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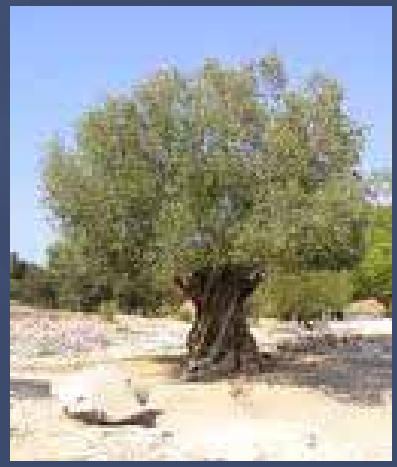
# Availability of renewable resources

Approximately 4 million tons of pine bark are annually available in Turkey, with a high potential to be utilized in various industries.

Olive trees are lopped between December and March, and all the lopped branches are used as fuel, likewise the remainings of cuttings for plantation.



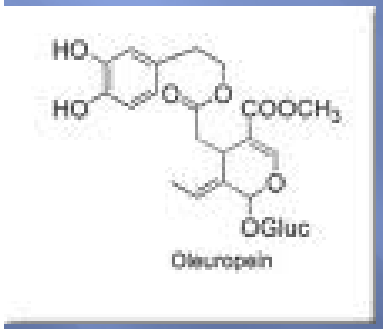


- have excellent radical scavenger properties
- accelerate wound healing processes
- a potent active ingredient for the treatment of minor injuries.


*In vitro* antioxidant activities of a commercial *P. radiata* bark extract, and fractions of ethanolic extracts of *P. Radiata* have been studied. Other Projects focused on antioxidant efficacies of 6 pine species (*P. pinea*, *P. brutia*, *P. radiata*, *P. halepensis*, *P. attenuata*, *P. nigra*), and a commercial *P. sylvestris* bark extract which was analyzed by a modified lipid peroxidation inhibition assay.



Oleuropein which possesses antiviral, anticarcinogenic properties is the most abundant secoiridoid in olive leaves. It also prevents cardiac diseases by protecting membrane lipid oxidation and improves the lipid metabolism.




Other biophenols present in olive leaves in high amounts are rutin, verbacoside, vanillin, vanillic acid and the glycosides of apigenin and luteolin. Recently, AIDS patients have begun to use olive leaf extract to strengthen the immune system, relieve chronic fatigue and boost the effects of anti-HIV medications.


# Aim of the study

**The aim of this study was to**

- **identify the potential usage of extracts obtained from waste bio-resources from various parts of Turkey,**
- **determine their antioxidant capacities and radical scavenging activities using different *in vitro* assays,**
- **evaluate the extent of oxidation in a model meat system.**

# Plant Material

Pine bark specimens were collected from 4 different locations in Turkey,

*P. nigra* from Bursa (N: 40° 29’ 44.7”, E: 29° 08’ 15.6”, altitude: 570 m),

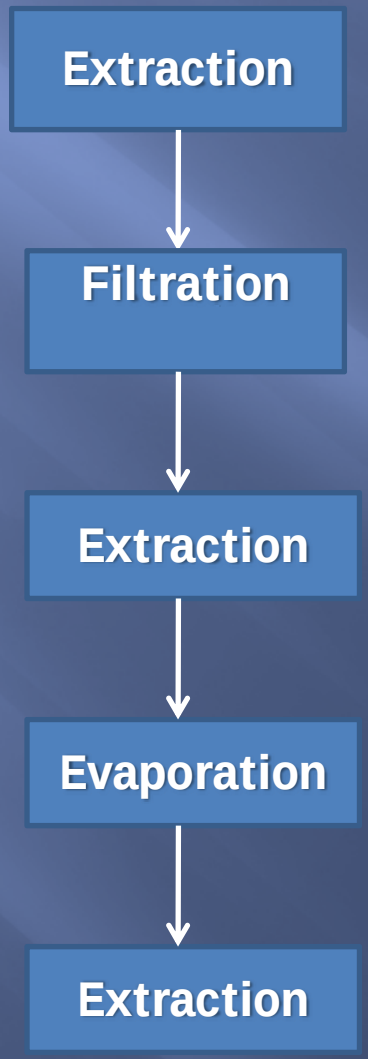
*P. pinea* from Aydın-Cine (N: 37° 32’ 30.1”, E: 28° 08’ 35.6”, altitude: 520 m),

*P. brutia* from Izmir-Deliomer (N: 38° 10’ 17.0”, E: 27° 03’ 46.7”, altitude: 120 m)

*P. sylvestris* from Eskisehir-Musaozu (N: 39° 41’ 46.2”, E: 30° 19’ 22.4”, altitude: 920 m) (June - August 2006).

# Preparation of pine bark extracts

**Patented method by Masquelier (1987)**



# Description of olive leaf extracts

Industrial extracts of olive leaves (olive leaf extract (OLE),

olive leaf juice (OLJ) olive mill wastewater pulp (OMWP) were kindly donated by Kale Natural Products.

Olive leaves were subjected to drying process at 5-10 °C under vacuum which, in turn, yields OLJ comprising approximately 50% of the loaded material. Dried leaves were extracted with ethanol at 70 °C, and subsequently transferred to a spray-dryer in order to obtain fine powder extract of olive leaf.

During oil processing, olive mill waste-water was received under nitrogen and directly transferred to the drying unit where OMWP was obtained as an output.

# Activity & oxidation analysis

**Antioxidant Assays**

- **Total phenol assay**
- **DPPH radical scavenging activity assay**
- ***β*-Carotene bleaching method**

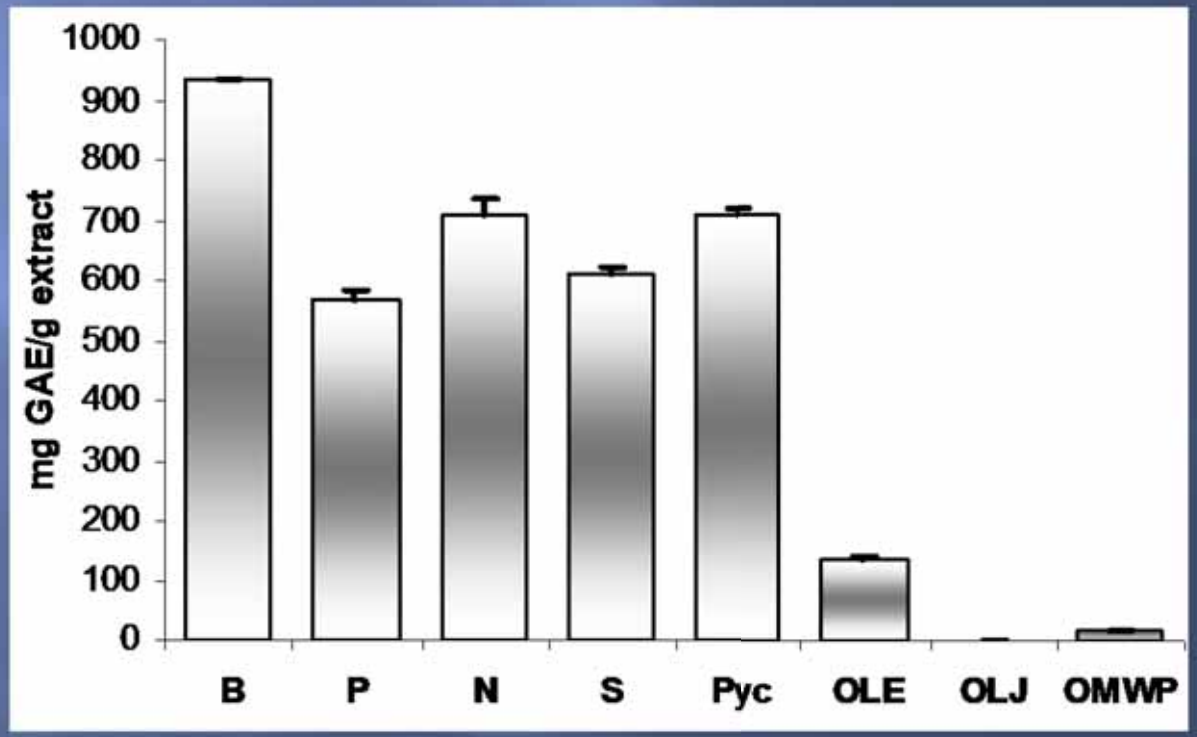
**Oxidation analysis of cooked meat**

- *TBA Analysis*
- **The 2-thiobarbituric acid (TBA) test modified by Shahidi et al. (1985), was used to determine the extent of oxidation in cooked turkey meat on days 0, 2, 5 and 7 of storage. Analyses were conducted in duplicate.**

# Results & Discussion

## Total Phenol Content

**Total phenols determined by the Folin-Ciocalteu method varied from 936.6 to 567.3 mg GAE/g extract for pine bark samples, whereas the values obtained from olive leaves were between 134.2 to 1.4 mg/g extract.**



# Antioxidant Efficiency

**$EC_{50}$ values**

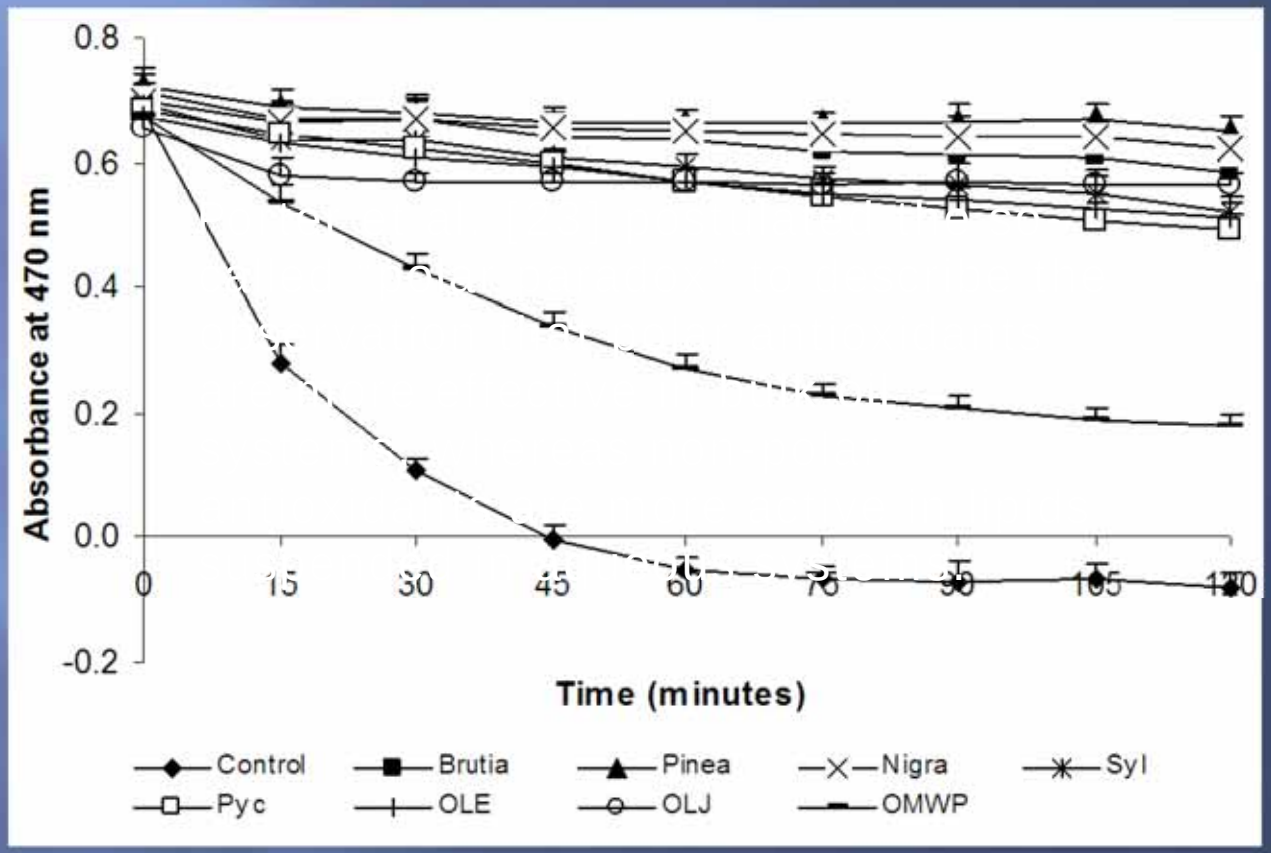
**The antioxidant activity at 200 ppm concentration by ß-carotene bleaching method in which ß-carotene was oxidized in the presence of linoleic acid.**

| Samples | $A_A$ (%) | $R_{OR}$ | $C_{AA}$ | $EC_{50}$ value (µg/ml) |
|---|---|---|---|---|
| *P. brutia* | 83.3 ± 1.6 | 0.167 | 871 | 8.1 ± 0.9 |
| *P. pinea* | 90.7 ± 2.8 | 0.093 | 958 | 8.7 ± 0.4 |
| *P. nigra* | 89.8 ± 0.7 | 0.102 | 918 | 9.8 ± 0.1 |
| *P. sylvestris* | 77.3 ± 0.0 | 0.227 | 790 | 10.9 ± 1.5 |
| Pycnogenol® | 74.8 ± 5.0 | 0.252 | 751 | 8.4 ± 1.2 |
| Olive leaf extract | 78.7 ± 5.8 | 0.213 | 777 | 23.0 ± 1.8 |
| Olive leaf juice | 81.1 ± 2.2 | 0.189 | 776 | > 8100[a] |
| Olive mill wastewater pulp | 35.1 ± 0.5 | 0.649 | 340 | 83.5 ± 0.6 |

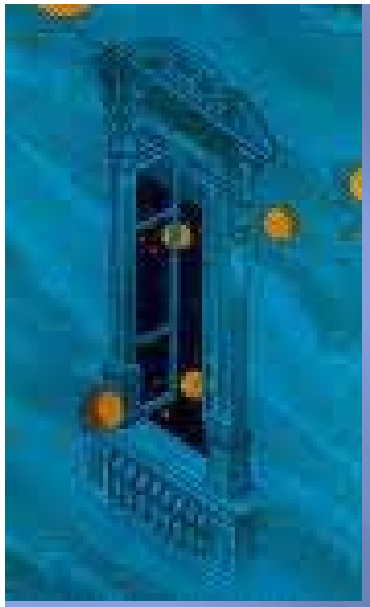
[a]showed 15.9% radical scavenging activity at 8153 µg/ml

**Antioxidant activity index is directly proportional to antioxidant activity coefficient whereas oxidation rate ratio is inversely correlated to the antioxidant activity index.**

# Antioxidant Efficiency




**Antioxidant activities of pine bark extracts, Pycnogenol® and olive leaf extracts by the ß-carotene bleaching method. Data points represent mean values of measurements (n=2) ± SEM.**



# Polar Paradox?

Porter et al. (1989) postulated the so-called “polar paradox” to describe the observation that polar antioxidants are more effective in bulk oil systems, whereas non-polar antioxidants are more active in lipids suspended in aqueous systems.

# Investigation of the polarity of OLJ

**explain its high antioxidant activity, particularly in ß-carotene linoleic acid model emulsified with Tween 20.**

**a TLC was conducted to examine the profile of OLJ and make a comparison with OLE.**

**The TLC profile ⇒ OLJ had both polar and non-polar UV active phenolic compounds .**

**So OLJ was extracted with ethyl acetate twice in 1:1 (v/v).**

**The non-polar fraction and OLJ were analyzed by ß-carotene bleaching assay and $A_A$ of the non-polar fraction was 1.2-fold higher than the activity of OLJ confirming that high $A_A$ of OLJ was associated with the non-polar compounds, most probably owing to formation of mixed micelles of linoleic acid and Tween 20**

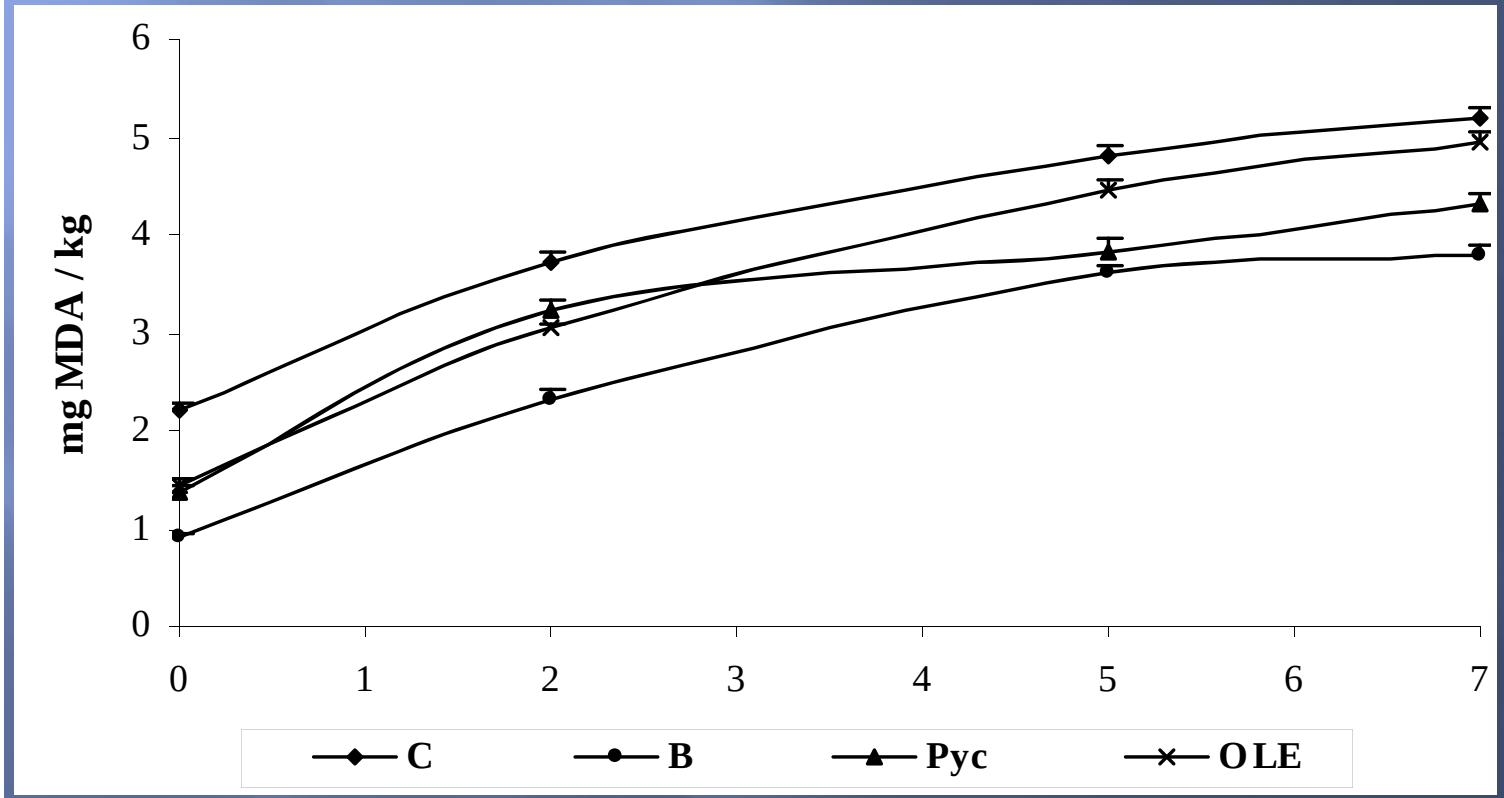
# Effects of Natural Extracts on Lipid Oxidation

Model food substrate → precooked meat due to relatively short shelf life.

Thiobarbituric acid-reactive substances were measured as an indicator of lipid peroxidation in cooked turkey meat during storage for 7 days at 4 °C

# Effects of Natural Extracts on Lipid Oxidation

| Samples | $A_{\Delta}$ (%) | Total Phenols | $EC_{50}$ value (µg/ml) |
|---|---|---|---|
| *P. brutia* | 83.3 ± 1.6 | 936.6 | 8.1 ± 0.9 |
| Pycnogenol® | 74.8 ± 5.0 | 361.5 | 8.4 ± 1.2 |
| Olive leaf extract | 78.7 ± 5.8 | 134.2 | 23.0 ± 1.8 |




Effect of selected extracts (C: Control; B: *P. brutia*; Pyc: Pycnogenol®; OLE: Olive leaf extract) on MDA formation in cooked turkey meat during storage for 7 days at 4°C. Data points represent mean values of treatments (n=2) SEM.

# Are one-dimensional methods sufficient to analyze multifunctional plant extracts?

**The extracts should be tested in food substrates.**

**Indeed, although one would have expected a better protection of Pycnogenol® in cooked turkey meat compared to OLE, as its $EC_{50}$ value was 2.7-fold lower and TFA value was 5.3-fold higher than OLE, both extracts showed similar effectiveness in reducing the lipid peroxidation.**

# How about other pytochemicals?

Tang and Cronin (2007) studied the effects of different concentrations of brined onion extracts on lipid oxidation in cooked turkey breast rolls for 7 days at 5 °C.

Meat enriched with the highest concentration of onion juice (50 %) showed the lowest oxidation (around 6.3 mg MDA/kg), whereas all 3 extracts tested in this study had much lower TBA values.

# How about other pytochemicals?

Mielnik et al. (2006) fortified cooked turkey meat with different concentrations of grape seed extract and reported a TBA value around 4.9 mg MDA/kg at 400 ppm concentration on day 6.

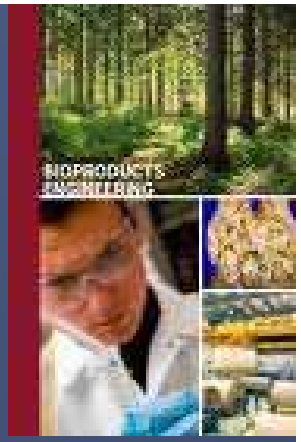
Similar TBA values were attained with OLE and Pycnogenol® treated samples on day 7, but with half the concentration of the grape seed extract.

# How about other pytochemicals?

**Antony et al. (2000) investigated the effect of different honey levels on oxidation in cooked turkey breast meat.**

**Oxidation of the meat sample supplemented with 50 mg/g honey was 60.9% lower compared to the control at the end of 48 h, whereas a similar level of protection was attained (62.2 %) with a *P. brutia* bark extract-supplemented sample on the second day of analysis, but the concentration tested was 250-fold lower indicating the superiority of *P. brutia* bark extract.**

# Conclusion



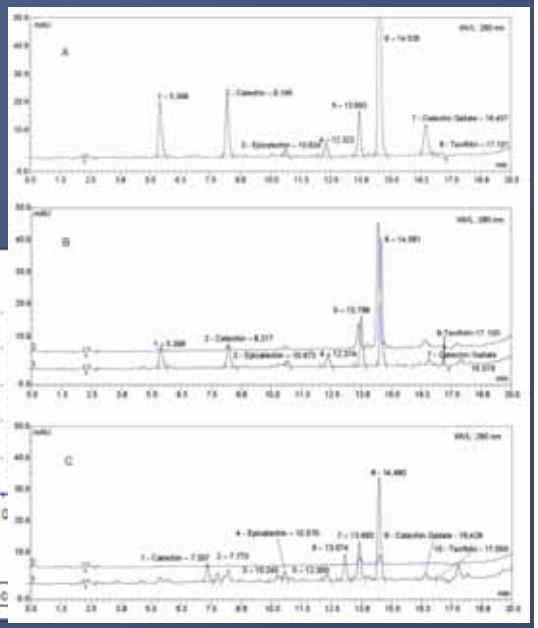

**Results clearly demonstrate high utilization potential of these bio-resources.**

**Particularly, *P. brutia* bark extract possesses the highest antioxidant activity and is a good functional supplement to precooked turkey meat products, both inhibiting the oxidation of fatty acids and enhancing the meat's quality in terms of health-promoting proanthocyanidins.**

**Additionally, OLJ can be utilized in a food emulsion, such as mayonnaise, due to its high activity in oil-in-water emulsion systems.**

# SUPERCRITICAL FLUID TECHNOLOGIES GROUP





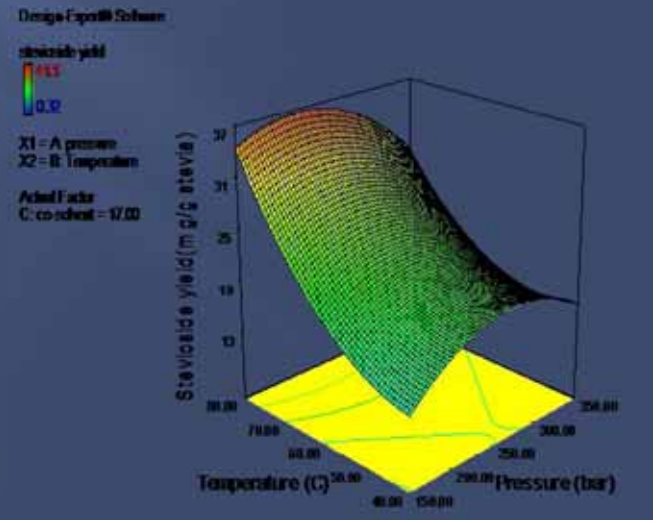
## Research areas

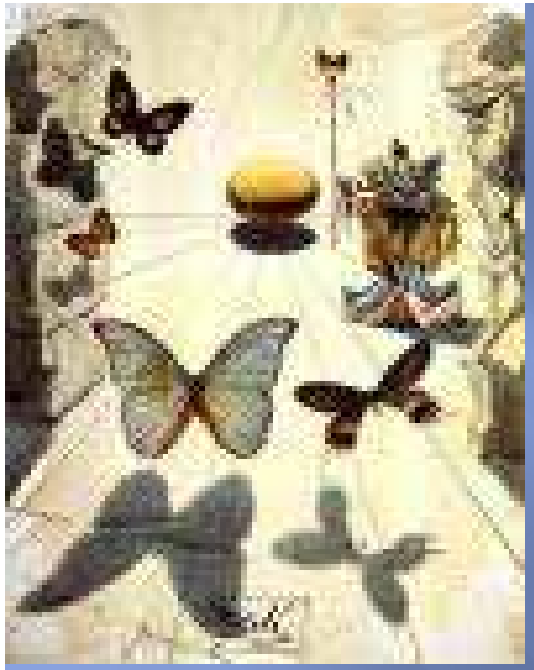
- **Extraction of different materials (*Stevia rebaudiana*, *Rosmarinus officinalis, Pinus brutia*, *P. pinea*, *P. nigra*, *P. sylvestris, Pistachia terebinthus, Arbutus unedo*, *Alkanna tinctoria*), optimization, determination of biological activities.**

- **Encapsulation of drug molecules with biopolymers to design micro- and nano particles for drug carrier systems**

- **Scale-up studies**

- **Utilization of industrial waste materials**





# Thank you