHERMAL

- High Hydrostatic Pressure
- Pulsed electric fields
- Ultrasound
- Ultraviolet
- Irradiation
- Cold Plasma
- DensePhase CarbonDioxide
- Ozone
- Chemicals

THERMAL

- Microwave
- Radiofrequency
- Ohmic Heating
- Induction Heating

Template graphic elements and format © 2013, Institute of Food Technologists.
All rights reserved. Slide content © 2013, by the presenter. All rights reserved.


Shelf Life Extension
Innovative Fresh Products
Unwanted OR Reduced Constituent
Clean-label Products
Unwanted Enzyme Inactivation
Pathogen Inactivation
EMERGING TECHNOLOGIES

In Nutrition Concept

NUTRITION & PUBLIC HEALTH

NUTRITION &FOOD QUALITY& INNOVATIVE PROCESSING

NUTRITION & PHYTOCHEMICAL BIOACTIVES & ANTIOXIDANTS

NUTRITION & CANCER & CLINICAL STUDIES

NUTRITION & NUTRITIONAL TOXICOLOGY

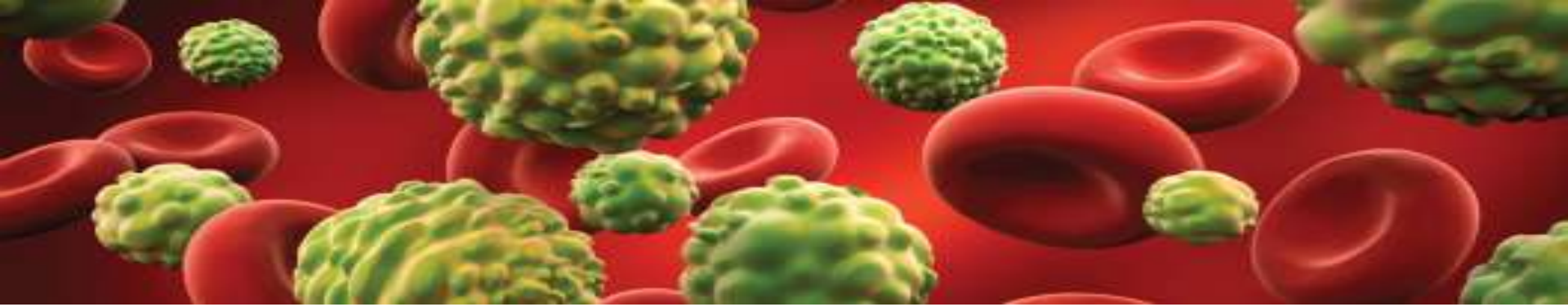
NUTRITION & OBESITY, and DIET and EXERCISE



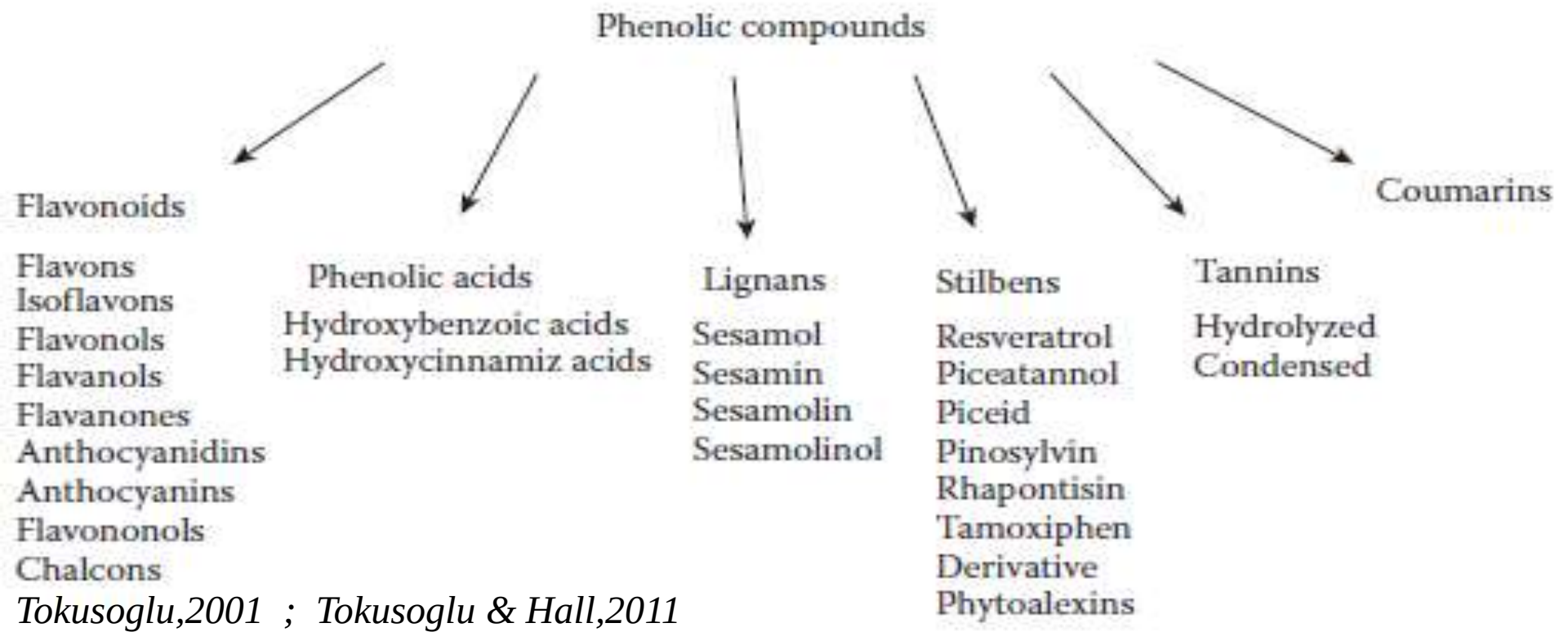
Innovative Technologies are effective on all concepts

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…



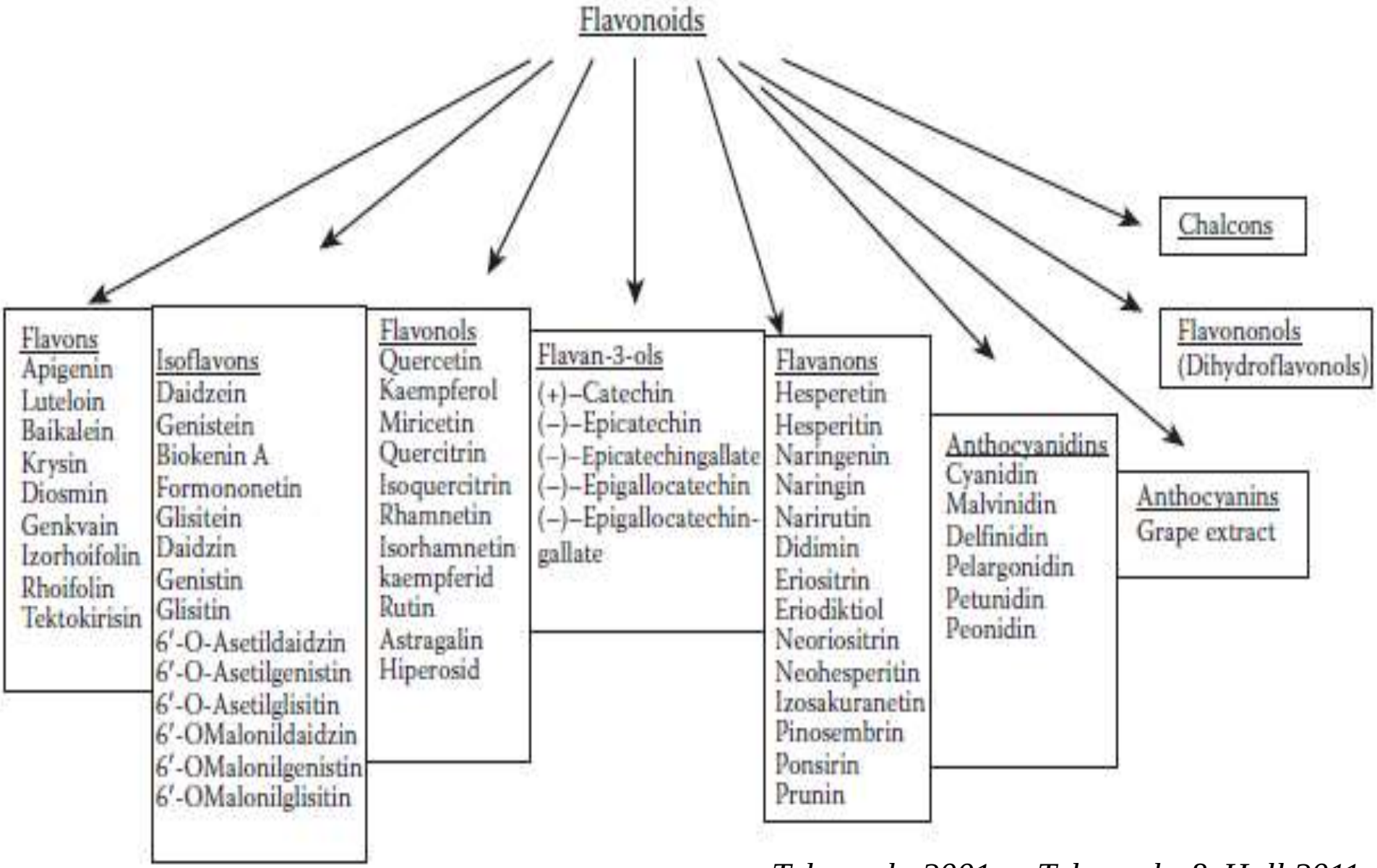


Polyphenols divide into several subgroups including majorly flavonoids….and especially anticarcinogen



Tokusoglu,2001 ; Tokusoglu & Hall,2011

Flavonoids Family in Food Plants…..



Tokusoglu,2001 ; Tokusoglu & Hall,2011

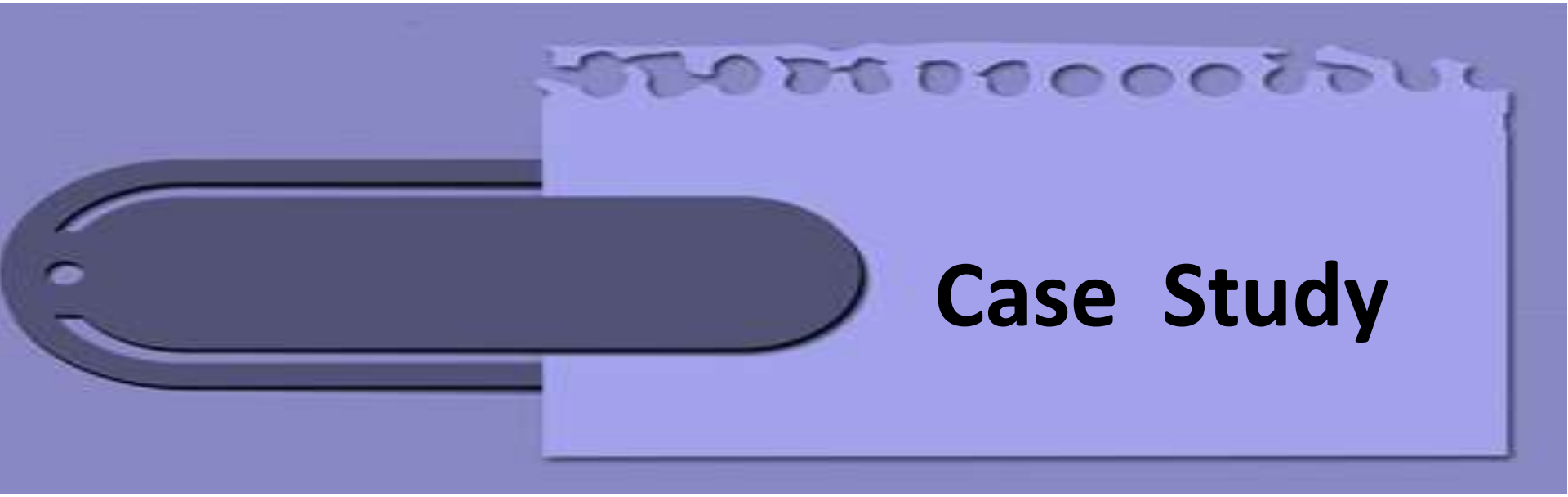
Chemoprevention & Phytochemicals…..

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 celluar 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 differantiation and apoptosis**
- **Inhibiting expression and functional activation of oncogenes**
- **Increasing expression of tumor-suppressor genes,**
- **Inhibiting angiogenesis and metastasis by modulating cellular signaling pathways**



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

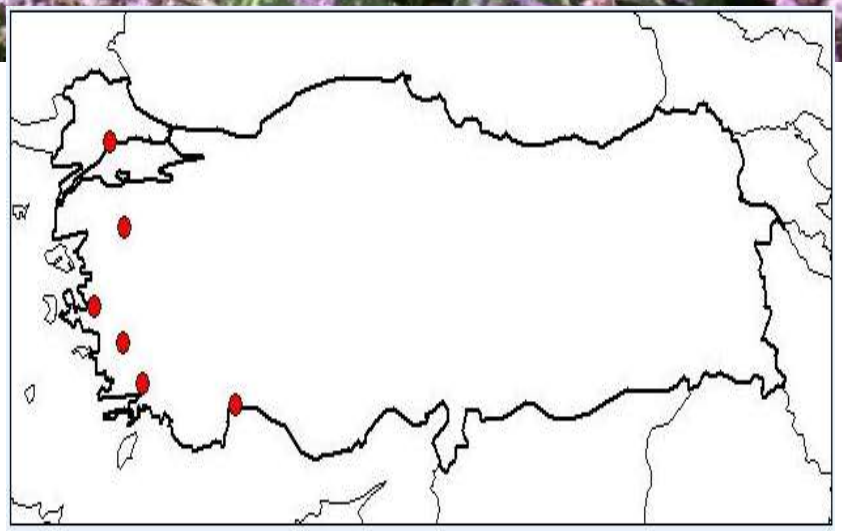
Aim of The Study

2-Material and Methods

3-Results and Discussion

4-Conclusion



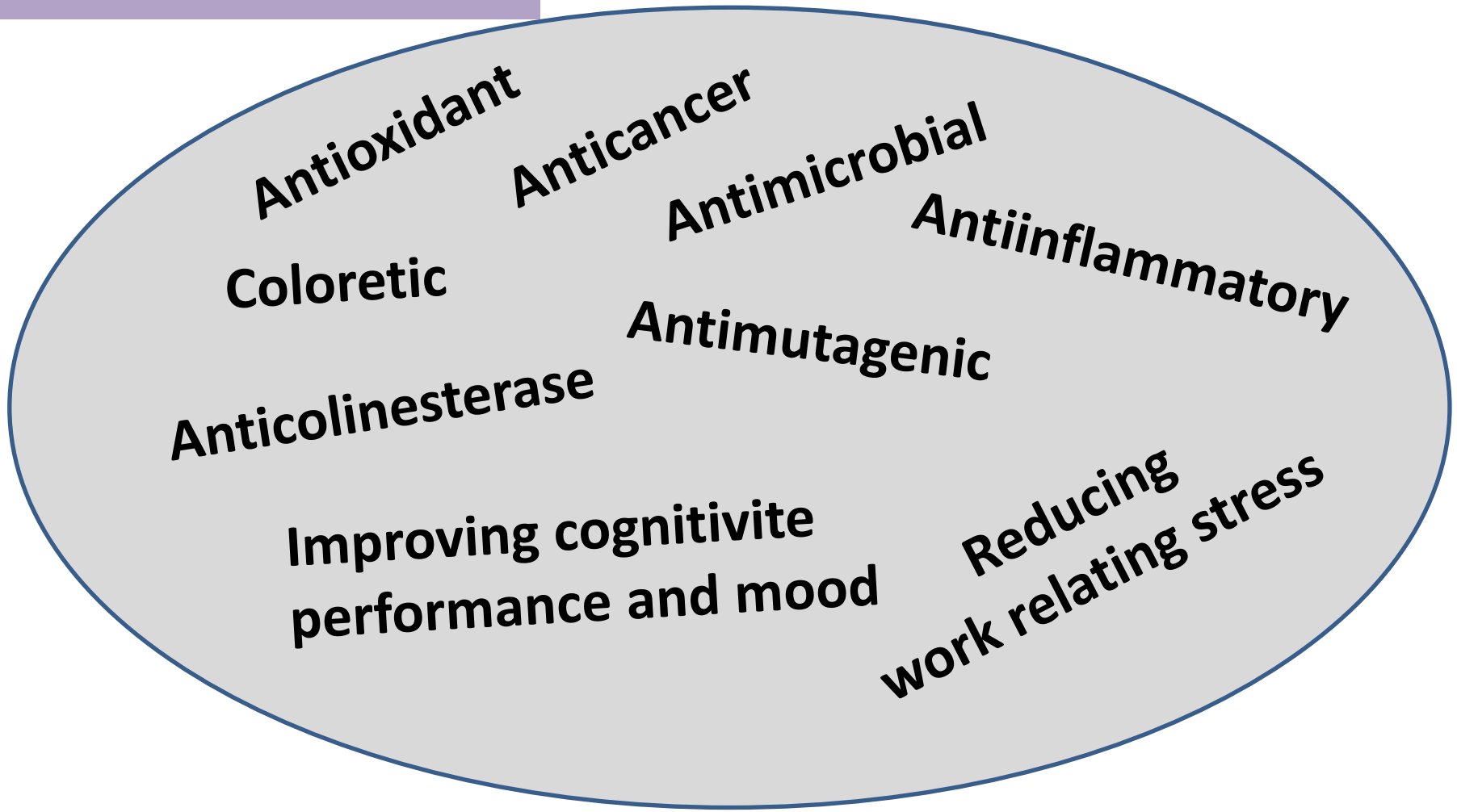


Taxonomy

Kingdom: Plantae

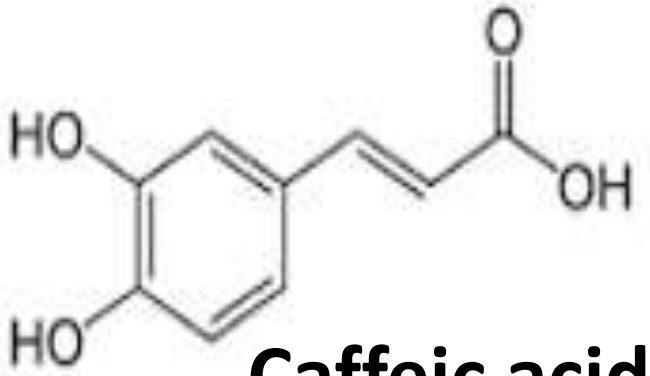
- **Subkingdom : Tracheobionta**
- **Division:Magnoliophyta**
- **Class: Magnoliopsida**
- **Subclass : Asteridae**
- **Order : Lamiales Family: Lamiaceae**
- **Genus : *Salvia***
- **Species : *S. fruticosa* Miller**

Salvia Genus------



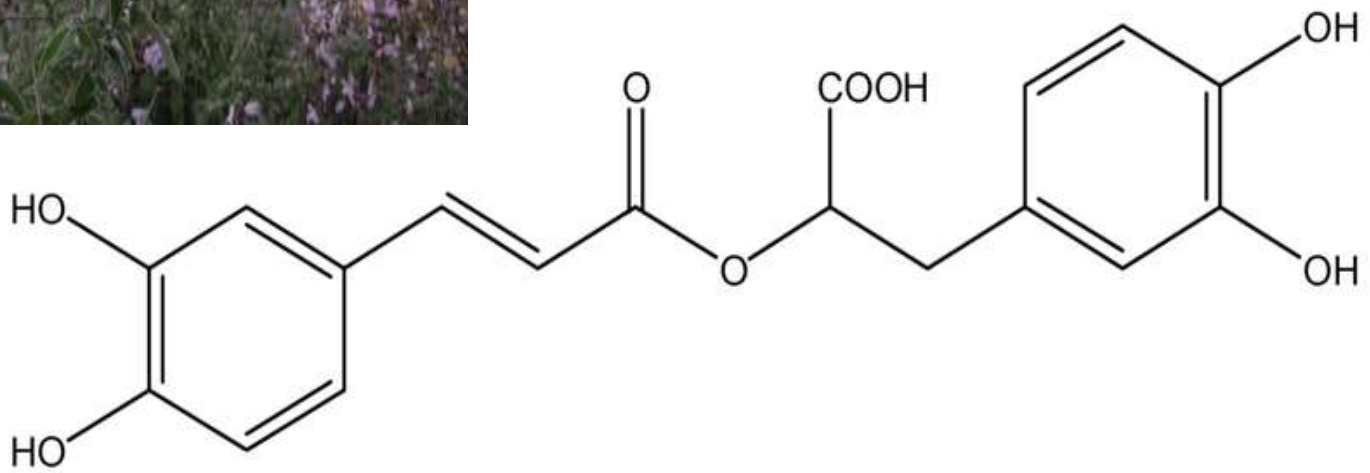
Phenolic Contents of *Salvia fruticosa (SF)*

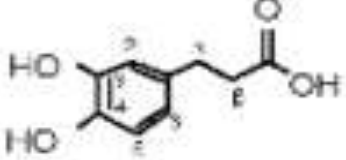




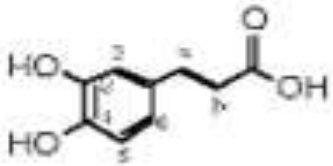
Caffeic acid

Rosmarinic acid

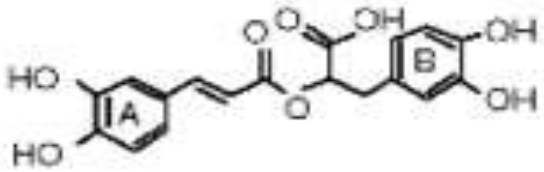




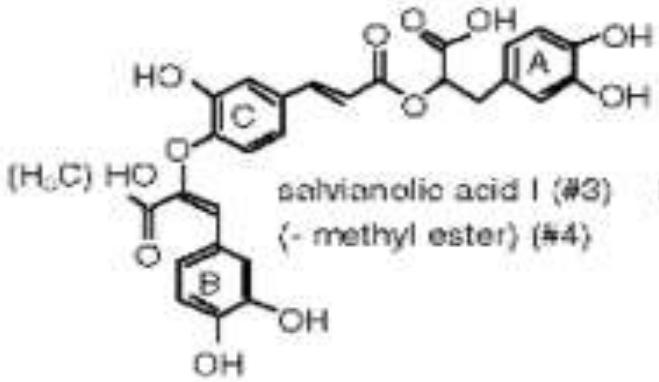
dihydrocaffeic acid (#1a)



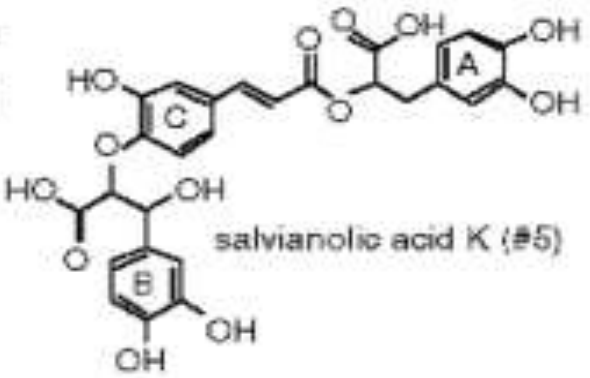
caffeic acid (#1)



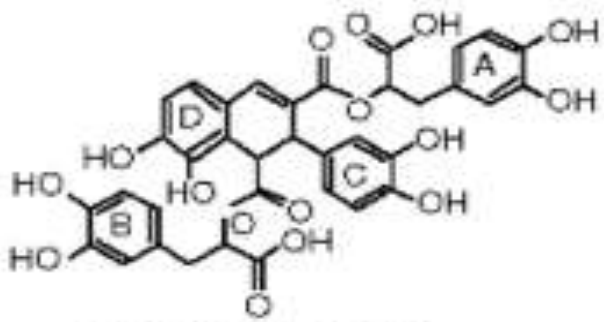
rosmarinic acid (#2)



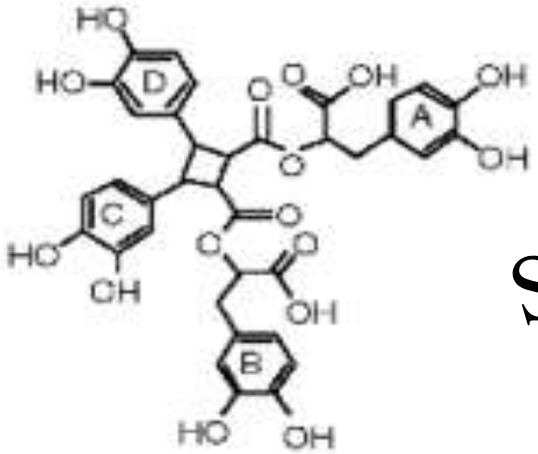
salvianolic acid I (#3)
(- methyl ester) (#4)



salvianolic acid K (#5)



salvianolic acid L (#6)

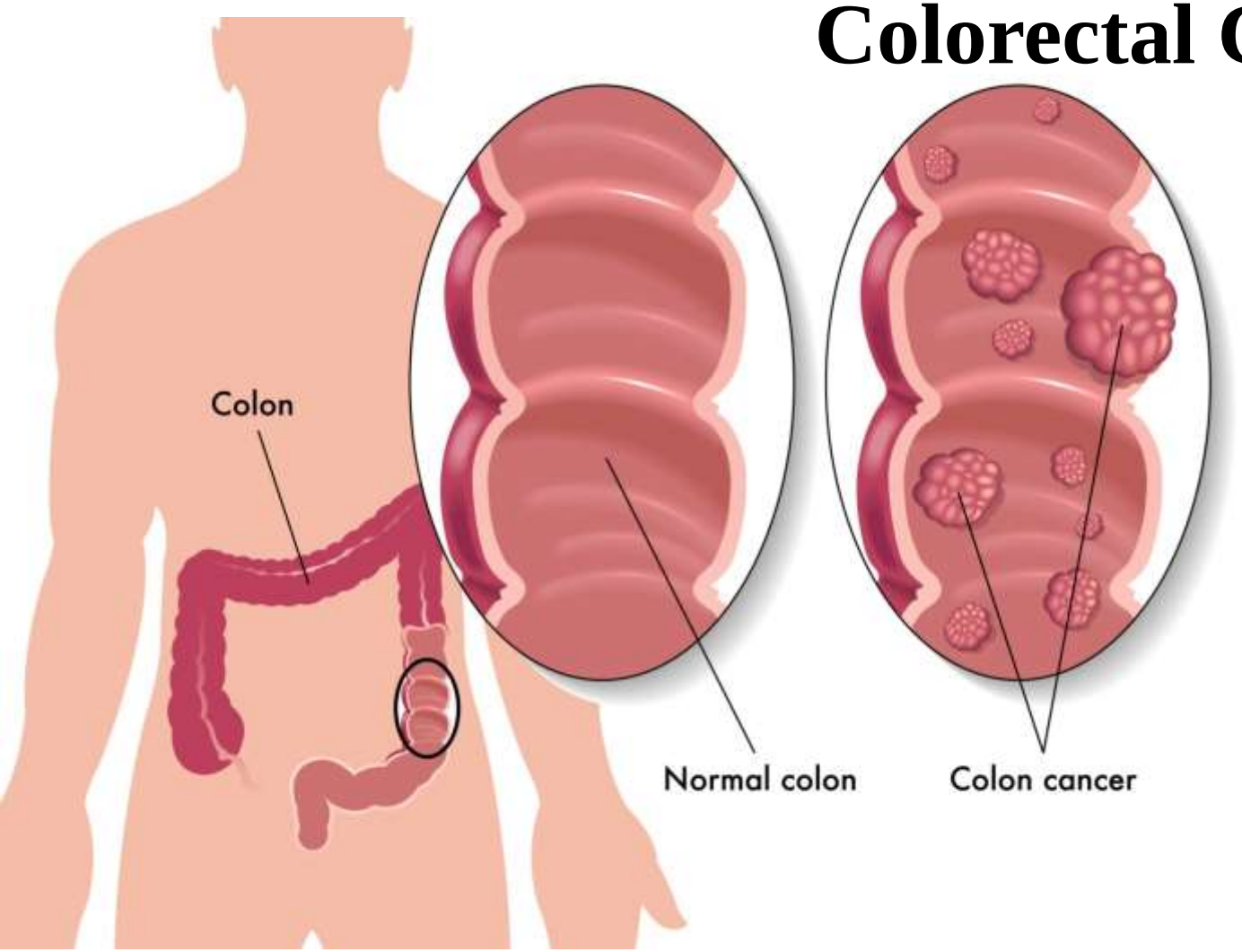


sagerinic acid (#7)

- **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

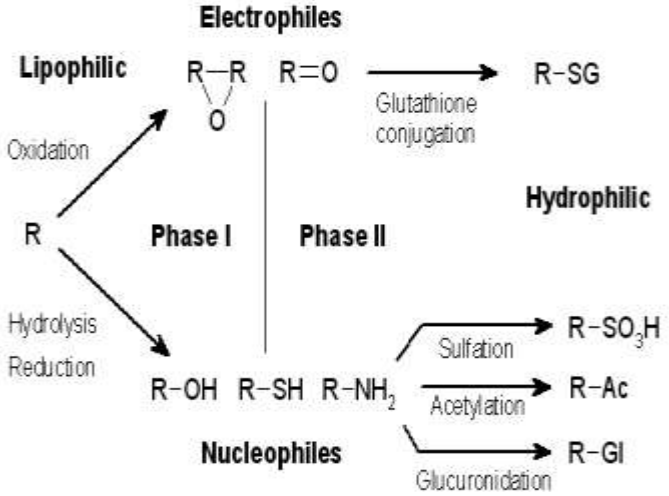
- **Oxidation**
  - Cytochrome P450 monooxygenase system
  - Flavin-containing monooxygenase system
  - Alcohol dehydrogenase and aldehyde dehydrogenase
  - Monoamine oxidase
- **Reduction**
  - NADPH-cytochrome P450 reductase
- **Hydrolysis**
  - Esterases and Amidase
  - Epoxide hydrolase

Xenobiotic Metabolism

- Occurs in two phases of reactions;

  Phase I reactions

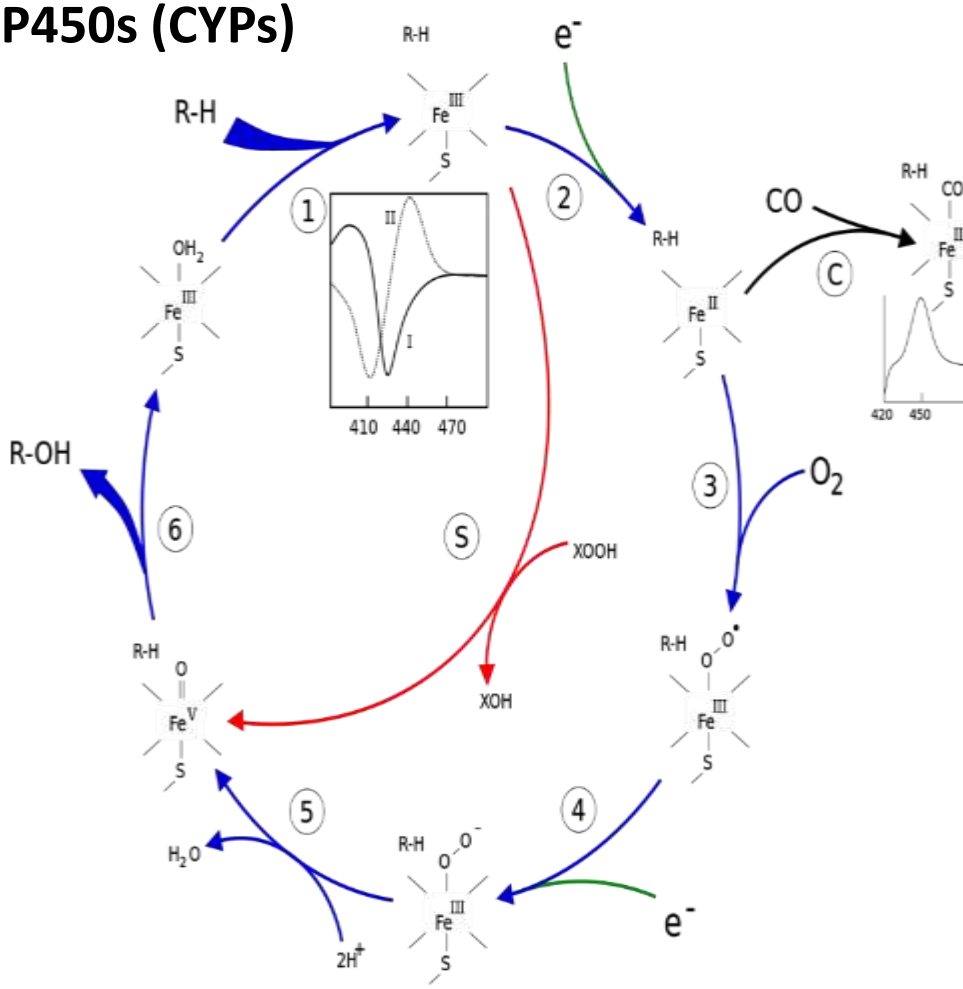
  Phase II reactions



- 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



$O_2 + NADPH + H^+ + RH \rightarrow NADP^+ + H_2O + ROH$

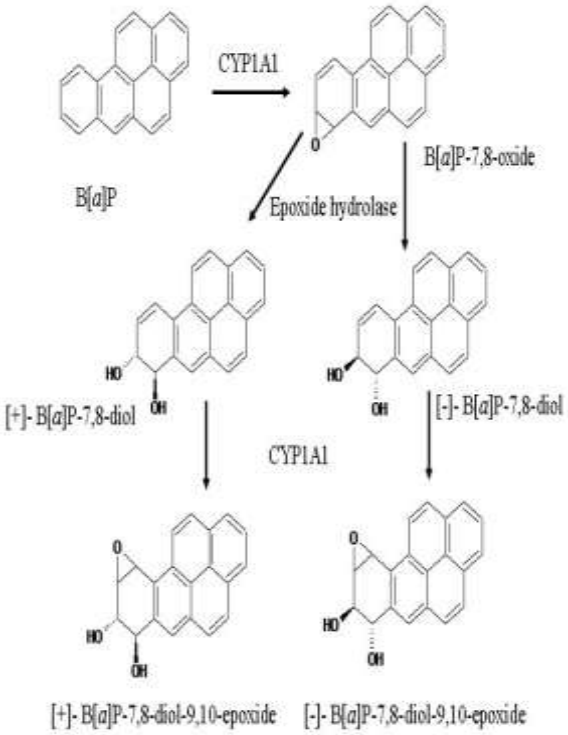
Human CYP Families	Names	Function
CYP1 (3 genes)	1A1, 1A2, 1B1	Xenobiotic metabolism
CYP2 (16 genes)	2A6, 2A7, 2A13, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1, 2F1, 2J2, 2R1, 2S1, 2U1, 2W1	Xenobiotic and steroid metabolism
CYP3 (4 genes)	3A4, 3A5, 3A7, 3A43	Xenobiotic and steroid metabolism
CYP4 (12 genes)	4A11, 4A22, 4B1, 4F2, 4F3, 4F8, 4F11, 4F12,	Fatty acid metabolism

Cytochrome P450 Genes

CYP1A1

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

- PAHs are metabolized to carcinogens by CYP1A1



CYP1A2

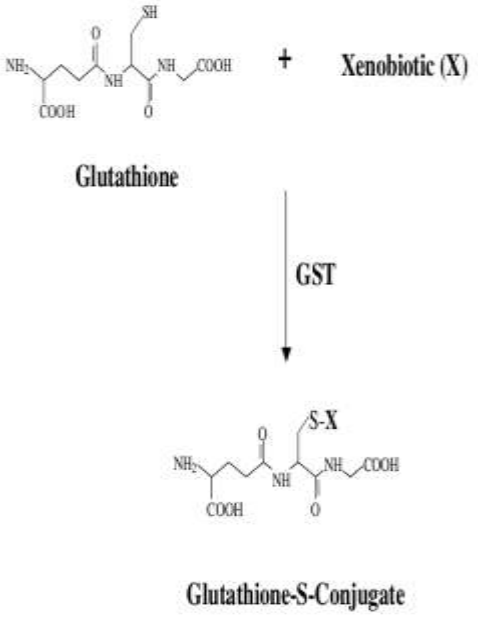
- Expression is induced by some PAHs
- Caffeine, aflatoxin B1, acetaminophen and cigarette smoke are the substrates
- Vegetables such as cabbages, cauliflower and broccoli are known to increase levels of CYP1A2

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

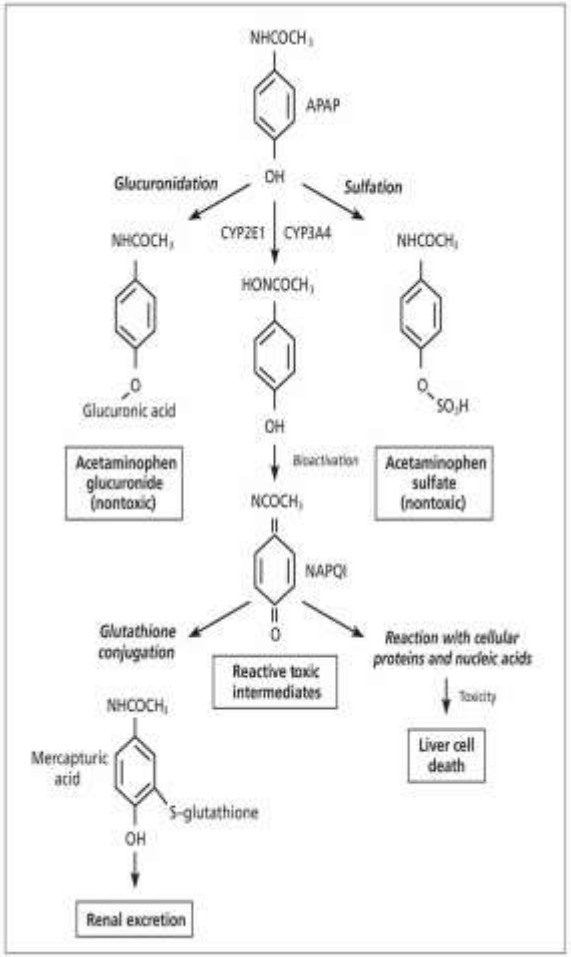
Phase II enzymes (GSTs)

- 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)



CYP3A4

- 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

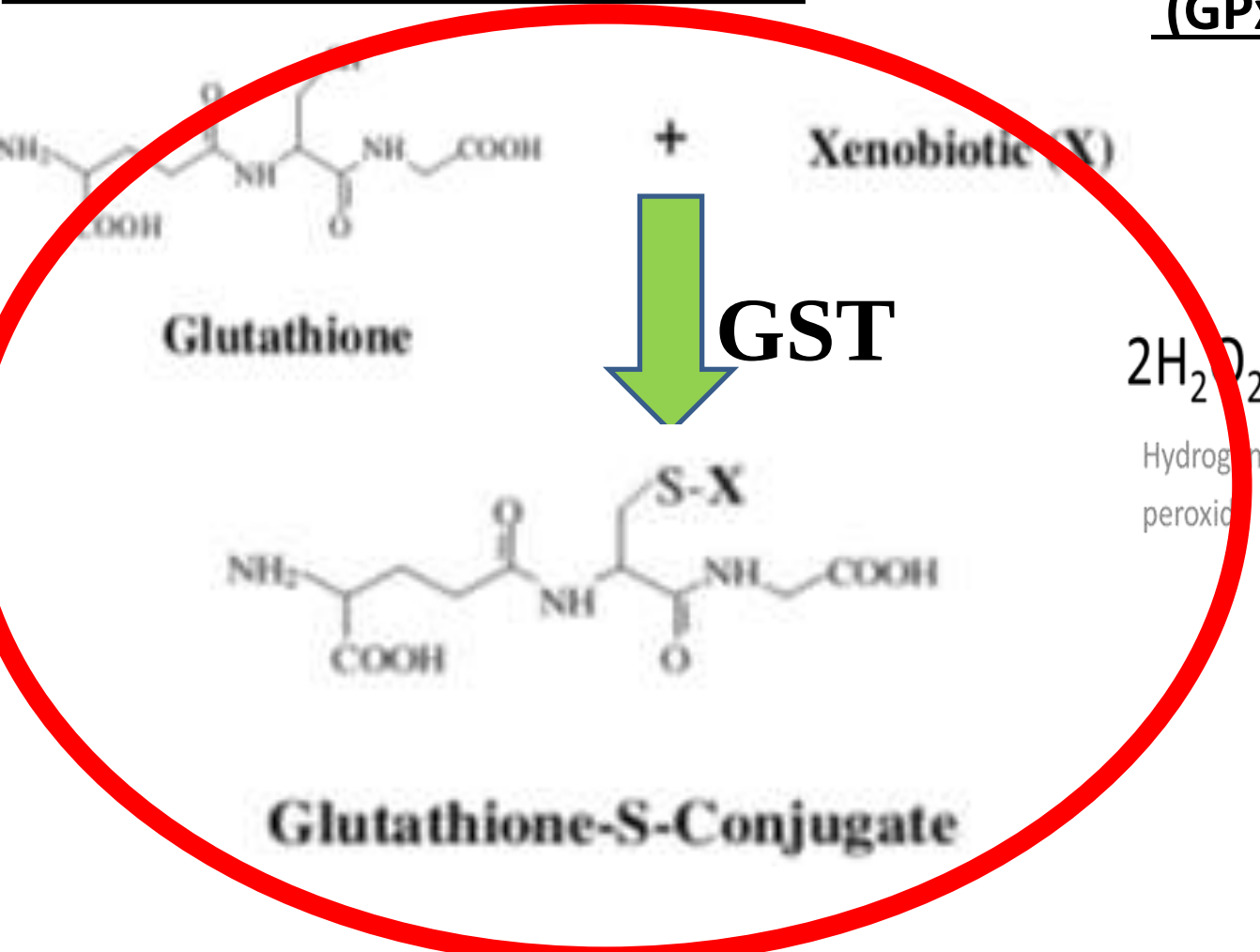
- They detoxify several anticancer drugs
- The known subtrates for GST Mu includes reactive epoxide intermediates
- Mutations of GST Mu gene have been linked with an increase in a number of cancers
- GST Pi have cellular protection against free radical and carcinogenic compounds due to its detoxifying 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)



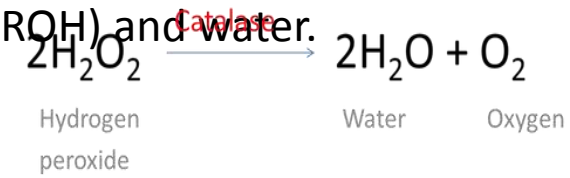
$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

Hydrogen peroxide — Water — Oxygen

Antioxidant Enzymes

Catalase

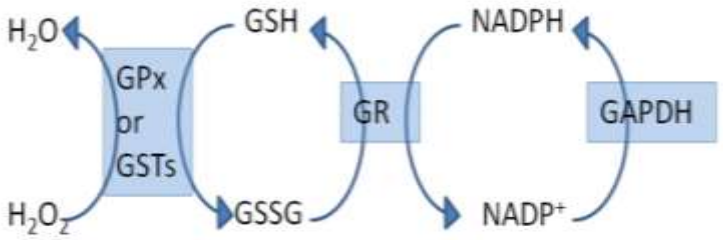
- found in most of all living organisms.
- catalyzes the decomposition of hydrogen peroxide to water and oxygen molecule
- have peroxidative function converting peroxides (ROOH) into alcohol (ROH) and water.



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.



RESEARCH ON
Salvia fruticosa (SF)

✓ **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**

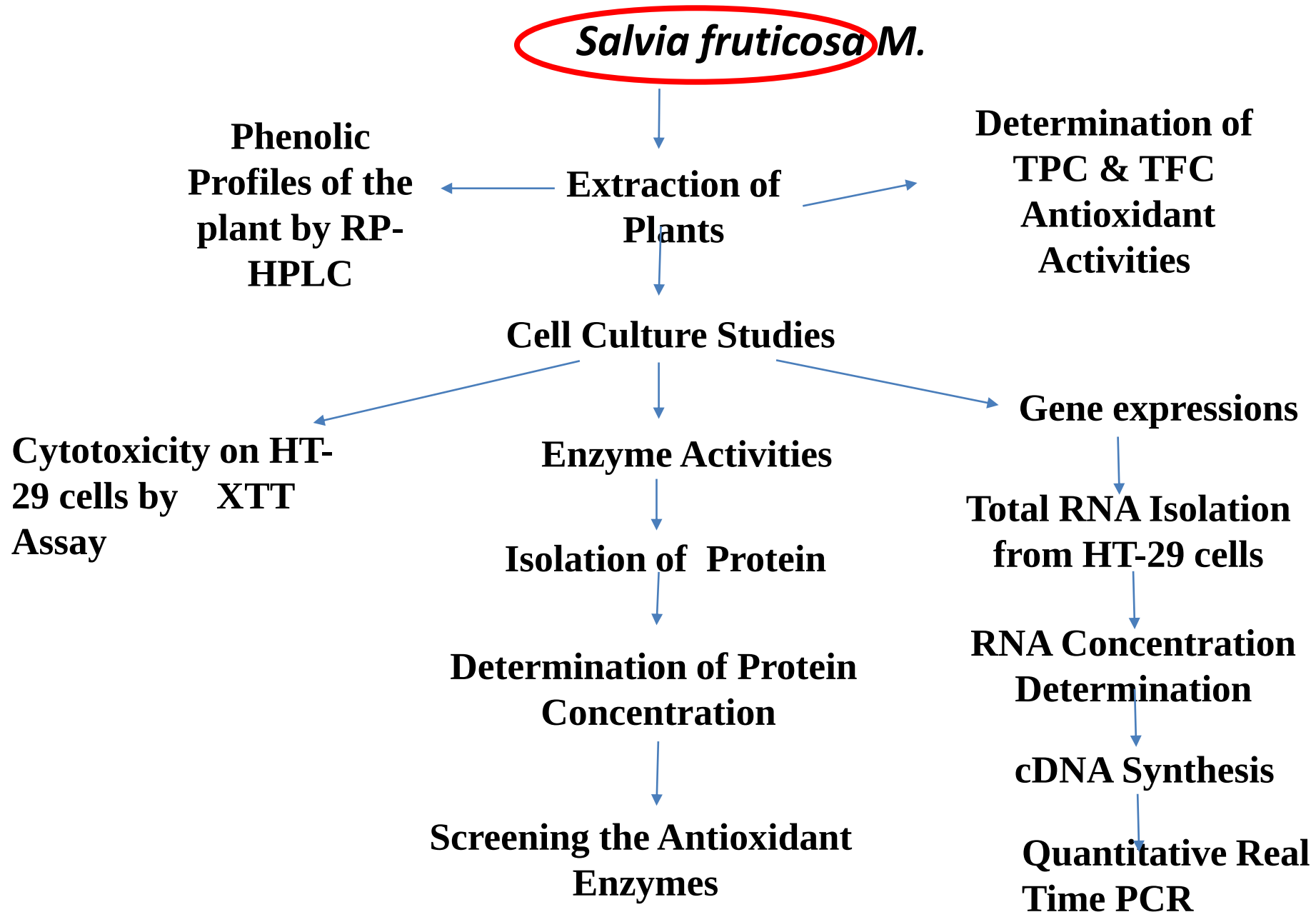
MATERIALS & METHODS

Salvia fruticosa M.

- *S. fruticosa M.* was collected from
- Germiyan Village, Çeşme, İzmir, Turkey.



MATERIALS & METHODS



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) = [Mass of SF (in extracted solution) / mass of total SF] X 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

mg GA /g dry extract mass= [(Abs(sample)- Abs(sample blank)) – Abs (blank)

Determination of TFC

- Total flavonoid content of the plant extract was determined according to **<u>Aluminum Chloride Colorimetric Assay</u>** (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 ($AlCl_3$)
- 6min 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

mg CE /g dry extract mass= [(Abs(sample)- Abs(sample blank)) – Abs (blank)

]/ slope X 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

RSA (Radical scavenging acitivity) % = $[(A_0 - A_1) / A_1] \times 100$

➢ 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

$$\text{Inhibition \%} = [(A_0 - A_1) / A_0] \times 100$$

➢ 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

$$ChA\ \% = [(A_0 - A_1) / A_0] \times 100$$

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.

Time (min.)	A%	B%	Flow
30	88	12	1 mL/min.
45	80	20	
60	70	30	

RP-HPLC-ANALYSIS

➢ Second

The other phenolic compounds; **(Soraia I. et al., 2010)**

A) 0.1% (v/v) formic acid in water

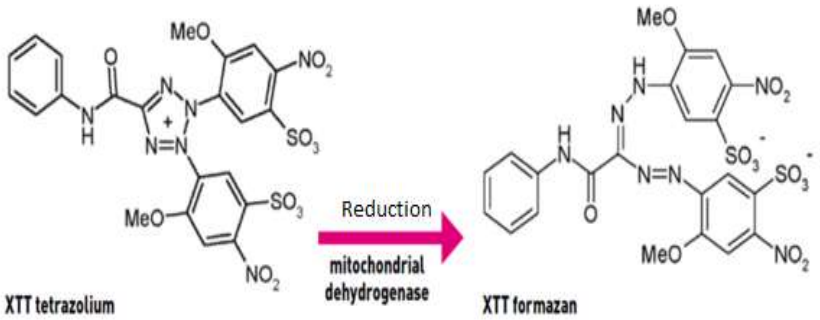
B) Acetonitrile

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

Time (min.)	A%	B%	Flow
0	80	20	1 mL/min.
10	70	30	
40	60	40	
60	40	60	
80	20	80	

Antiproliferative effects of *Salvia fruticosa*

- The cytotoxic effects of water extract of *S. futicosa* 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 mithocondrial 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)

Components	Reaction Volume (µl)
SYBR Green PCR master mix	5 µL
Forward Primer (10µM)	0.2 µL
Reverse Primer (10 µM)	0.2 µL
cDNA	1,6 µL
RNAse free water	3 µL
Total volume	10 µL

	Cycles	Segment		Temperature (°C)	Time
Preincubation		1		95	10 min
Amplification	40	Denaturing		95	20 sec.
		Annealing	GAPDH	58	30 sec
			CYP1A1	60	
			CYP1A2	60	
			CYP2E1	61.5	
			CYP3A4	65	
			GSTM1	60	
			GSTP1	60.5	
			GPx4	60	
			Catalase	59.5	
		Elongation		72	20 sec

Determination of mRNA expression

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

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

In order to evaluate the results, ΔΔCt method was used.

$\Delta Ct = Ct\ (target) - Ct\ (normalizer)$

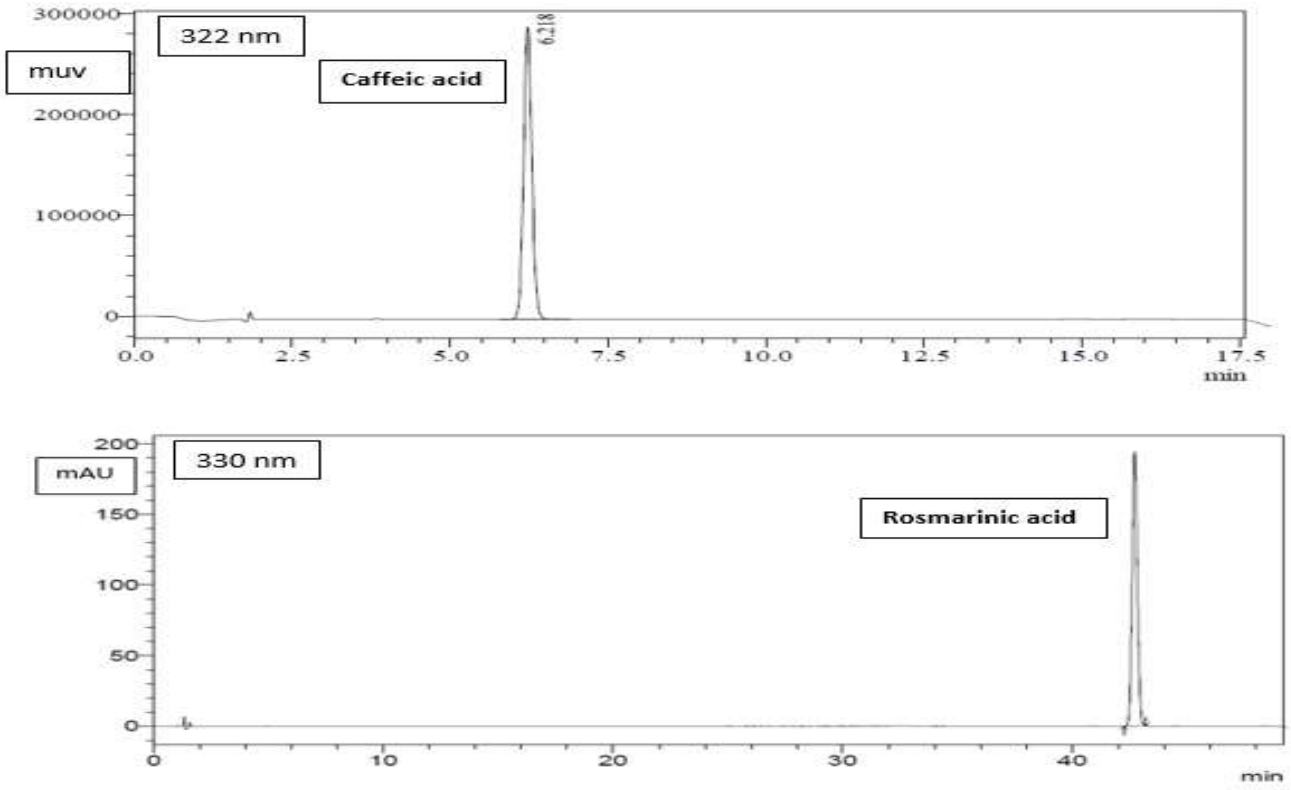
ΔCt is the difference between the CT values of the target and the normalizer that is GAPDH .

$\Delta\Delta C = \Delta Ct\ (sample) - \Delta Ct\ (reference)$

Comparative expression level = $2^{-\Delta\Delta Ct}$

RESULTS

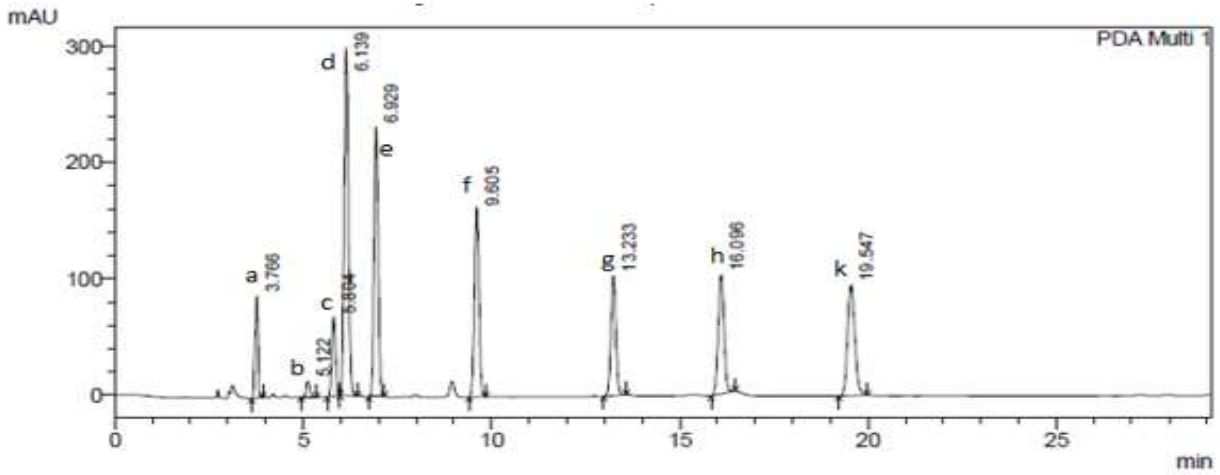
➢ *Phenolic contents of S. fruticosa*



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

RESULTS

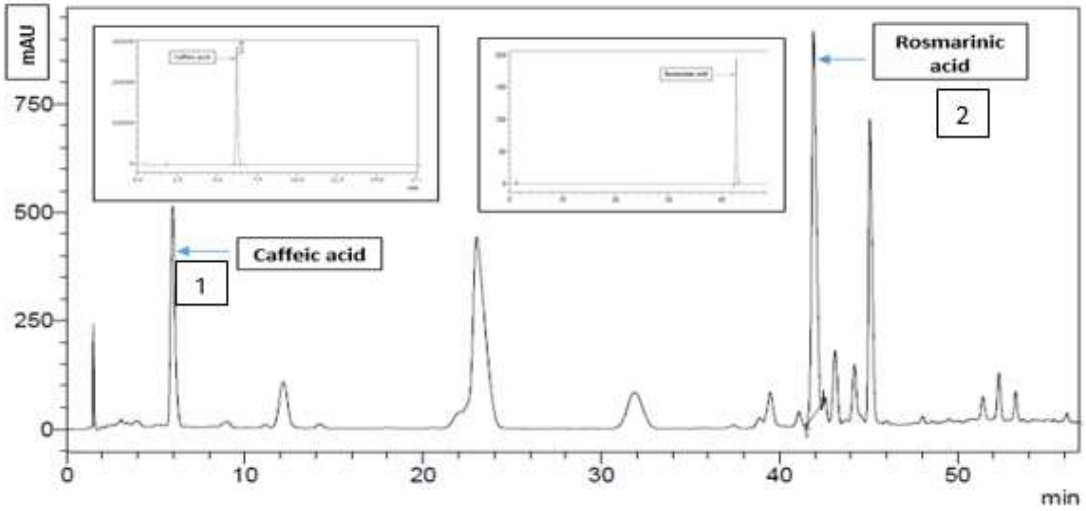
Phenolic contents of *S. fruticosa*



HPLC chromatogram of phenolic standarts 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.

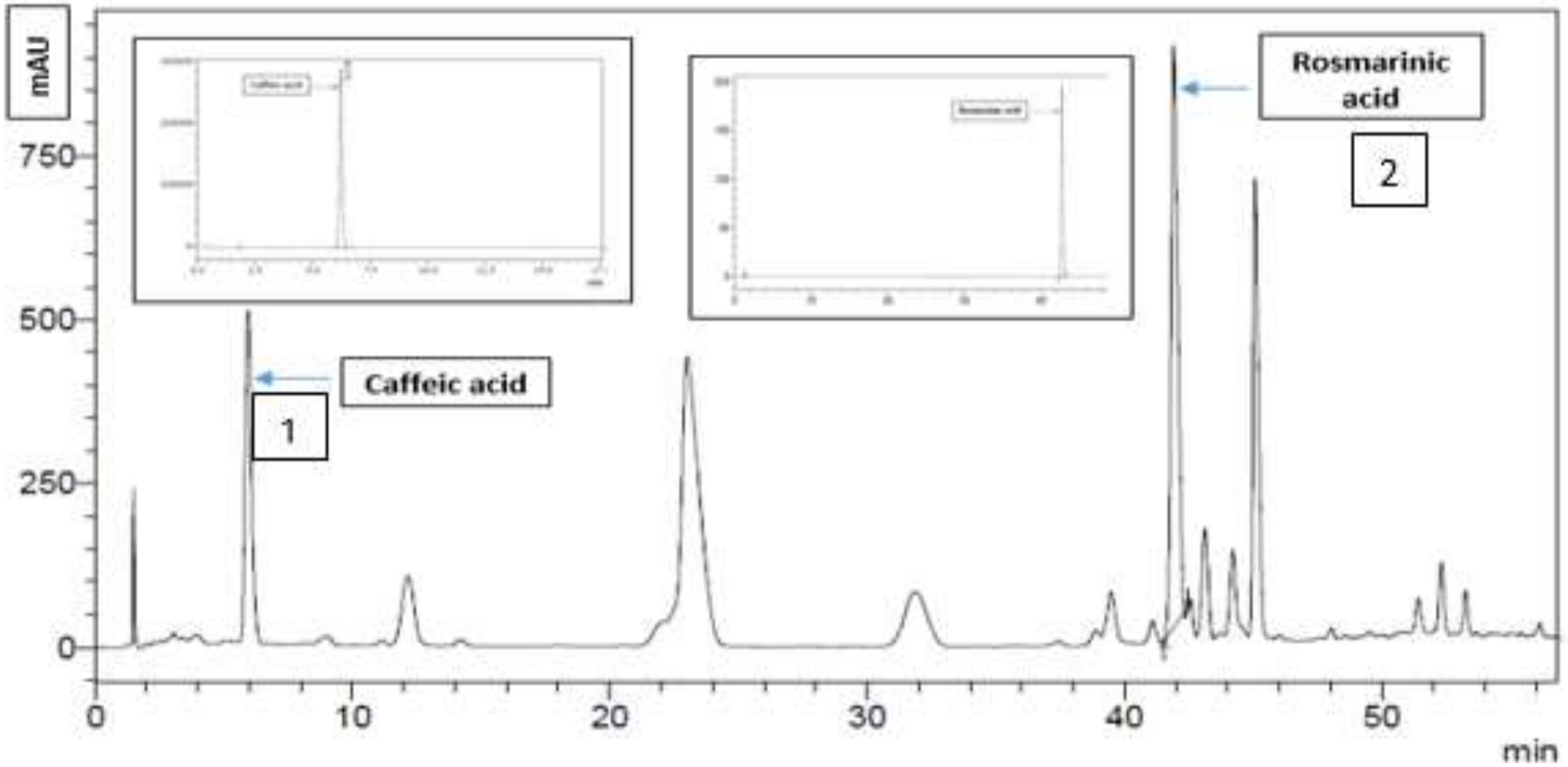
MATERIALS & METHODS

Salvia fruticoso M.

Phenolic Profiles of the plant by RP-HPLC

Extraction of Plants

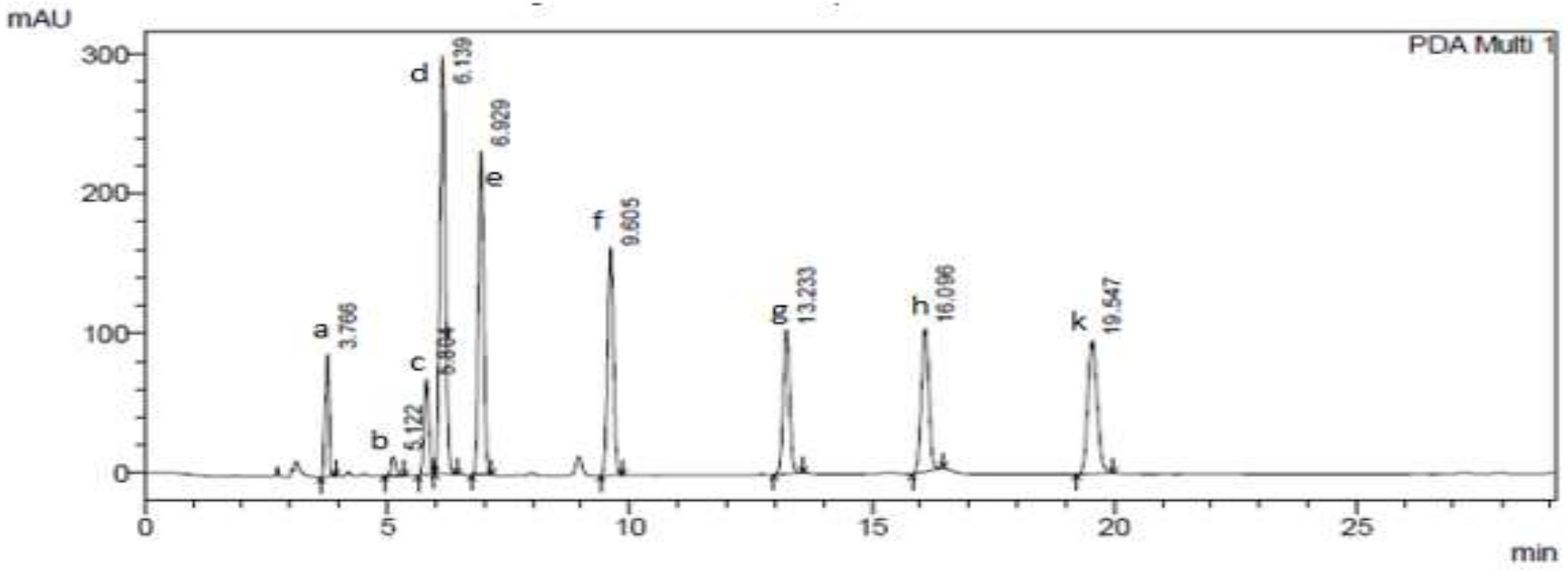
Determination of TPC & Antioxidant Activities



MATERIALS & METHODS

Salvia fruticoso M.

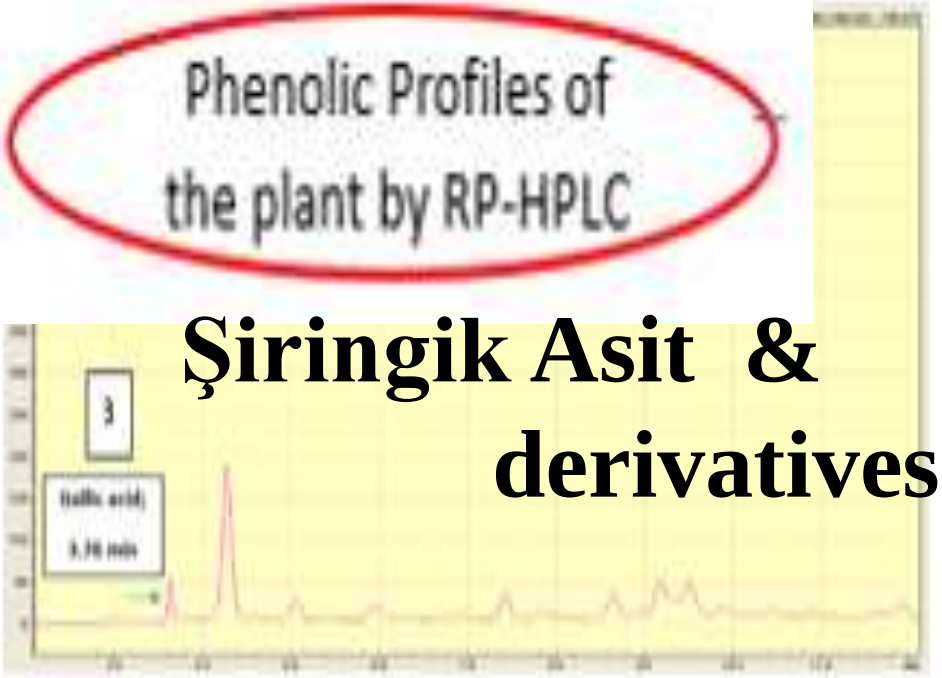
Phenolic Profiles of the plant by RP-HPLC ← **Extraction of Plants** → **Determination of TPC & Anticoxidant Activities**



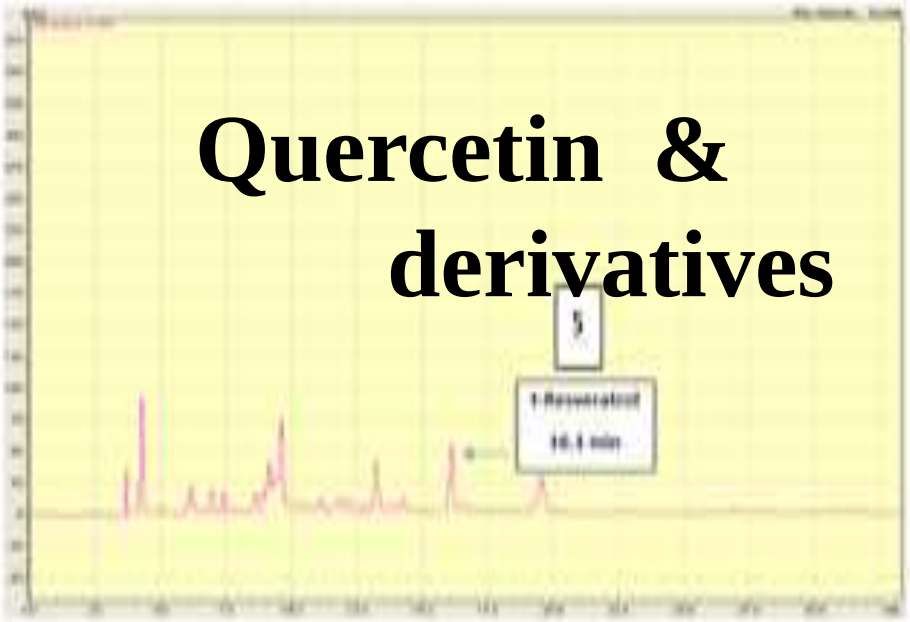
(a; gallic acid, b; (+)catechin, c; (-) epicatechin, d; epigallocatechin gallate, e; syringic acid, f; p-coumaric acid, g; rosmarinic acid, h; t-resveratrol, k; quercetin).

Phenolic Profiles of the plant by RP-HPLC

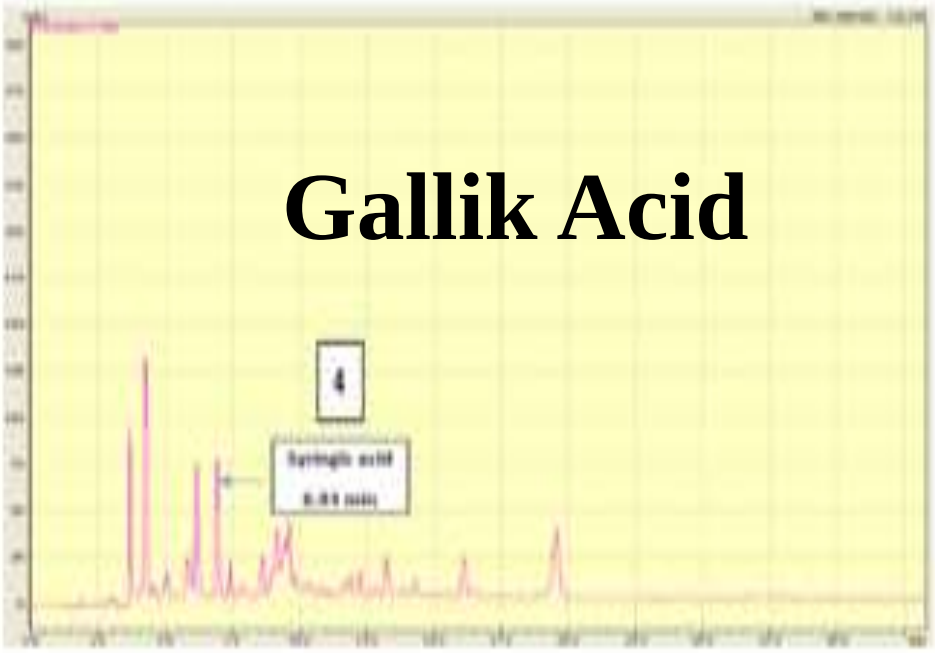
Şiringik Asit & derivatives



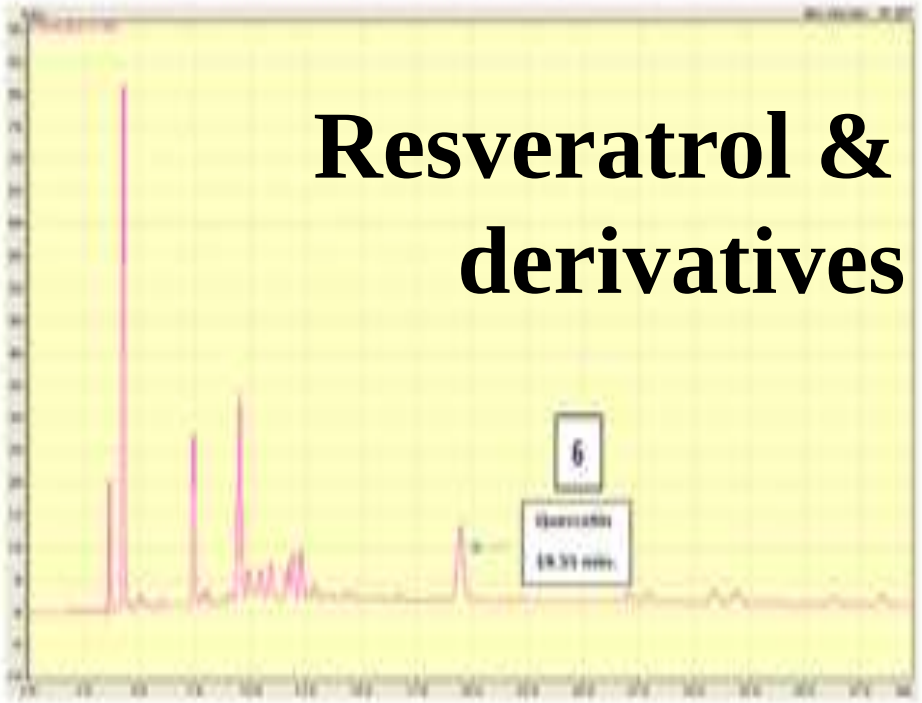
Quercetin & derivatives

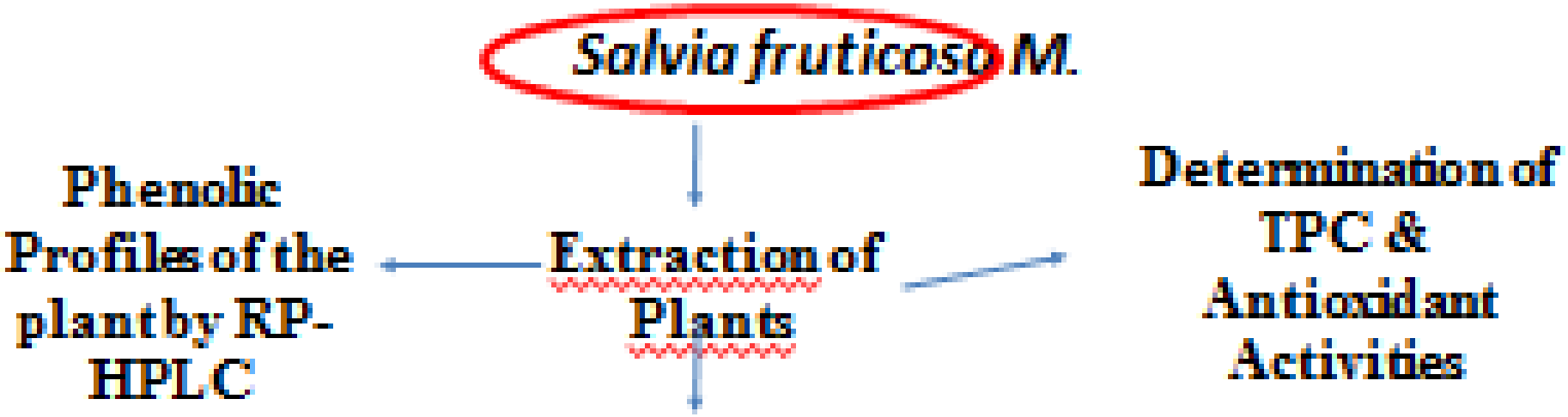


Gallik Acid



Resveratrol & derivatives





- 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

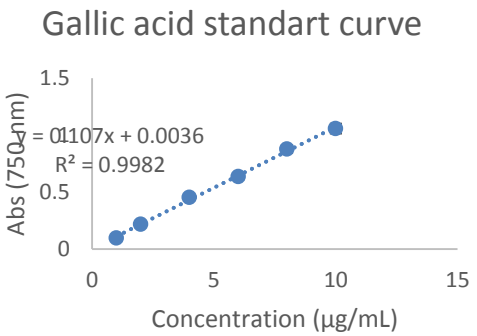
- *Salvia fruticosa extraction*

Extract	% Yield
Water extract of *S. fruticosa*	16.5 ± 1.5

RESULTS

- TPC of *Salvia fruticosa*

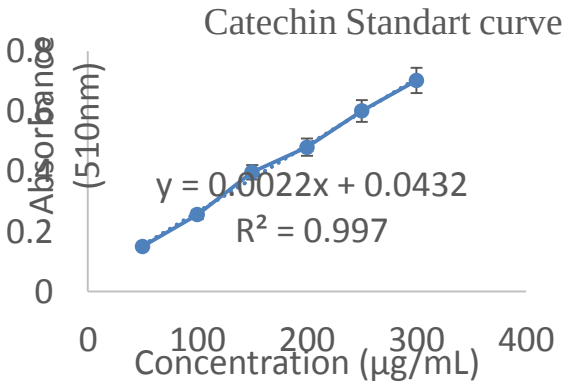
Concentration (mg/mL)	Total Phenol Content µg GAE / mg dry extract + SD
0.25	178.84 ± 2.15
0.5	171.66 ± 2.61
Average	175.2 ± 5.07



RESULTS

- TFC of *Salvia fruticosa*

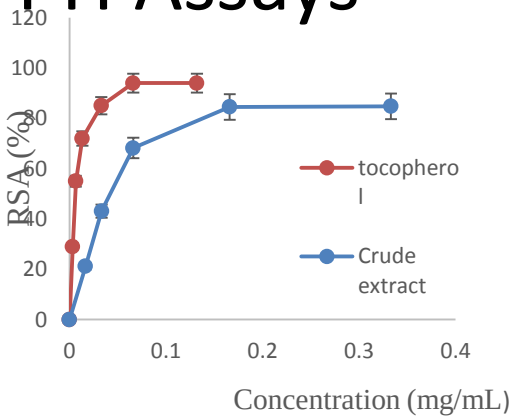
Concentration (mg/mL)	Total Flavonoid Content (µg CE / mg dry extract + SD)
0.25	105.4 ± 3.12
0.5	108.9 ± 2.61
Average	107.14 ± 2.47



RESULTS

- Free radical scavenging capacity of *S. futicosa* by DPPH Assays

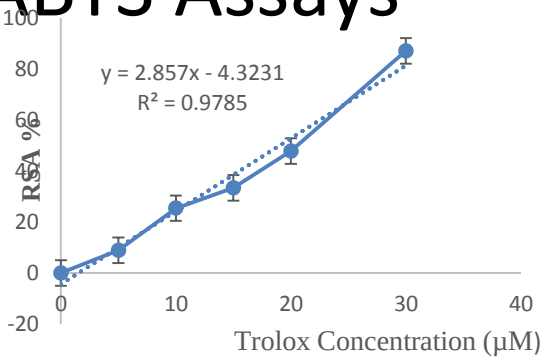
Sample	Antioxidant Activity	Maximum RSA (%)
	IC50 µg/ mL ± SD	RSA % ± SD
S. futicosa extract	34.9 ± 0.009	84,8 ± 0.85
α-tocopherol	17.1 ± 0.014	94 ± 1.02



RESULTS

- Free radical scavenging capacity
  y ABTS Assays

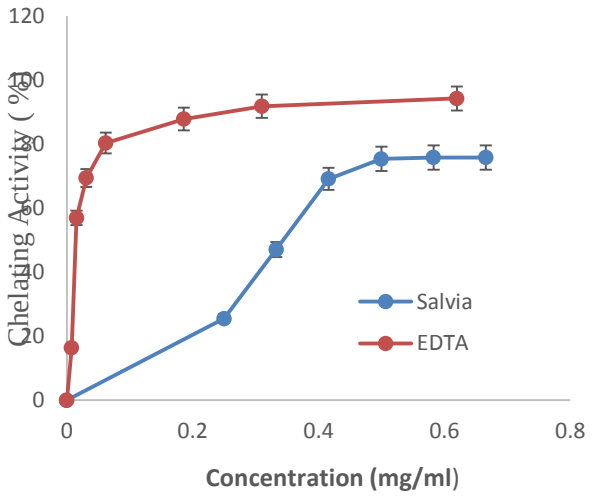
Concentration (µg/mL)	TEAC value (mmol TE/ g extract ± SD)
2,5	1.23 ± 0.0043
5	1.31 ± 0.0052
10	1.15 ± 0.0046
Average	1.23 ± 0.1900



RESULTS

- Metal chelating activity of *S.*

Sample	Fe^{+2} Chelating Activity	Maximum RSA (%)
	IC50 mg/ mL ± SD	RSA % ± SD
S. futicosa extract	0.43 ± 0.0035	75.8 ± 0.92
EDTA	0.107 ± 0.002	94.3 ± 1.08

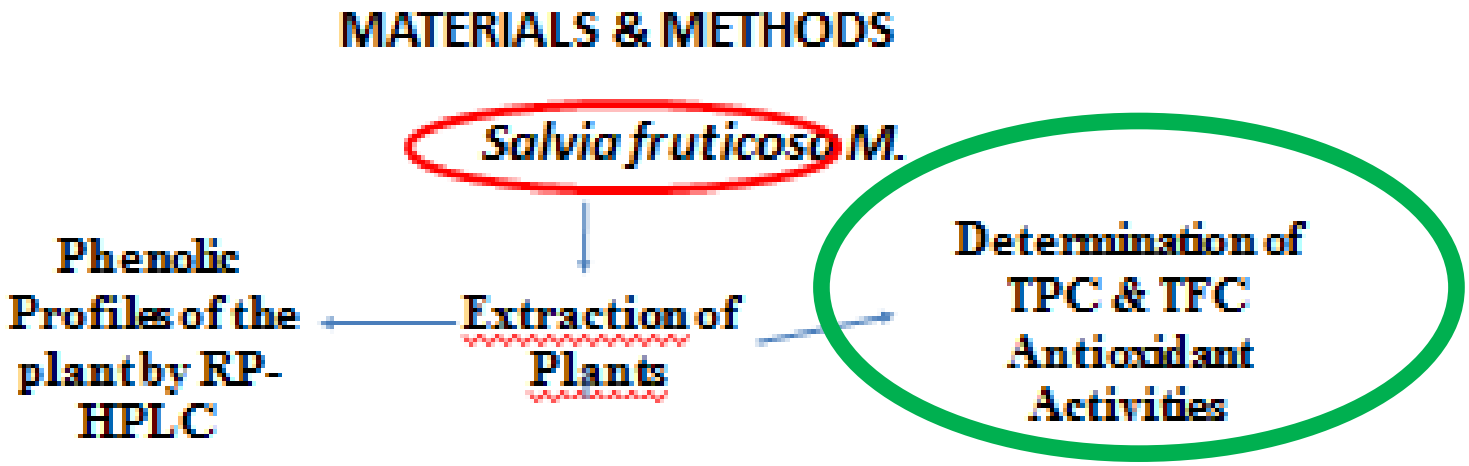


Phenolic Profiles

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

Phenolic compounds	mg/g dry extract ± SD
Rosmarinic acid	59.3 ± 3.82
Caffeic acid	15.16 ± 0.4
Gallic acid	0.56 ± 0.024
Syringic acid	0.74 ± 0.0104
Trans resveratrol	0.17 ± 0.06
Quercetin	0.73 ± 0.04

Total Phenolics & Flavonoids Profile

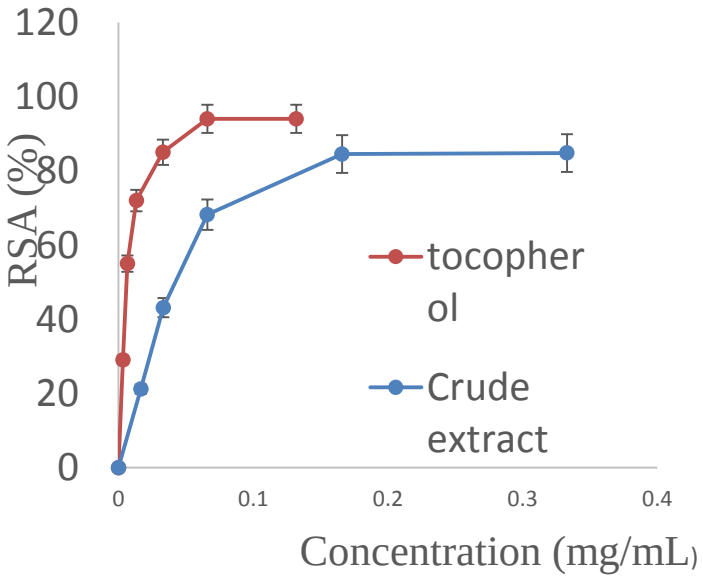


Concentration (mg/mL)	Total Phenol Content µg GAE / mg dry extract + SD	Total Flavonoid Content (µg CE / mg dry extract + SD)
0.25	178.84 ± 2.15	105.4 ± 3.12
0.5	171.66 ± 2.61	108.9 ± 2.61
Average	175.2 ± 5.07	107.14 ± 2.47

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

Sample	Antioxidant Activity	Maximum RSA (%)
	IC50 µg/mL ± SD	RSA % ± SD
S. futicosa extract	34.9 ± 0.009	84,8 ± 0.85
α-tocopherol	17.1 ± 0.014	94 ± 1.02

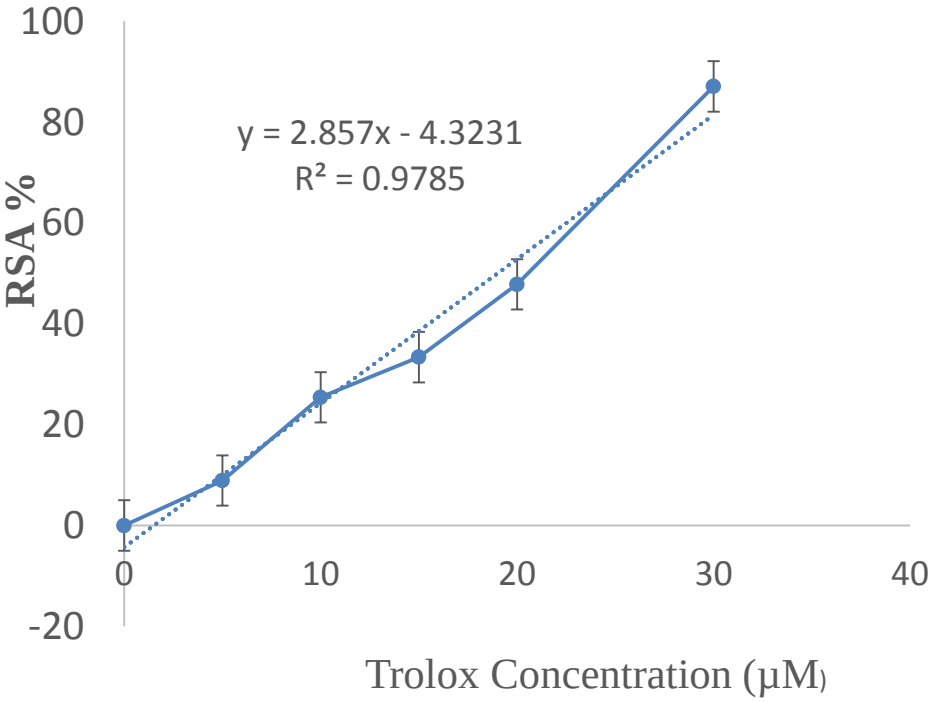
RESULTS

Salvia fruticosa M.

Phenolic Profiles of the plant by RP-HPLC

Extraction of Plants

Determination of TPC and TFC & Antioxidant Activities



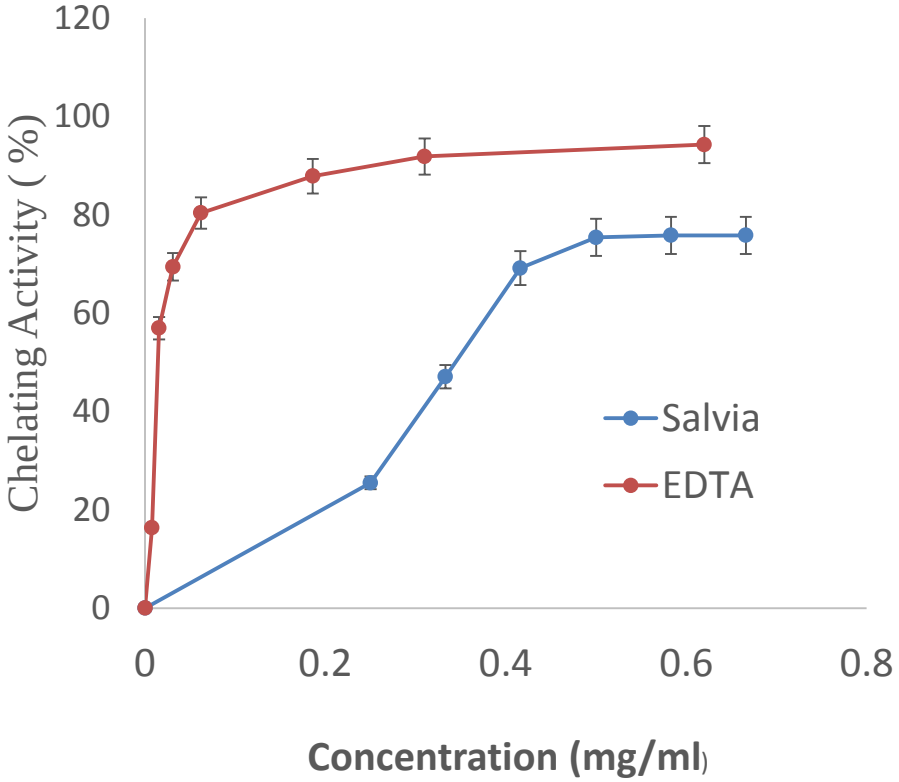
Antioxidant Activity by ABTS Method

Concentration (µg/mL)	TEAC value (mmol TE/ g extract ± SD)
2,5	1.23 ± 0.0043
5	1.31 ± 0.0052
10	1.15 ± 0.0046
Average	1.23 ± 0.1900

RESULTS

Salvia fruticosa M.

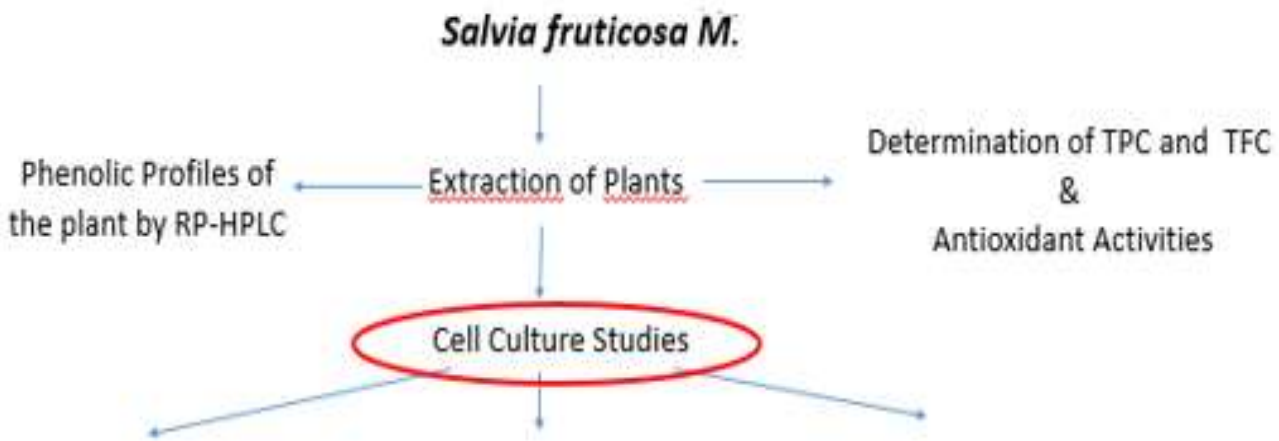
Phenolic Profiles of the plant by RP-HPLC ← Extraction of Plants → Determination of TPC and TFC & Antioxidant Activities

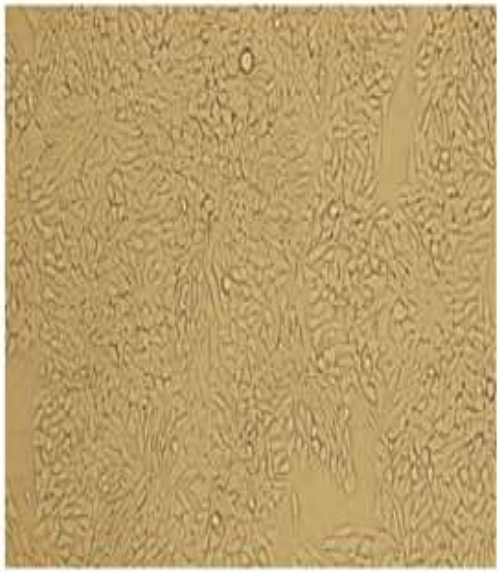


Antioxidant Activity by Metal Chelating Activity

Sample	Fe^{+2} Chelating Activity	Maximum RSA (%)
	IC50 mg/ mL ± SD	RSA % ± SD
S. futicosa extract	0.43 ± 0.0035	75.8 ± 0.92
EDTA	0.107 ± 0.002	94.3 ± 1.08

MATERIALS & METHODS





Cell Culture Studies

- HT-29 Cell line : Colorectal adenocarcinoma
- Type : Adherent, epithelial like
- Origin : female, 44 years
- Medium in cell culture: McCoys'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% CO_2.

MATERIALS & METHODS

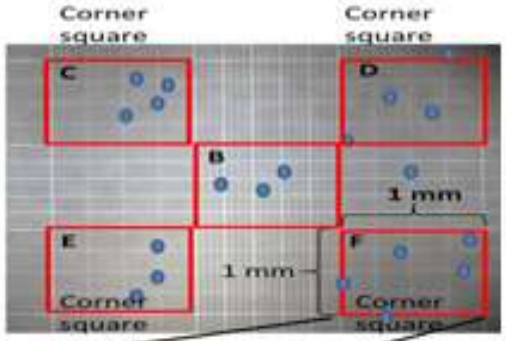
Salvia fruticosa M.

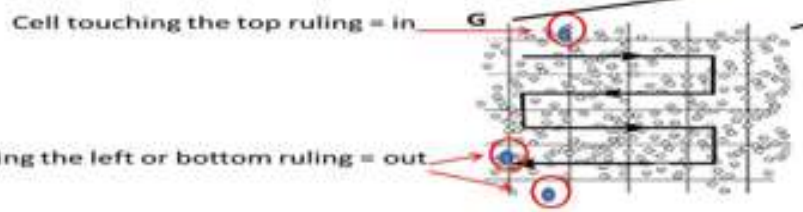
Phenolic Profiles of the plant by RP-HPLC ← Extraction of Plants → Determination of TPC and TFC & Antioxidant Activities

Extraction of Plants → Cell Culture Studies

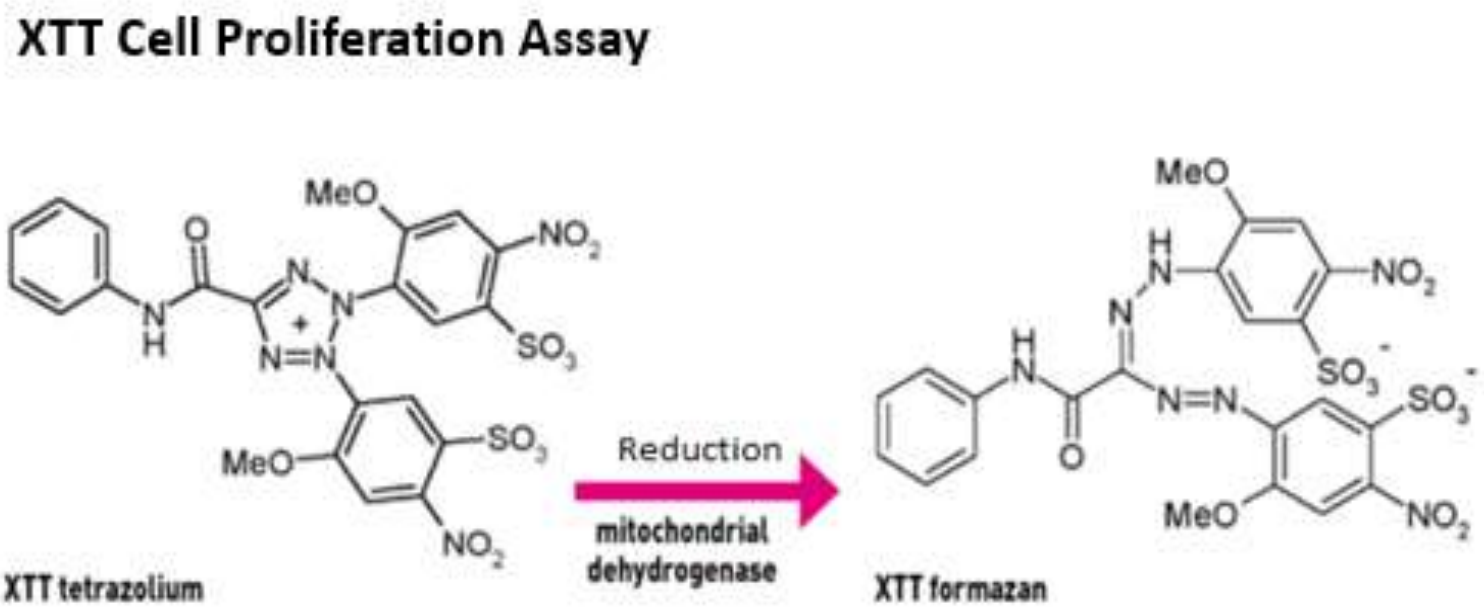
➢ **Viable Cell Counting**





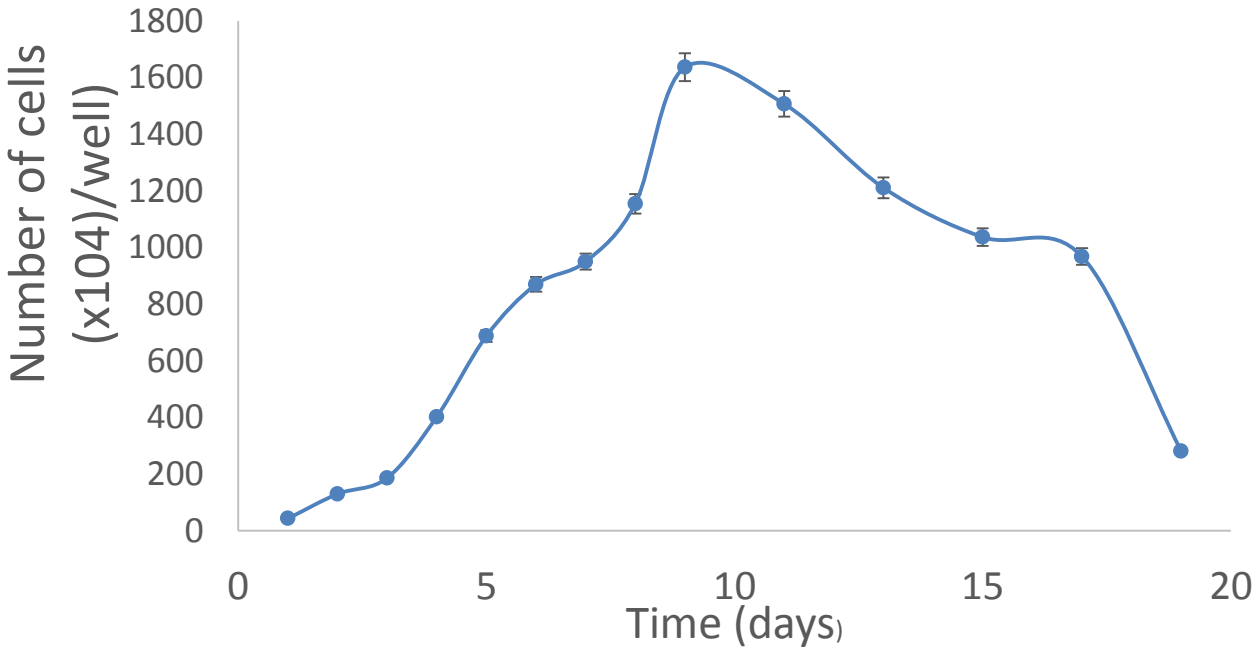


Cytotoxicity determination on HT-29 colon cancer cells



RESULTS

HT-29 Growth curve



RESULTS

- Cytotoxicity *of S. fruticosa* on

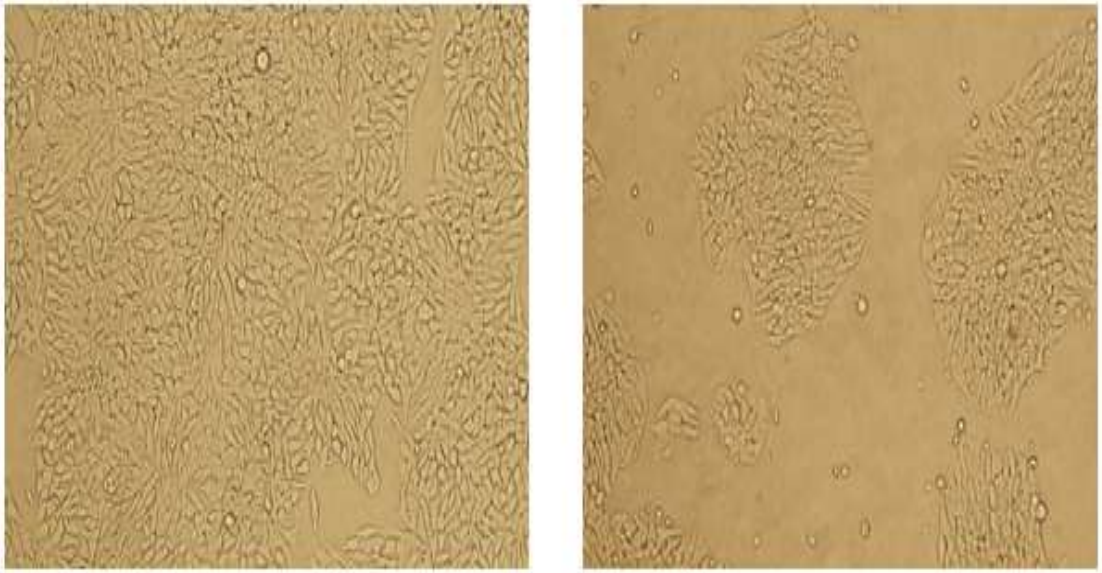




HT-29 cells. Left: 48 hours 0,1 % DMSO treated control. Right: 48 hours IC_{50} concentration (0.174 mg/mL) *S. fruticosa* treated.

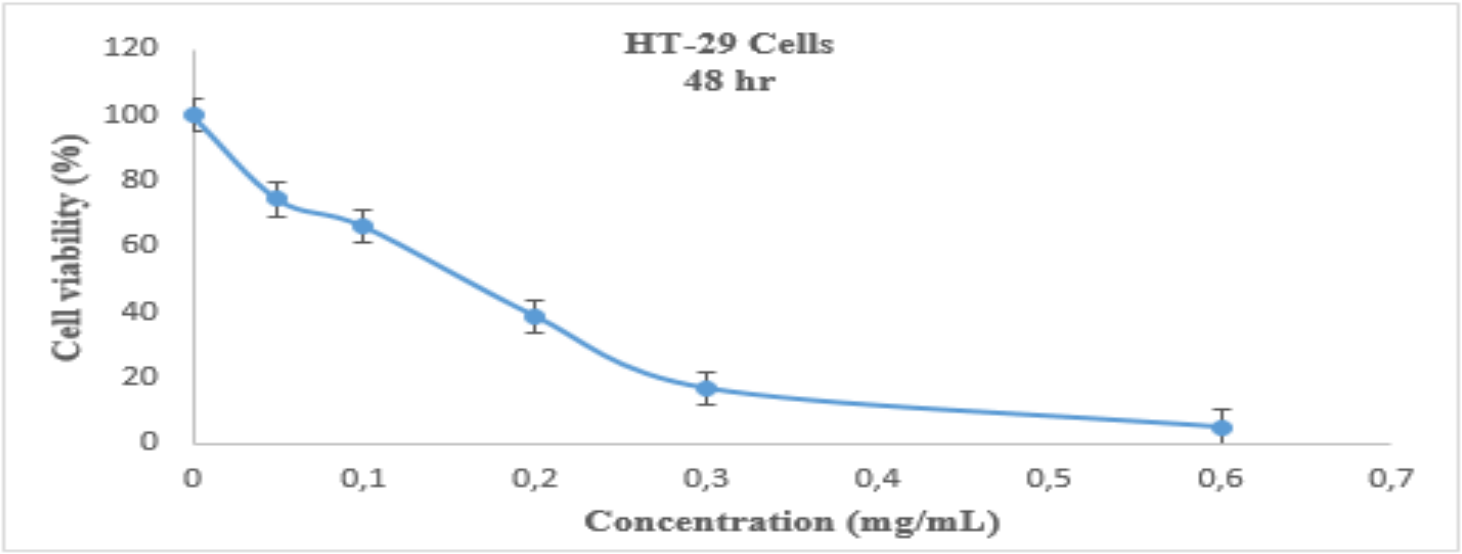
RESULTS

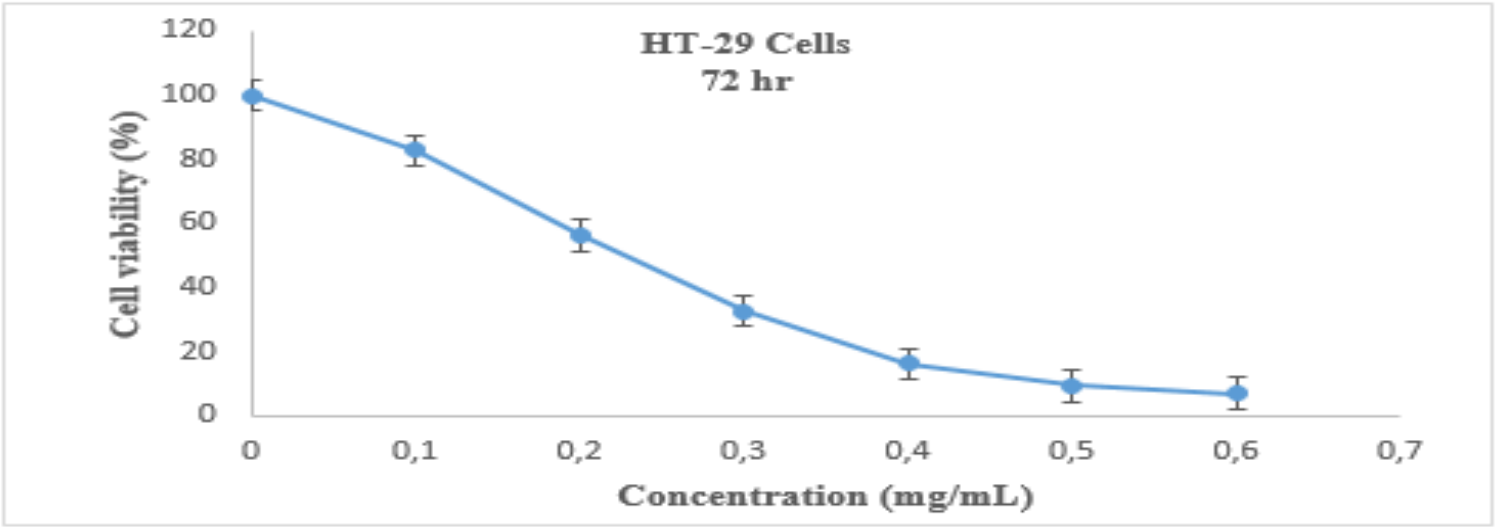
- Cytotoxicity *of S. fruticosa* on HT-29 Cells



HT-29 cells. Left: 72 hours 0,1 % DMSO treated control. Right: 72 hours IC_{50} concentration (0.228 mg/mL) *S. fruticosa* treated.

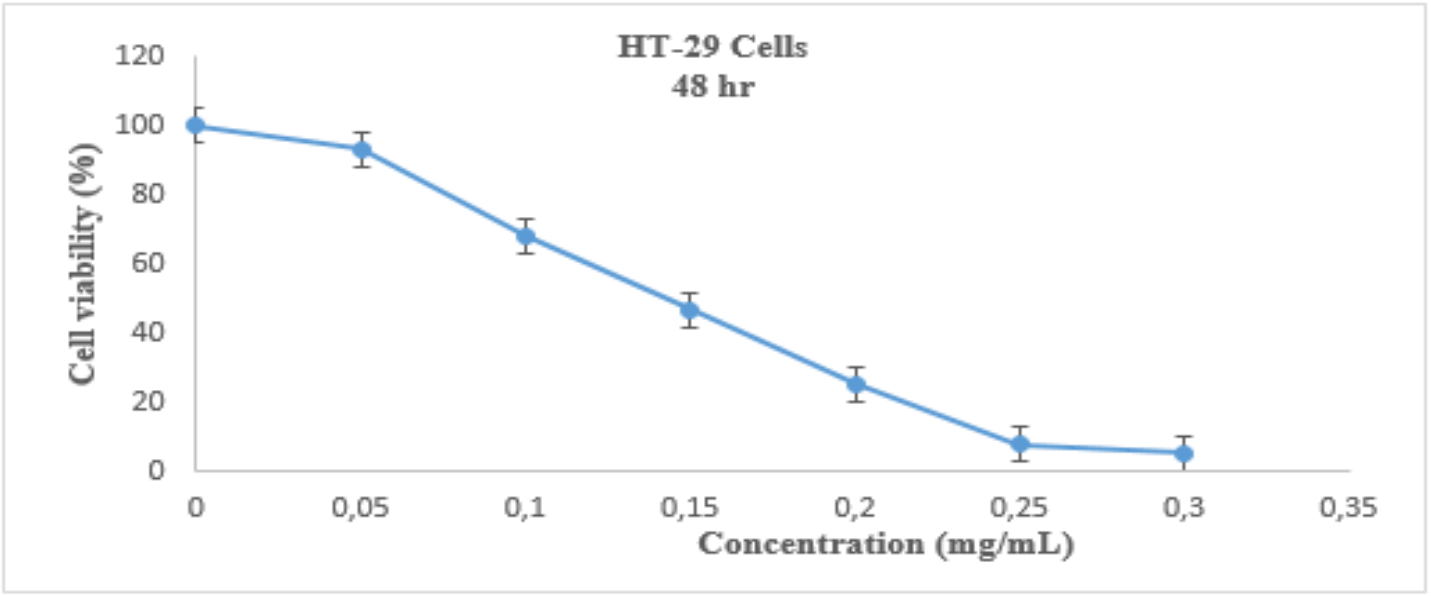
- Cytotoxicity *of S. fruticosa* on HT-29 Cells

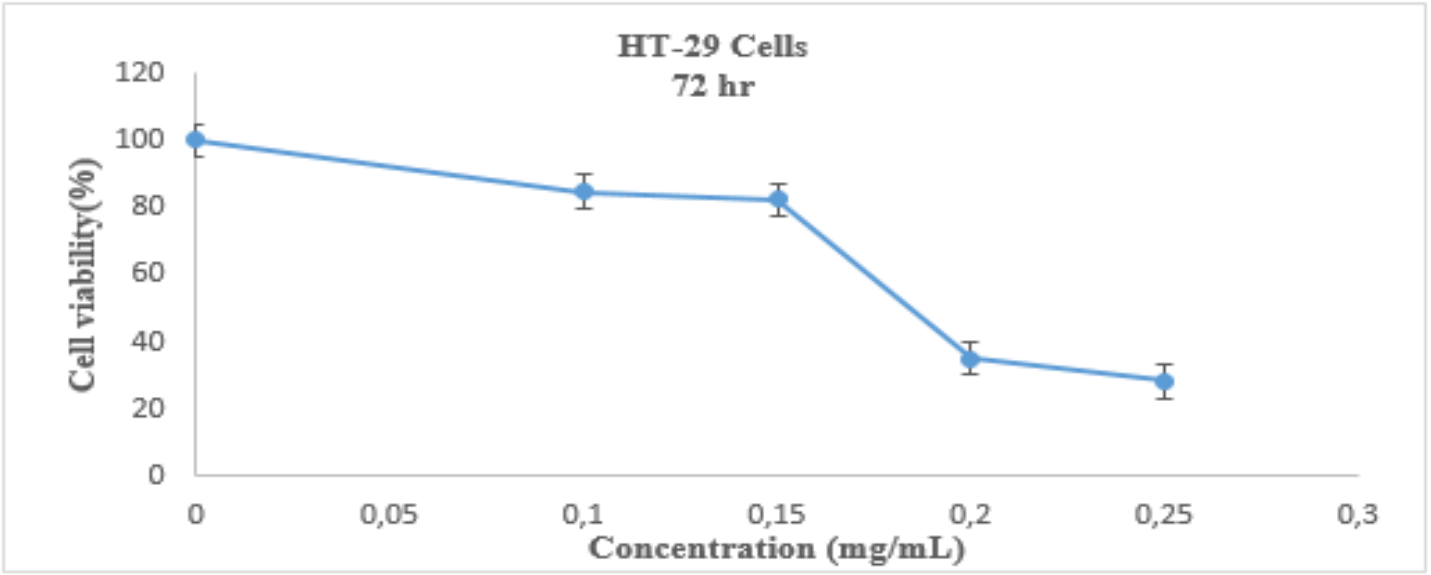




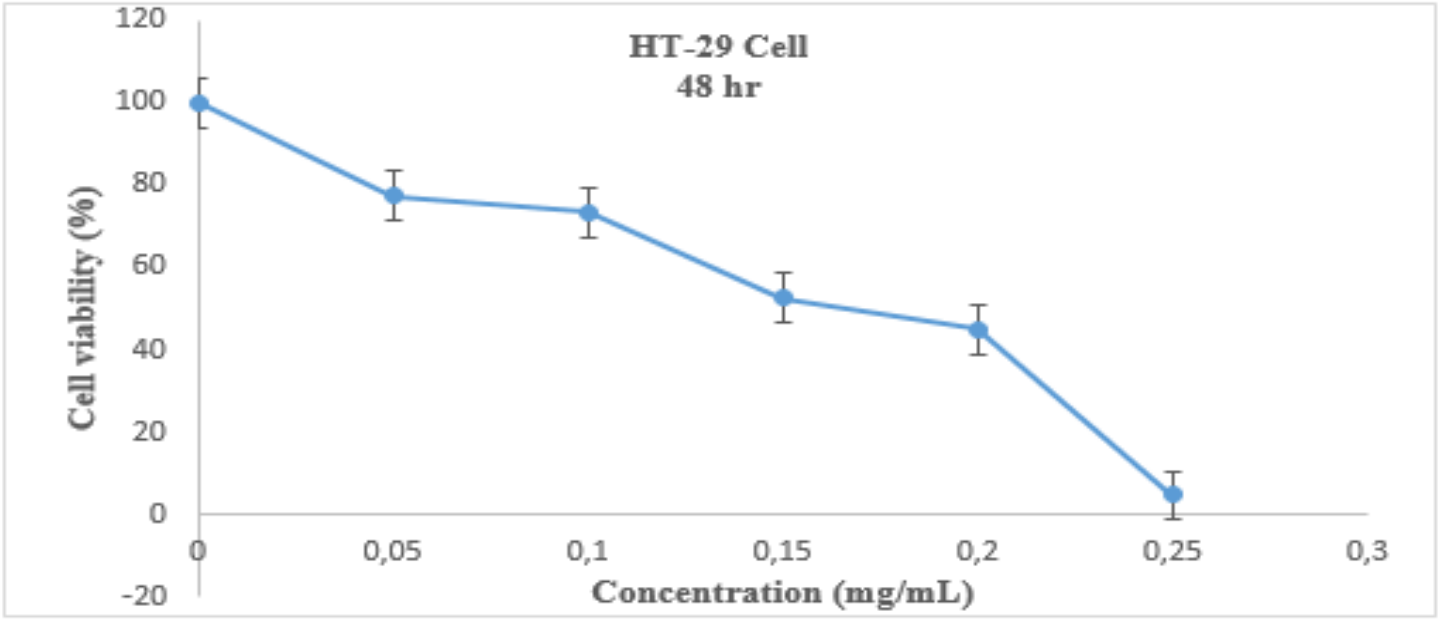
Viabilities of HT-29 cells in response to dose and time dependent treatment of *S. futicosa*

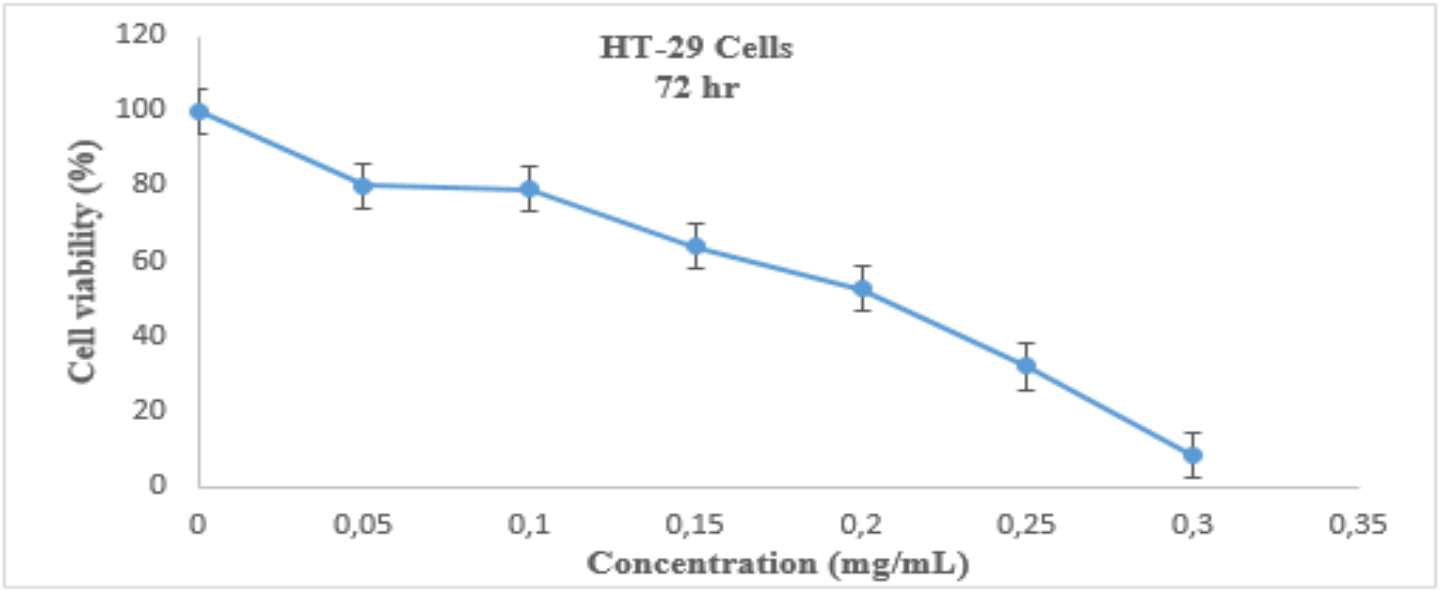
➢ Cytotoxicity of rosmarinic acid on HT-29 Cells





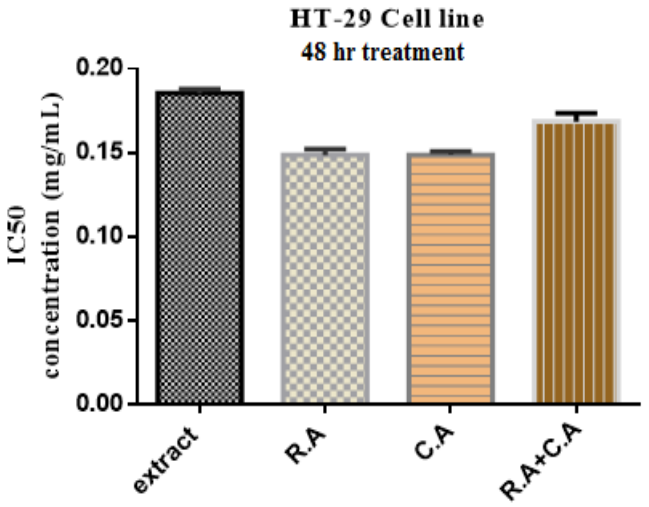
➢ Cytotoxicity of caffeic acid on HT-29 Cells

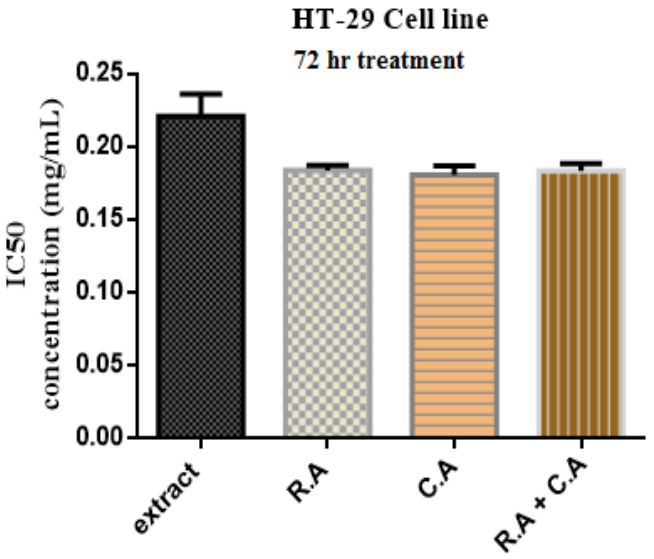




➢ Cytotoxicity of S. fruticosa, rosmarinic acid and caffeic acid on HT-29 Cells

IC_{50} (mg/mL) ± S.D		
Agents	Incubation	
	48 hr	72 hr
Crude extract	0.185 ± 0.0025	0.229 ± 0.0148
Rosmarinic acid (R.A)	0.148 ± 0.0036	0.184 ± 0.0033
Caffeic acid (C.A)	0.149 ± 0.0021	0.181 ± 0.0063
R.A + C.A	0.169 ± 0.005	0.184 ± 0.0076

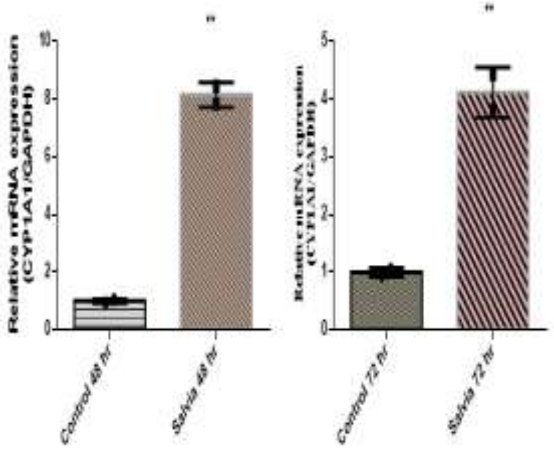


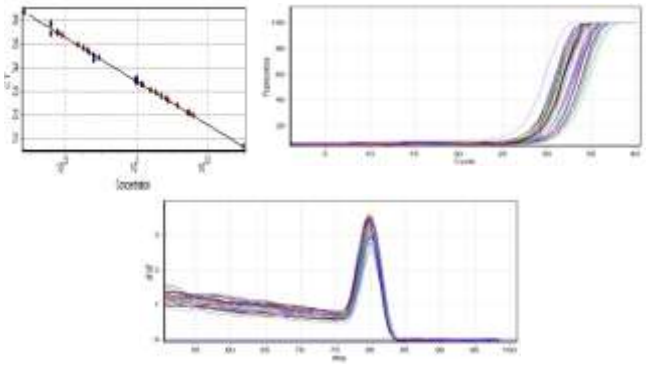


RESULTS

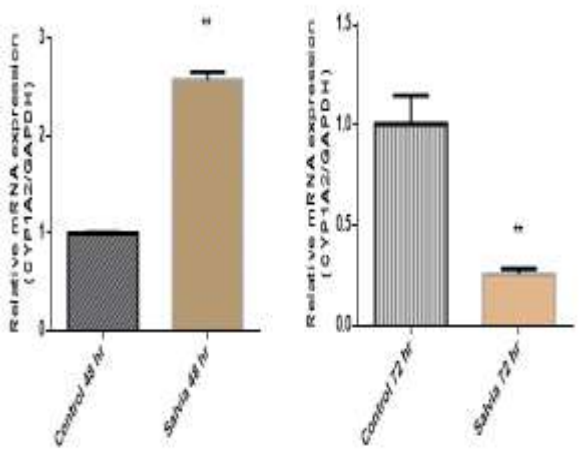
EFFECTS OF *Salvia fruticosa* on CYP1A1, CYP1A2, CYP2E1 AND CYP3A4 mRNA EXP

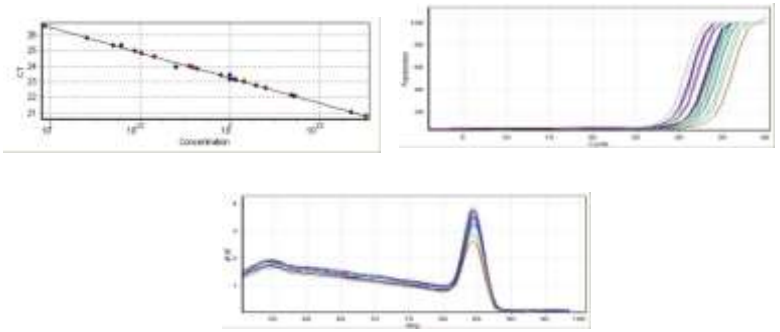
CYP1A1





CYP1A2

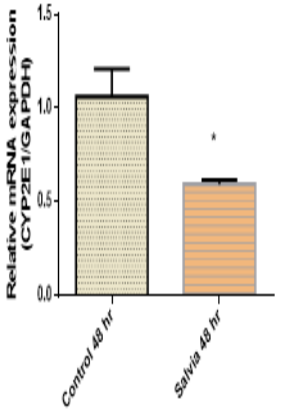


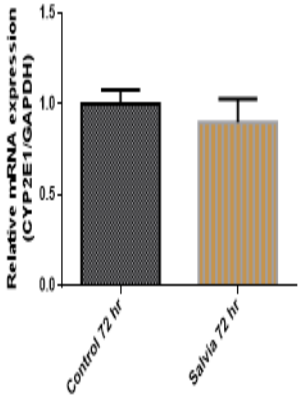


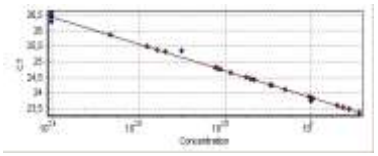
RESULTS

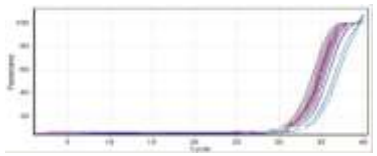
EFFECTS OF *Salvia fruticosa* on CYP1A1, CYP1A2, CYP2E1 AND CYP3A4 mRNA EXPRESSION

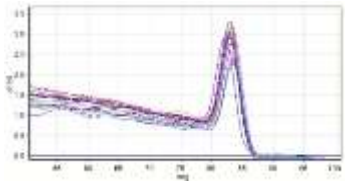
CYP2E1



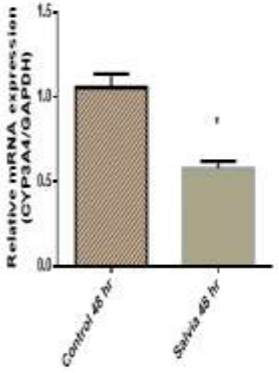


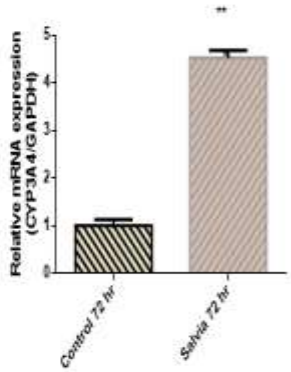


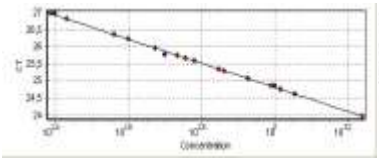


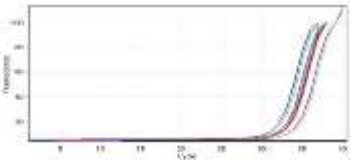


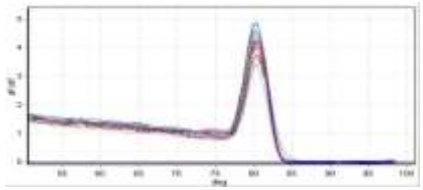
CYP3A4











RESUL
TS

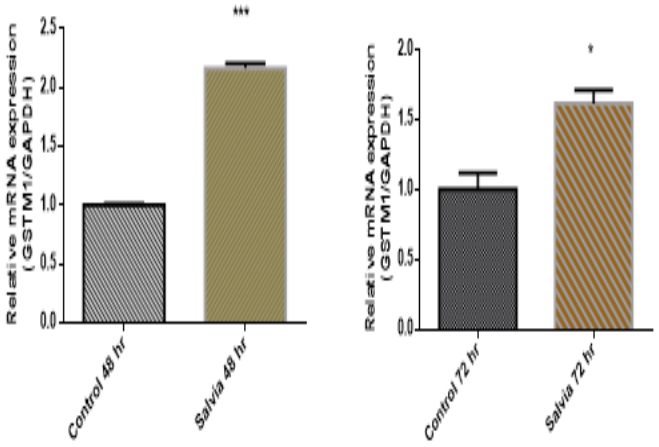
FOLD CHANGES OF CYP1A1 CYP1A1, CYP1A2, CYP2E1 AND CYP3A4 mRNA EXPRI

HT-29 Cell line		
Genes	48 hr incubation	72 hr incubation
CYP1A1	8.14 ↑	4.12↑
CYP1A2	2.57 ↑	3.89 ↓
CYP2E1	1.72 ↓	NS
CYP3A4	1.75 ↓	4.02 ↑

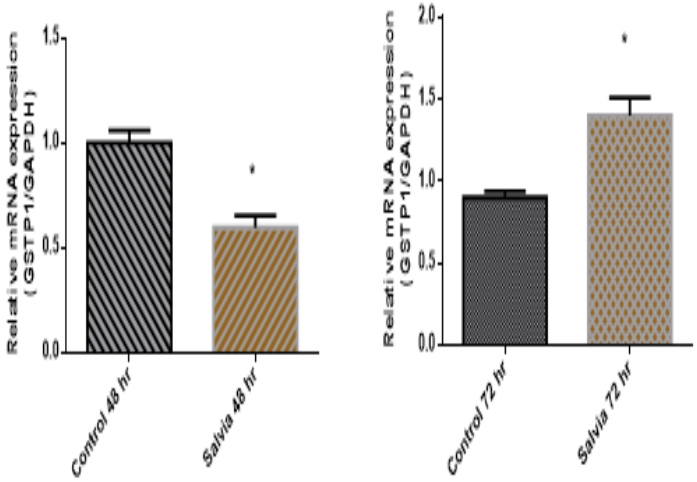
RESULTS

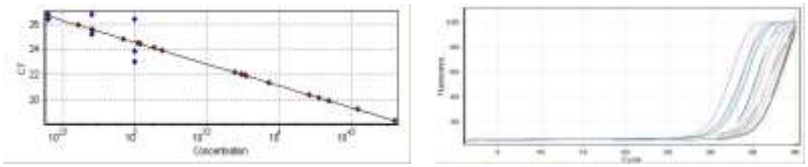
EFFECTS OF *Salvia fruticosa* on GST M1, GST P1, GPx4 AND CATALASE mRNA EXPRESSION

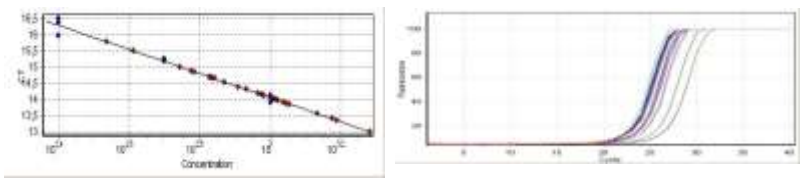
GST M1

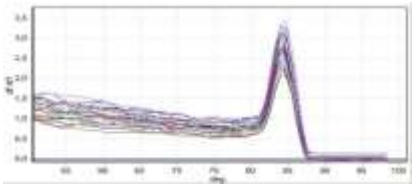


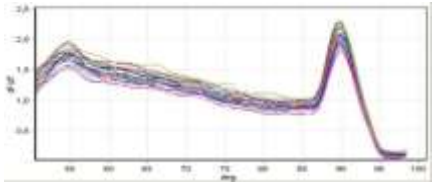
GSTP1







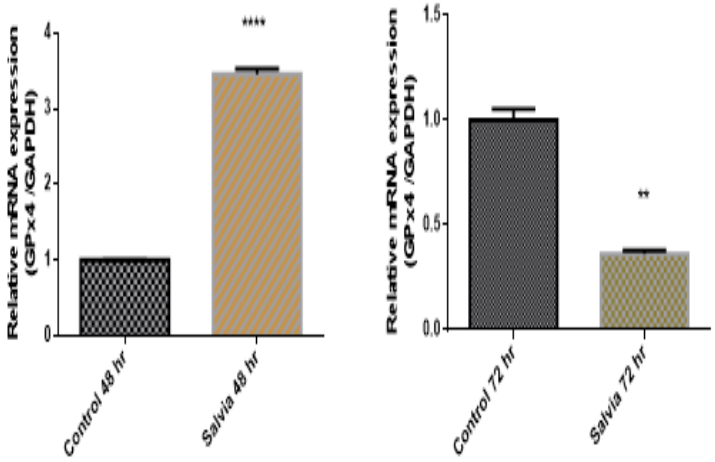


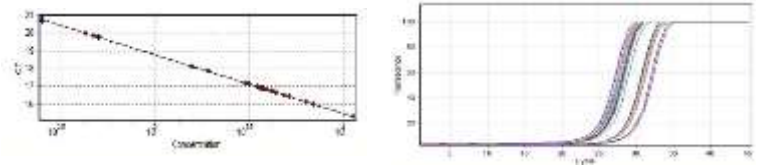


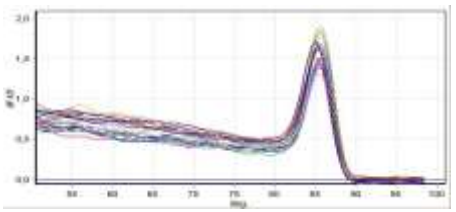
RESULTS

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

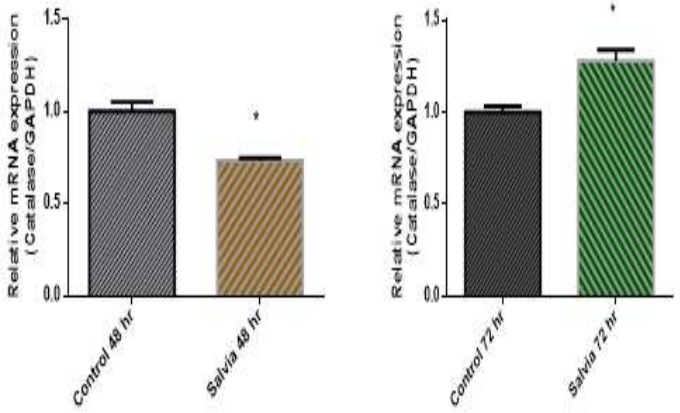
GPx4

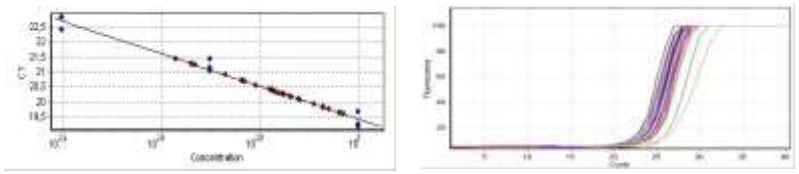


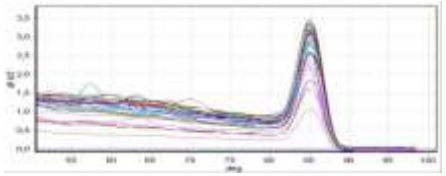




CATALASE





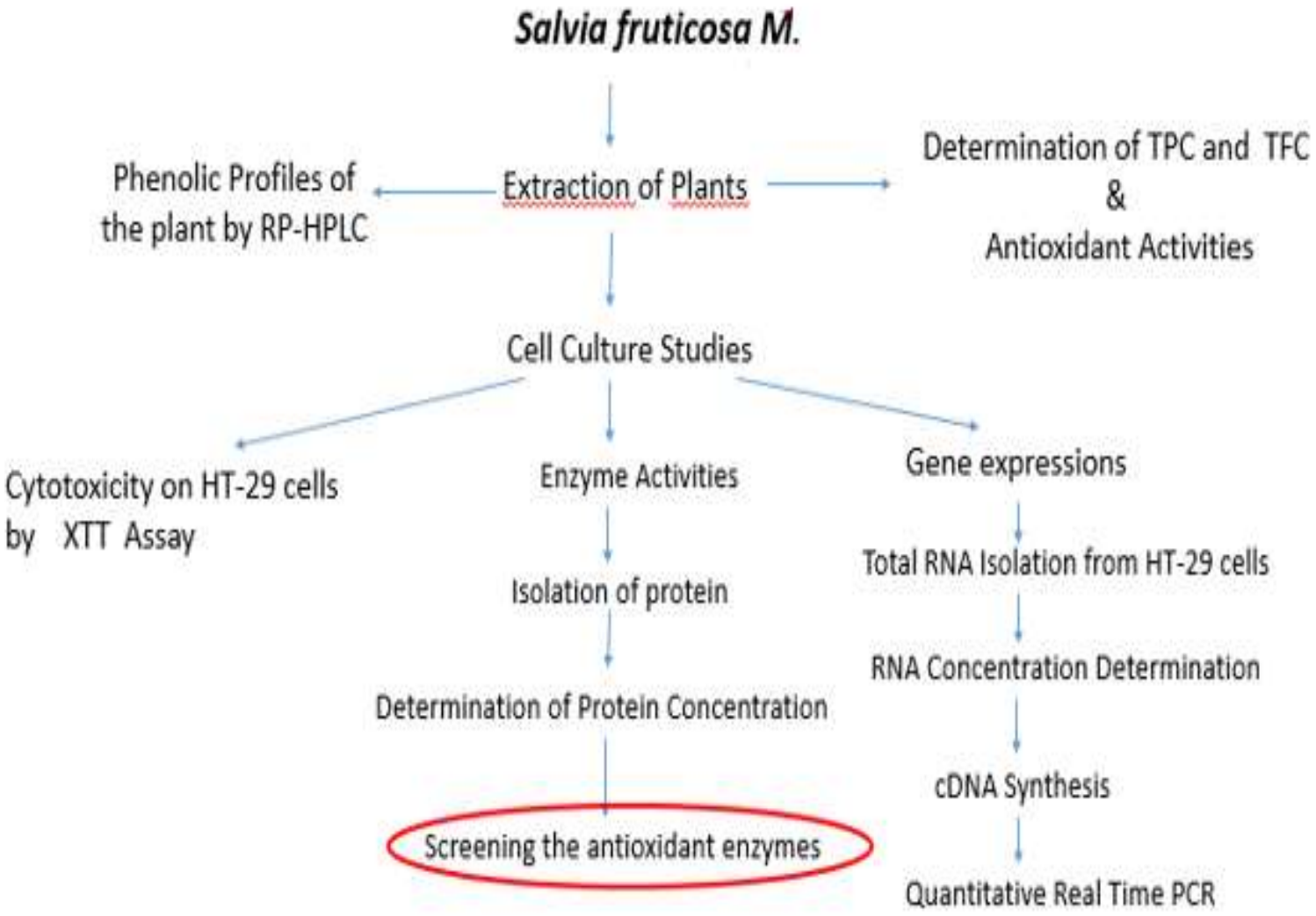


RESULTS

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

HT-29 Cell line		
Genes	48 hr incubation	72 hr incubation
GST M1	2.17 ↑	1.62 ↑
GST P1	1.68 ↓	1.3 ↑
GPx4	3.5 ↑	2.17 ↓
CATALASE	1.37 ↓	1.28 ↑

RESULTS


Salvia fruticosa M.
Extraction of Plants
Phenolic Profiles of the plant by RP-HPLC
Determination of TPC and 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

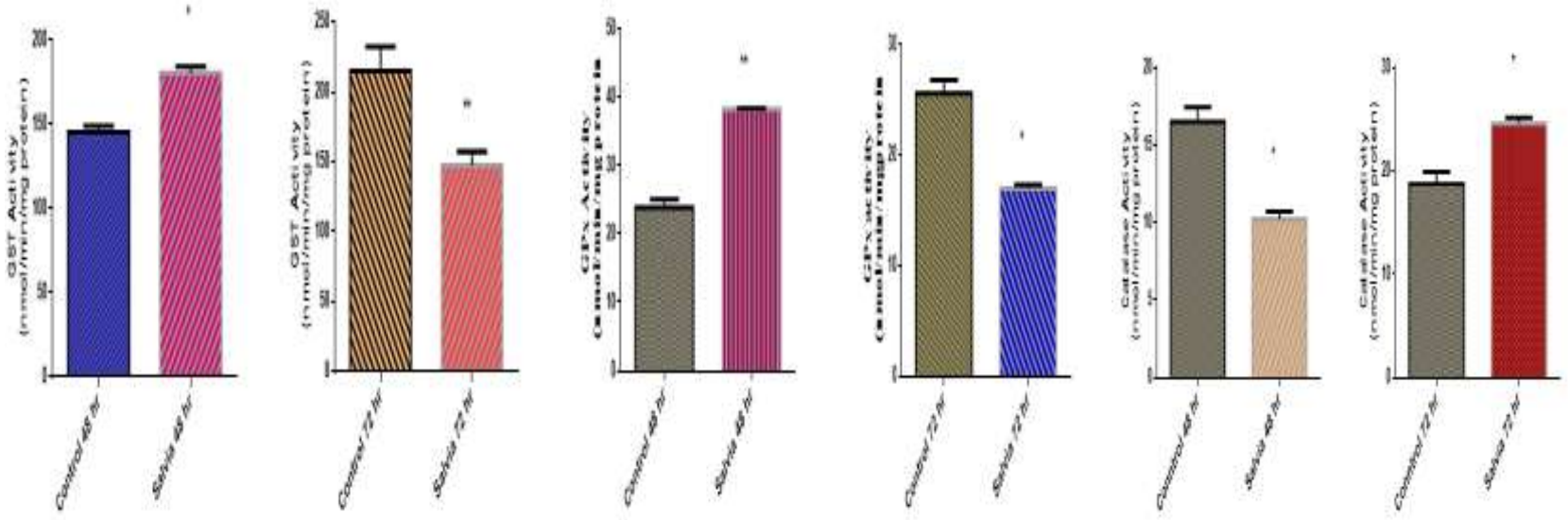
RESULTS

EFFECTS OF *Salvia fruticosa* on Total GSTs, GPx4 AND CATALASE ACTIVITIES

Total GSTs

GPx4

CATALASE



(Statistically significant, $p < 0.05$)

RESULTS

CHANGES OF Total GSTs, GPx4 AND CATALASE ACTIVITIES

HT-29 Cell line		
Enzymes	48 hr incubation	72 hr incubation
GSTs	↑	↓
GPx4	↑	↓
CATALASE	↓	↑

DISCUSSION

Avtioxidant Activity

Sample	Antioxidant Activity	Maximum RSA (%)
	IC50 µg/ mL ± SD	RSA % ± SD
S. futicosa extract	34.9 ± 0.009	84,8 ± 0.85
α-tocophero	17.1 ± 0.014	94 ± 1.02

Concentration (µg/mL)	TEAC value (mmol TE/ g extract ± SD)
2,5	1.23 ± 0.0043
5	1.31 ± 0.0052
10	1.15 ± 0.0046
Average	1.23 ± 0.1900

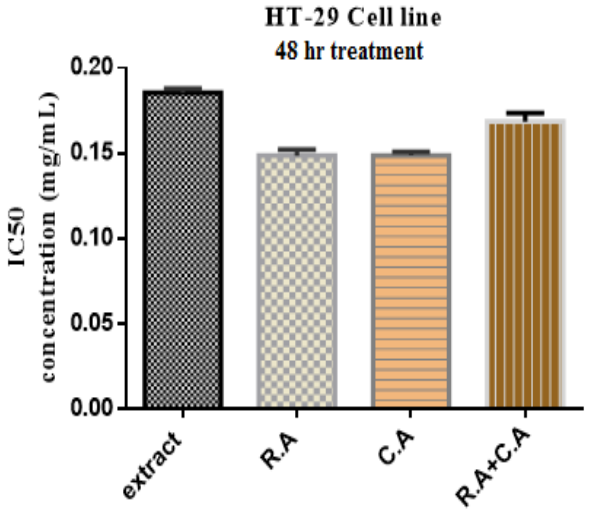
Sample	Fe^{+2} Chelating Activity	Maximum RSA (%)
	IC50 mg/ mL ± SD	RSA % ± SD
S. futicosa extract	0.43 ± 0.0035	75.8 ± 0.92
EDTA	0.107 ±	94.3 ± 1.08

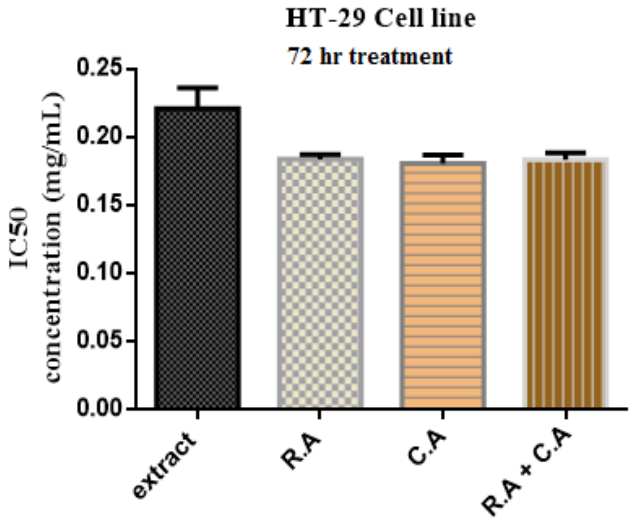
Concentration (mg/mL)	Ratio of TFC/TPC
0.25	0.59
0.5	0.63
Average	0.61

DISCUSSION

Cytotoxicity of *Salvia fruticosa*

IC_{50} (mg/mL) ± S.D		
Agents	Incubation	
	48 hr	72 hr
Crude extract	0.185 ± 0.0025	0.229 ± 0.0148
Rosmarinic acid (R.A)	0.148 ± 0.0036	0.184 ± 0.0033
Caffeic acid (C.A)	0.149 ± 0.0021	0.181 ± 0.0063
R.A + C.A	0.169 ± 0.005	0.184 ± 0.0076





DISCUSSION

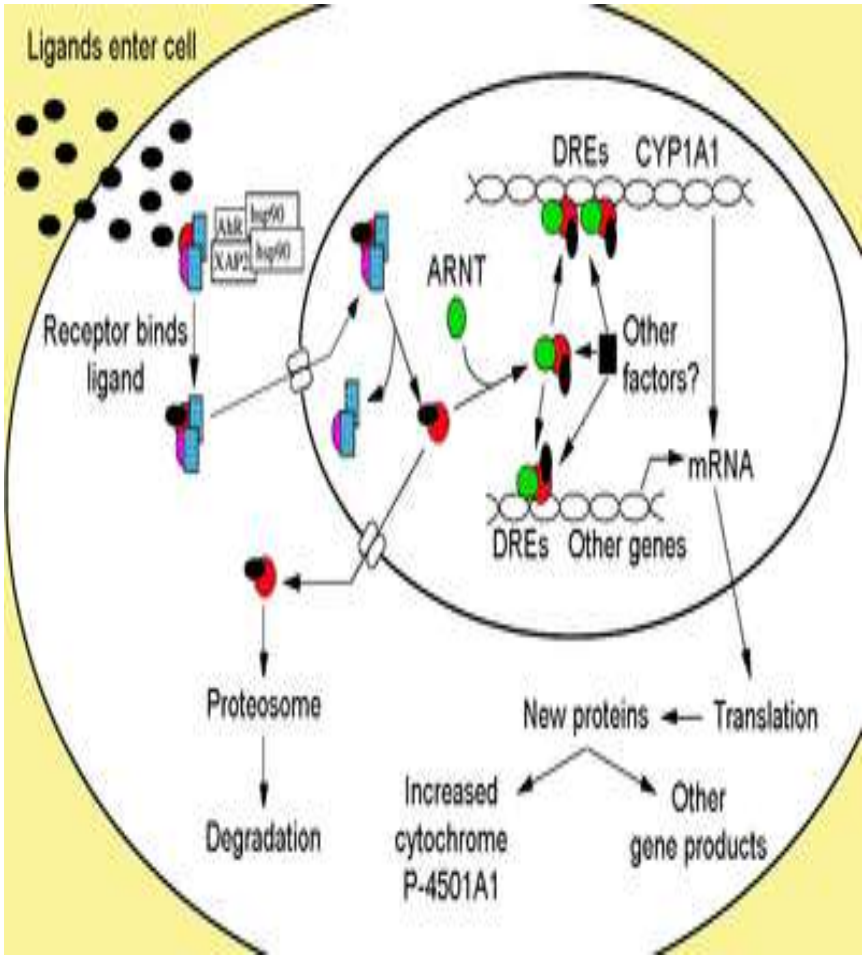
Analysis of *Salvia fruticosa on* mRNA Expression and Antioxidant Enzyme Results

HT-29 Cell line		
Genes	48 hr incubation	72 hr incubation
CYP1A1	8.14 ↑	4.12↑
CYP1A2	2.57 ↑	3.89 ↓
CYP2E1	1.72 ↓	NS
CYP3A4	1.75 ↓	4.02 ↑
GST M1	2.17 ↑	1.62 ↑
GST P1	1.68 ↓	1.3 ↑
GPx4	3.5 ↑	2.17 ↓
CATALASE	1.37 ↓	1.28 ↑

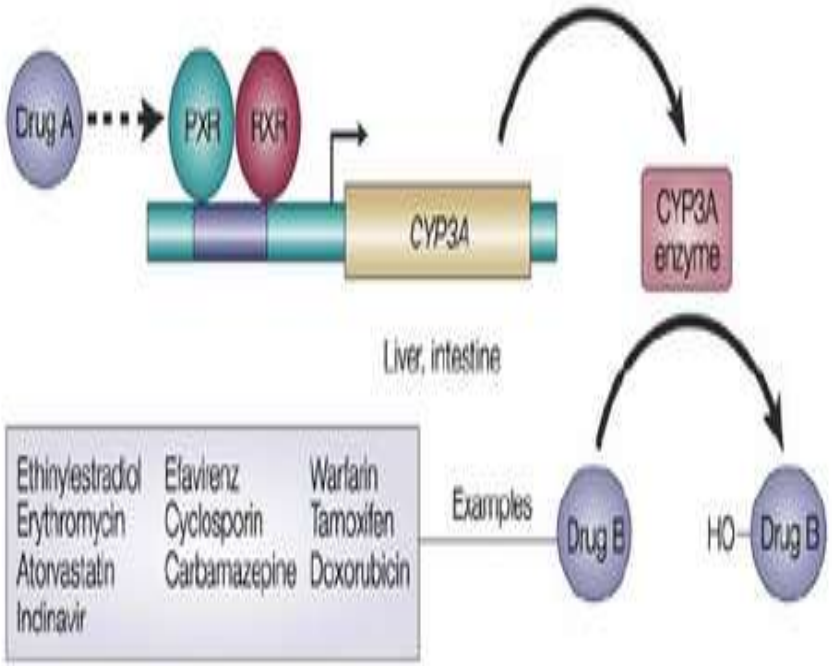
HT-29 Cell line		
Enzymes	48 hr incubation	72 hr incubation
GSTs	↑	↓
GPx4	↑	↓
CATALASE	↓	↑

DISCUSSION

AHR Pathway



PXR-CYP CASCADE



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 compunds (flavonoids), depending on their structures, are either beneficial antioxidants or acting as harmful pro-oxidant comqounds**

THANK YOU FOR YOUR INTERESTS

GREETINGS FROM




MANISA


TURKEY –İZMİR
AYKUT FIRAT



İZMİR- KARŞIYAKA

WISHING SUCCESFULL OMICS CONFERENCES

SEE YOU NEXT MEETING



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