Marina Čobanov
University of Novi Sad

Extraction of aubergine peel by subcritical water: chemical characterization and bioactivity assays

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1. **Advancing pedagogical methods** for industrial agri-food waste valorisation **based on business-academia synergies**.
2. **Integrating citizen science** in bio-economy-enhanced waste valorisation as a means of **civic engagement and environmental advocacy**.

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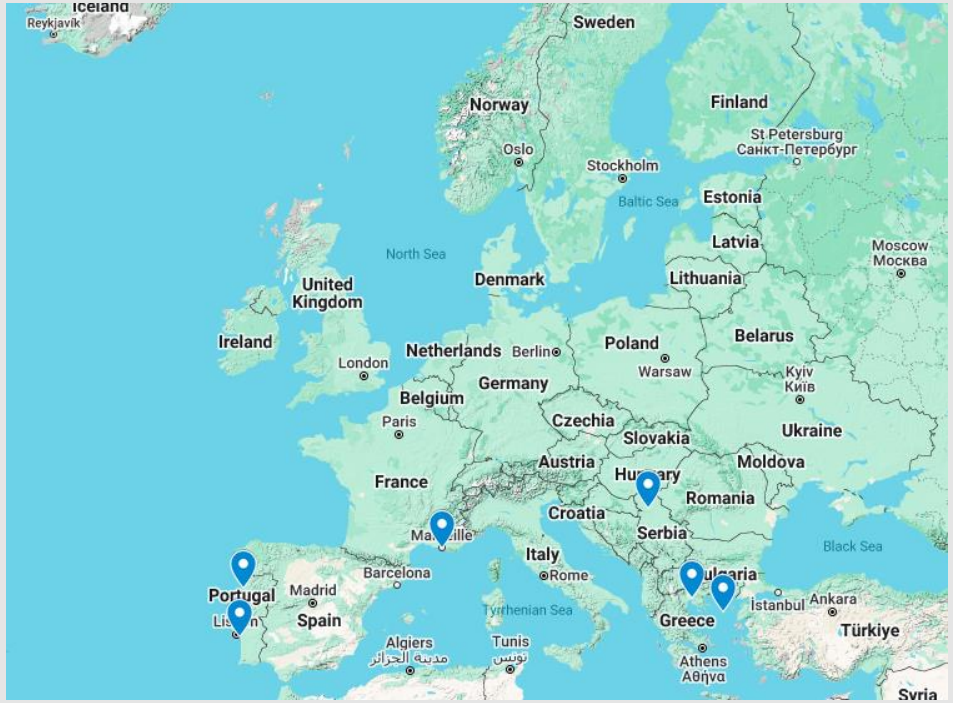

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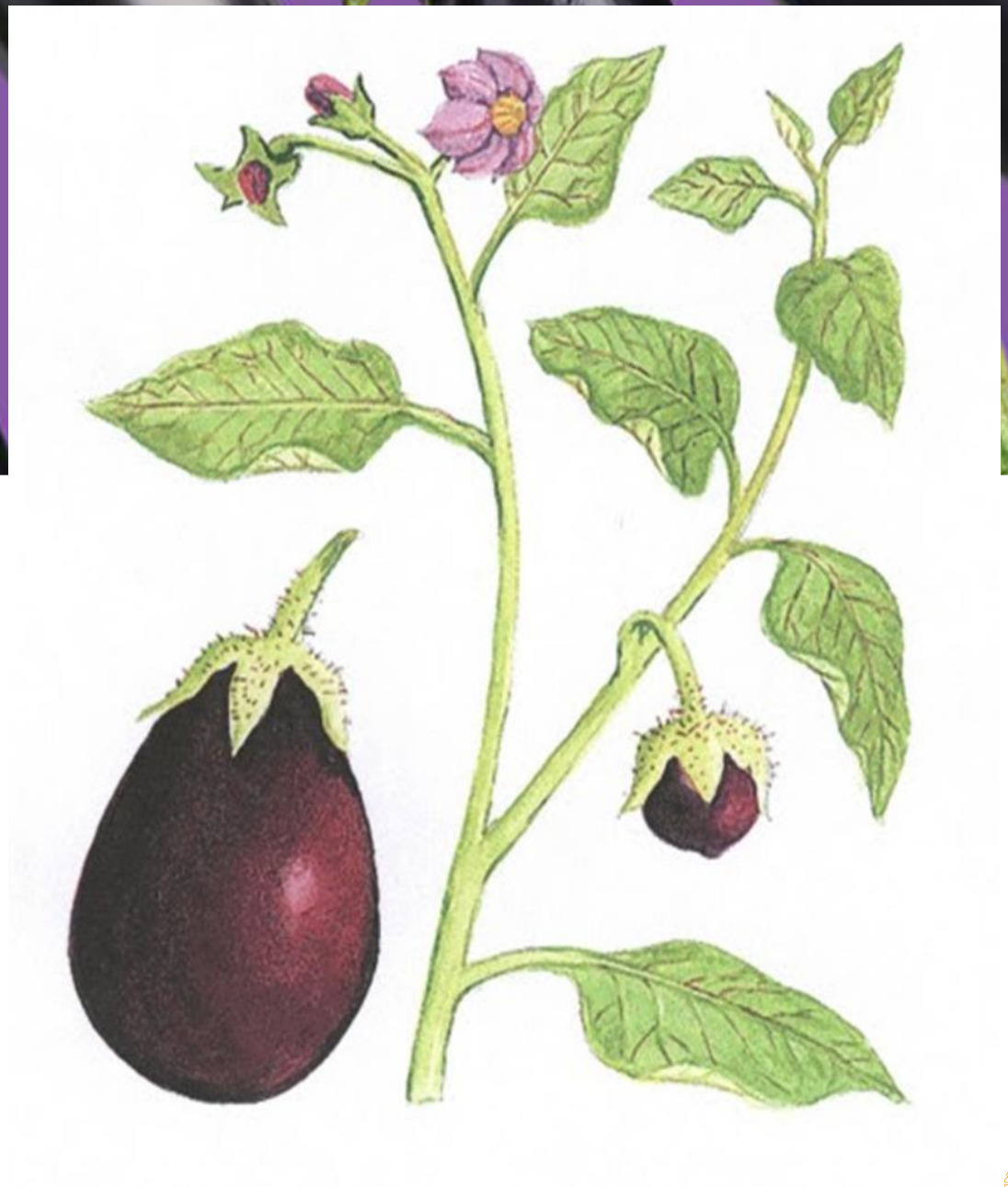
ORIGIN AND VARIETIES

AUBERGINE

- lat. *Solanum melongena*
- A plant from the nightshade family *Solanaceae* (alongside the tomato, chili pepper, and potato)
- It was first cultivated in India
- It was introduced to Europe through the Iberian Peninsula
- During the Middle Ages it was primarily used among Muslim and Jewish communities, known by its Arabic name al-batinjan (*al-bāḏhinjān*)



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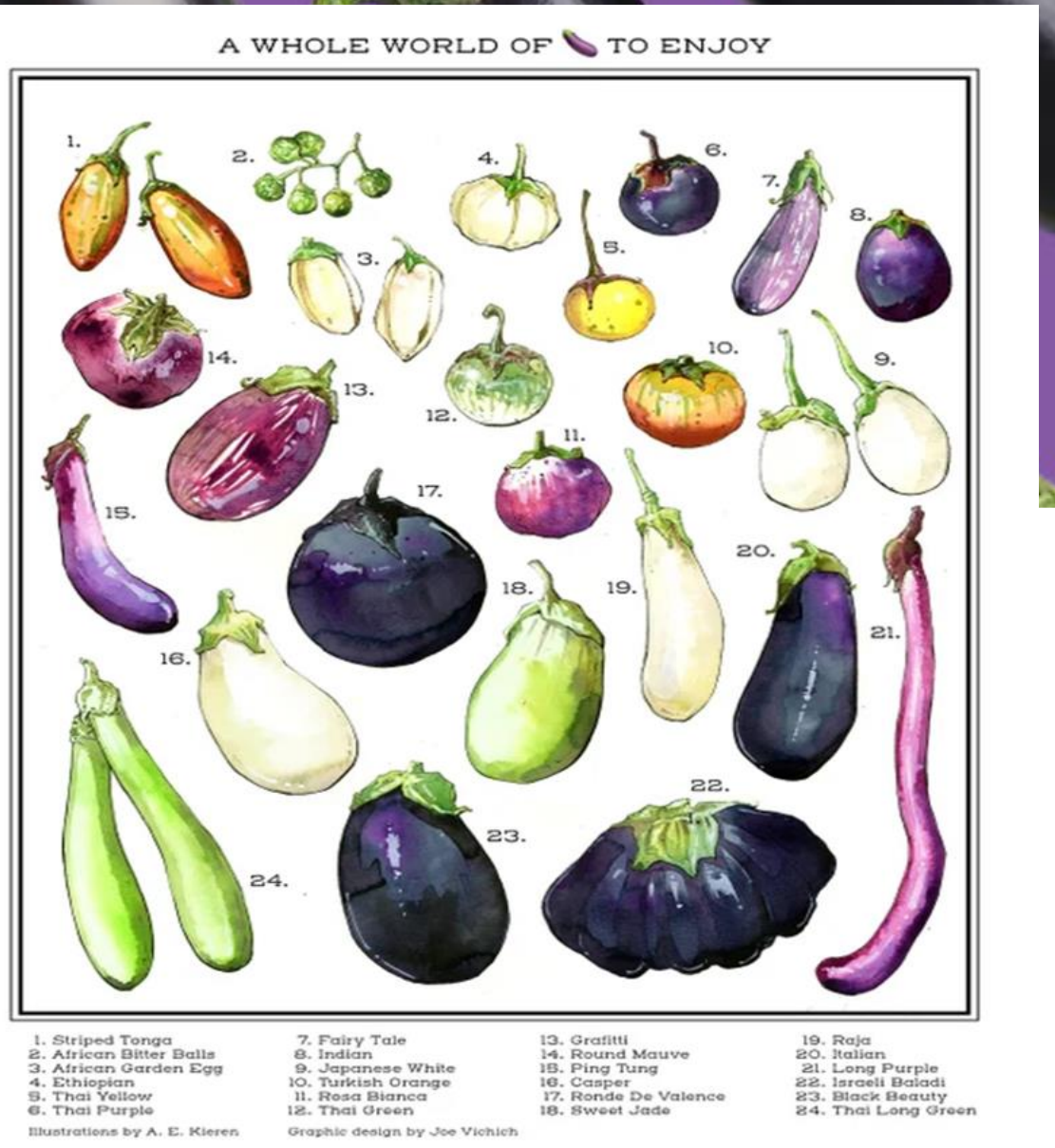
ORIGIN AND VARIETIES

AUBERGINE

- Usually dark purple, but can be found in different colours
- It can be found in several shapes:
  - Egg-shaped (lat. *S. melongena* var. *esculentum*)
  - Elongated and thin (lat. *S. melongena* var. *serpentinum*)
  - Dwarf types (lat. *S. melongena* var. *depressum*)



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MANAGING BIOWASTE

AUBERGINE

- 55,197,878 tonnes - annual global production
- 10,134,330 tonnes - annual by-product waste accumulation (with industrial consumption being dominant)

- If not properly utilized, waste can cause:
  - Greenhouse gas emission
  - Emission of foul odors
  - Attraction of vermin

- Aubergine skin - valuable source of value-added chemicals

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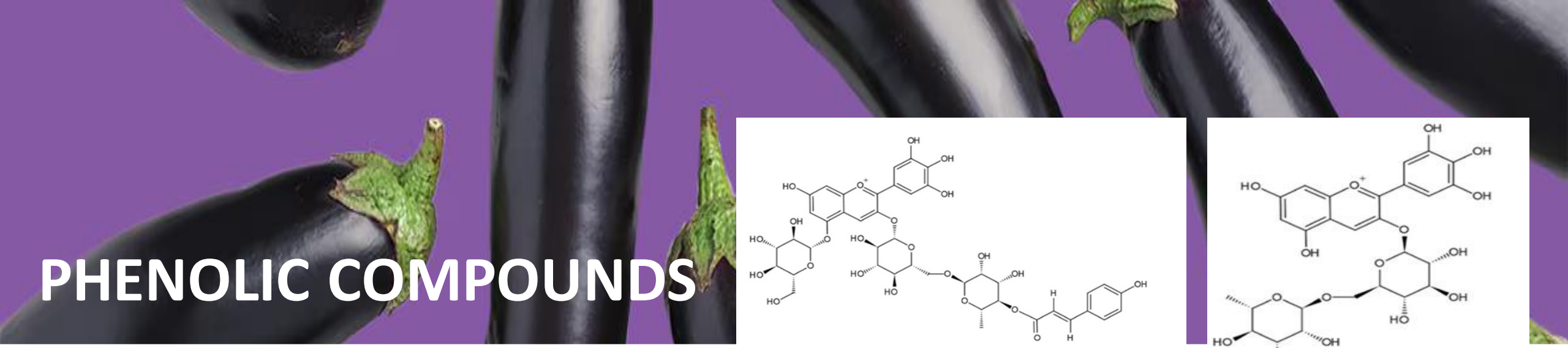
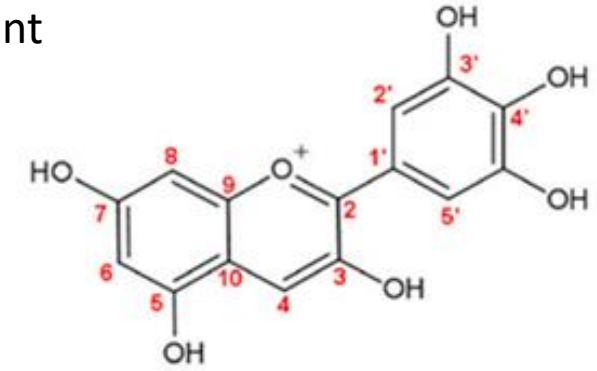


PHENOLIC COMPOUNDS

AUBERGINE

- Plant secondary metabolites
- Dark skin indicates an abundance of flavonoids
- With delphinidin-derived anthocyanins being the most abundant



- Two delphinidin-based anthocyanins are particularly abundant:
  - Delphinidin-3-rutinoside
  - Delphinidin-3-(p-coumaroylrutinoside)-5-glucoside (nasunin)

- Other delphinidin-based anthocyanins:
  - Delphinidin-3-rutinoside-5-glucoside
  - Delphinidin-3-glucoside
  - Delphinidin-3-rutinosyl-glucoside

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PHENOLIC COMPOUNDS

AUBERGINE

- Polyphenols are known for their biological activities, including:
  - Anticarcinogenic effects – through inhibition of kinases (MAPK and PKC) and transcription factors (NF-κB and AP-1)
  - Antimicrobial activity – by disrupting ATP formation and inhibiting human immunodeficiency virus (HIV)
  - Antioxidant properties – acting as free radical scavengers and reducing oxidative stress

- Phenolic composition can be significantly different depending on:
  - Agronomic conditions of the plant
  - Environmental factors

- In the study conducted by García-Salas et al. (2014):
  - Purple-striped eggplant - highest diversity of phenolic compounds
  - Long eggplant - highest quantity of phenolic compounds

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AUBERGINE

- High-molecular-weight carbohydrate polymer
- Present in plants as a structural component of cells
- Structural chain - galacturonic acid units (GalA) linked by α-1,4 glycosidic bonds
- Substitutions:
  - methyl esters
  - acetyl, ammonia or some neutral sugars
- The Food and Agriculture Organisation (FAO) sets a minimum of 65% GalA in pectin structure to permit its use as a food additive E440
- Degree of esterification (DE) – another important parameter that influences the texturizing, gelling and emulsifying properties of this compound
- Pectin extracted from aubergine skin - GalA content in the range of 66.8-69.7 % GalA, making it a high-quality pectin
- Aubergie skin – excellent source of pectin, with the extraction yields reaching 26.10-33.64%

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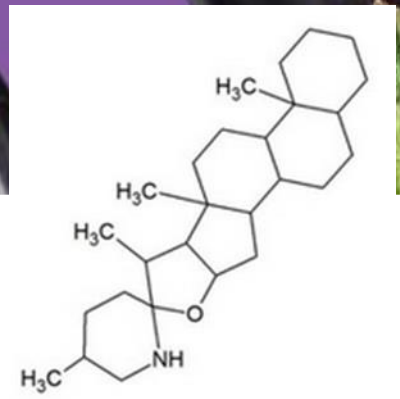
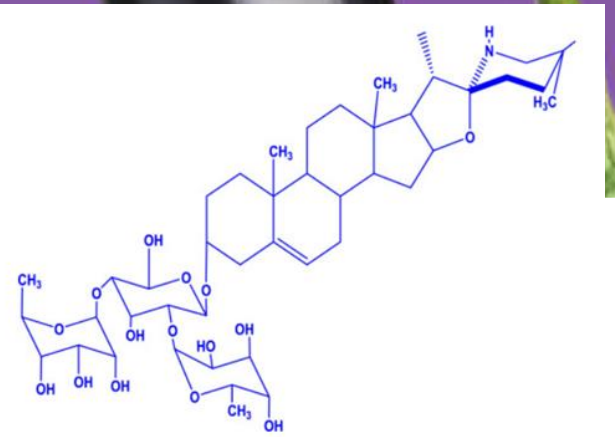
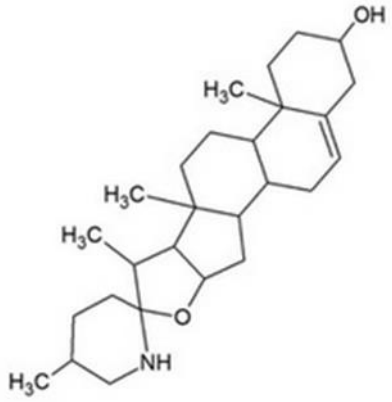
GLYCOALKALOIDS





AUBERGINE

- Nitrogen-containing plant secondary metabolites
- Defense against pests
- Toxicity - antinutritional effects
- Could be used for treating diseases such as:
  - Alzheimer's disease (AD)
  - Myasthenia gravis and
  - Glaucoma



- They have also been reported in peppers, where they have antiproliferative activity
- They are more common in aubergine flesh and seeds, whereas in the skin they are only found in small amounts
- Two glycoalkaloids are most common:
  - Spirosolane-type glycoaglicone solasonine
  - Solamargine

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EXTRACTION METHODS

- Extraction methods for aubergine skin peel include:
  - Solvent extraction
  - Ultrasound-assisted extraction (UAE)
  - Microwave-assisted extraction (MAE)
  - Supercritical fluid extraction (SFE)
  - Pulsed electric field extraction
  - Ohmic heating-assisted extraction

- The main aim is to develop more efficient extraction methods by:
  - ✓ Improving extraction time and yield
  - ✓ Reducing solvent consumption
  - ✓ Employing green and GRAS solvents
  - ✓ Simplifying concentration and purification steps

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AUBERGINE SKIN PEEL - SUBCRITICAL WATER EXTRACTION (SWE)

INSIDE THE LAB

- First-time implementation of SWE method for aubergine skin peel
- This extraction method uses water as a solvent for extracting semi-polar compounds (due to dielectric constant change)
- Heated to a temperature below critical point and compressed to prevent evaporation
- Under these conditions, water acts as an efficient solvent with lower viscosity, density, and surface tension compared to room temperature water
- However, this process can be energy consuming and can also cause compound degradation through hydrolysis
- On the other hand, it supports the principles of green chemistry
  - ✓ Extraction parameters: T=130 °C; t=30 min; p=40 bar N2; 500 rpm
  - ✓ Sample solution: 8.3g dried plant material + 250ml distilled water

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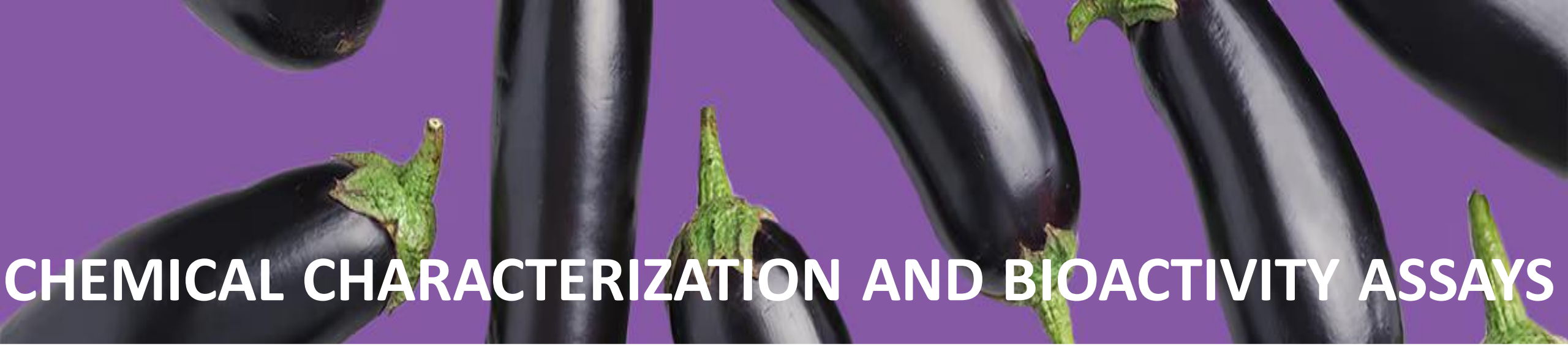

CHEMICAL CHARACTERIZATION AND BIOACTIVITY ASSAYS

INSIDE THE LAB

- ✓ Quantitative analysis with UV/Vis spectrophotometry
  1. Total phenolic content (TPC) - the Folin-Ciocalteu method
  2. Total flavonoid content (TFC) - the aluminum-chloride method
  3. Total anthocyanin content (TAC) - the pH differential method
  4. Total reducing sugars - the phenol-sulfuric acid method

- ✓ Quantitative analysis via gravimetry
  1. Pectin content – alcoholic precipitation

- ✓ Phytochemical Screening for Qualitative Analysis

- ✓ Bioactivity assays
  1. Total antioxidant activity - the phosphomolybdate method
  2. DPPH radical scavenging activity assay
  3. ABTS antioxidant capacity assay

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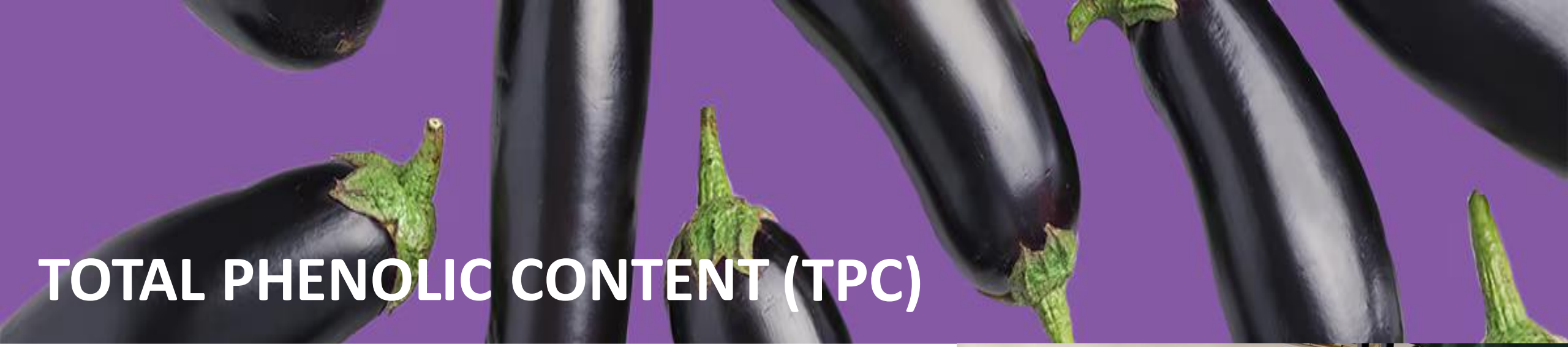

TOTAL PHENOLIC CONTENT (TPC)

INSIDE THE LAB

- The Folin-Ciocalteu method
- This metod is based on an oxidation-reduction reaction, where mixture of acids in Folin's reagent is reduced proportionally to the total phenolic content, producing a blue-colored chromophore
- The absorbance of this chromophore is measured at wavelengths between 725 and 765 nm
- Quantification is performed using a calibration curve of absorbance versus concentration, expressed as gallic acid equivalents (GAE)

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TOTAL FLAVONOID CONTENT (TFC)

INSIDE THE LAB

- The aluminum-chloride method
- Phenolic compounds exhibit the property of forming chelate complexes with metal ions such as Al^{3+}, during which Al(III)-flavonoids are formed
- These products absorb electromagnetic radiation in the wavelength range of 410 to 440 nm
- Quantification was performed using a calibration curve based on absorbance versus concentration, expressed as rutin equivalents (RE)

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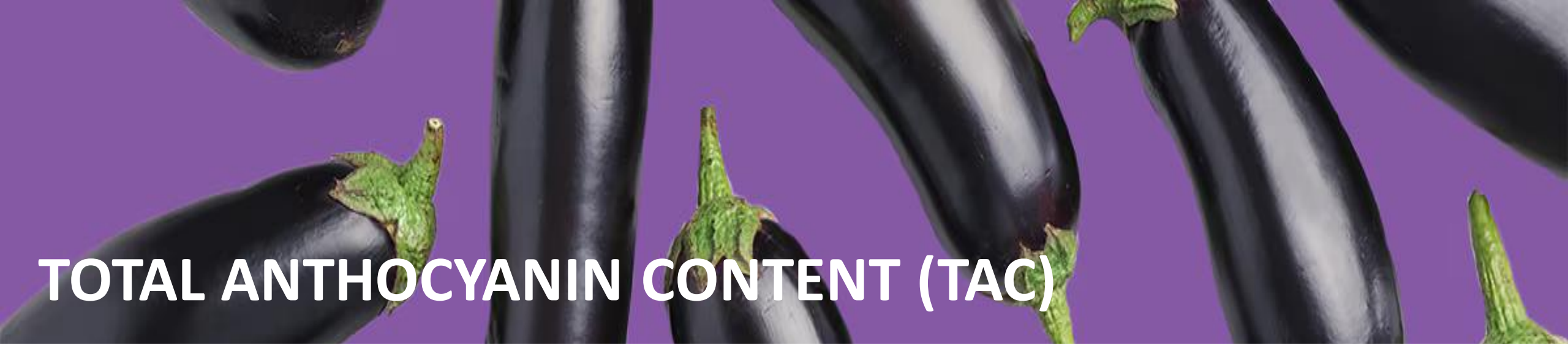

TOTAL ANTHOCYANIN CONTENT (TAC)

INSIDE THE LAB

- The pH differential method
- This method is based on the fact that anthocyanins change their chemical structure depending on pH
- At pH 1 (0.025 mol/L KCl buffer), anthocyanins exist predominantly in their colored flavylium cation form and will absorb light at around 520 nm
- At pH 4.5 (sodium acetate buffer), anthocyanins are in the colorless chemiketal form and will not absorb light at 700 nm
- For quantification, the Lambert-Beer equation was used

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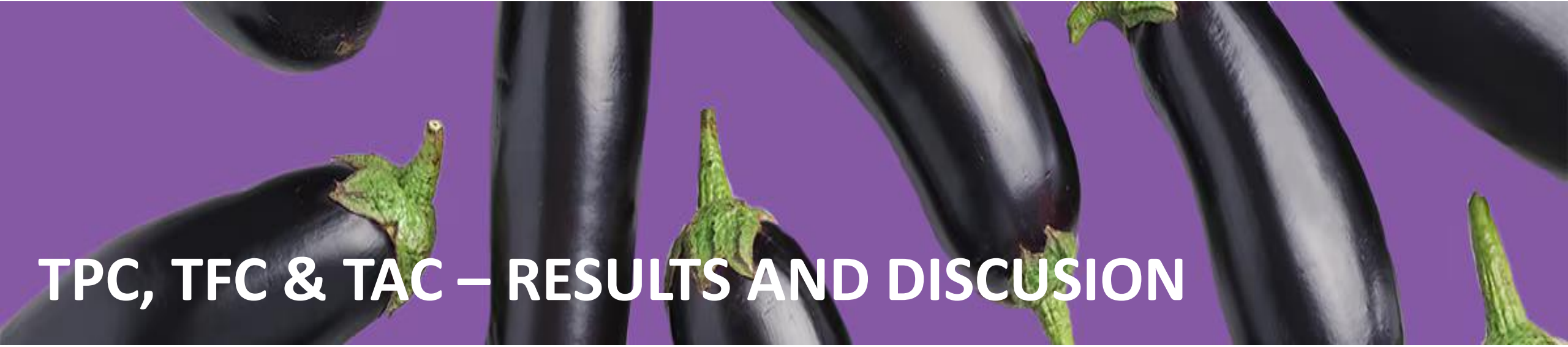
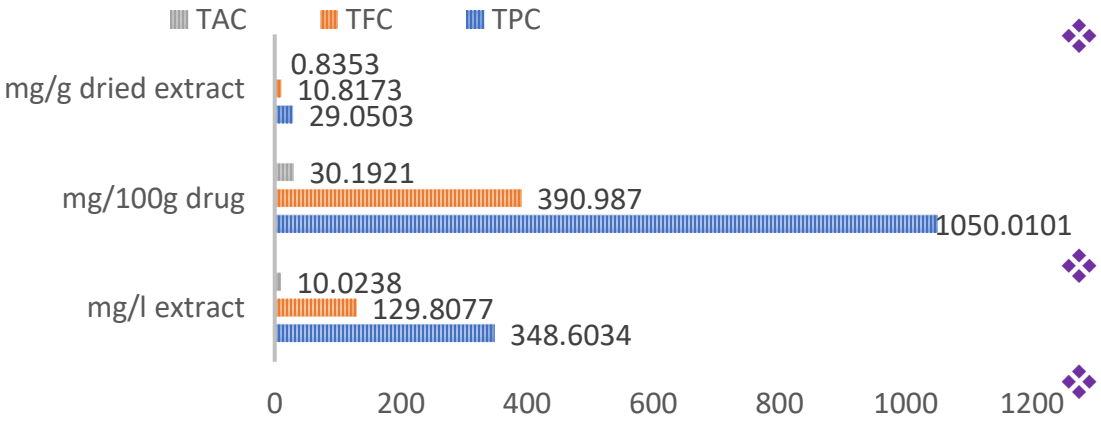


TPC, TFC & TAC – RESULTS AND DISCUSION

	mg/l extract	mg/100g drug	mg/g dried extract
TPC (GAE)	348.6034 ± 1.98	1050.0101 ± 5.95	29.0503 ± 0.16
TFC (RE)	129.8077 ± 3.33	390.9870 ± 10.03	10.8173 ± 0.28
TAC (C-3-G)	10.0238	30.1921	0.8353



- Enzyme-assisted extraction conducted by Amulya and Islam (2023), found TAC in the range of 114.3 -585.3 mg C3G/l, depending on the operating conditions
- Anthocyanin degradation – thermolabile compounds (oxidation, hydrolysis)
- Flavonoids - more stable than anthocyanins

- High total phenolic compound concentration – subcritical water as a good solvent for extracting polar compounds
- These findings are in correlation to other studies
- Kadoglidou et al. (2022) – 19 aubergine genotypes were used – ultrasound assisted extraction – TPC in the range of 6.33 - 12.74 mg GAE/g dw

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BIOACTIVITY ASSAYS

INSIDE THE LAB

- The total antioxidant capacity (TAC) was assessed using the phosphomolybdate method
- This method relies on the reduction of molybdenum(VI) to molybdenum(V) by the sample extract, leading to the formation of a green phosphate/Mo(V) complex under acidic conditions
- This complex can absorb light at 695 nm

- The ABTS assay involves the production of the blue/green ABTS•+ chromophore with the addition of the reagent
- This chromophore has an absorption maximum at 734 nm
- When present, antioxidants will stabilize this radical, reducing its absorbance
- ✓ ABTS (uncolored) + Reagent → ABTS•+(blue/green) (λmáx= 734 nm)
- ✓ ABTS•+(blue/green) + AOH → ABTS (uncolored) +AO

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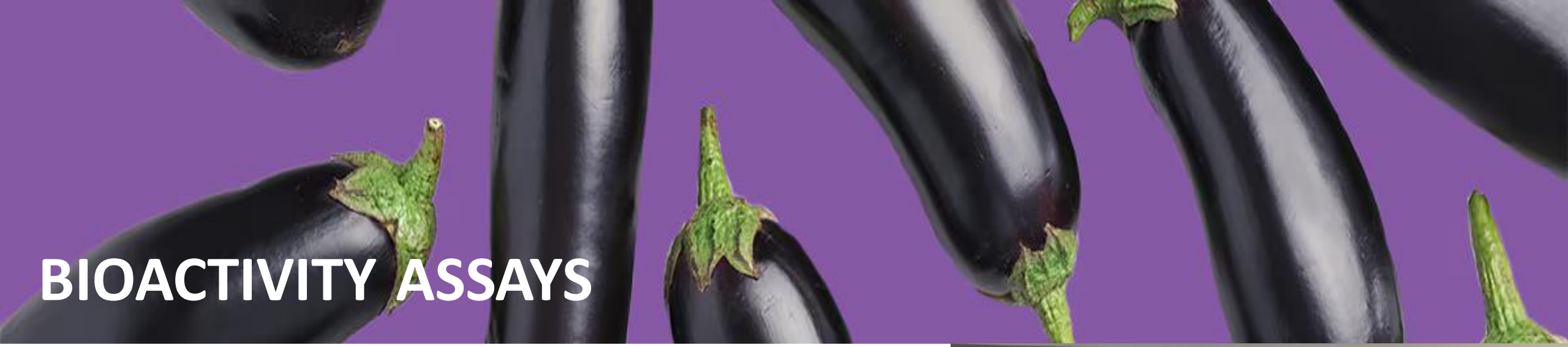

BIOACTIVITY ASSAYS

INSIDE THE LAB

- The DPPH assay is used to measure total antioxidant capacity
- In its radical form, DPPH• imparts a dark purple color to the solution
- When scavenged by an antioxidant through donating a hydrogen atom, it becomes colorless (DPPH-H)
- The absorbance is measured at 517 nm
- Instead of Trolox, which is used in the original method, ascorbic acid was used as the reference substance in our study

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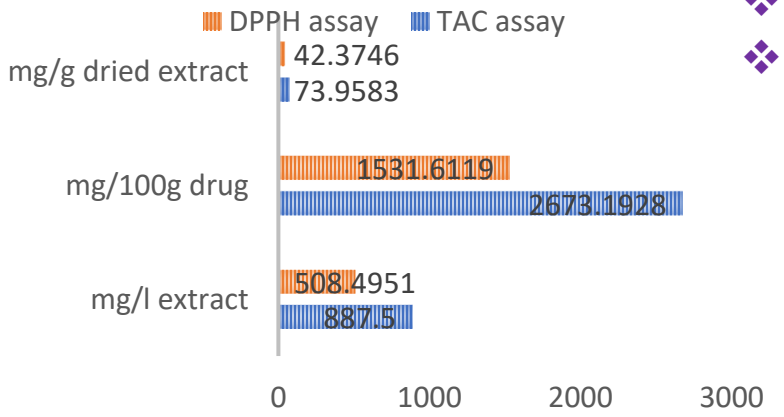
BIOACTIVITY ASSAYS – RESULTS AND DISCUSION

	mg/l extract	mg/100g drug	mg/g dried extract
TAC assay (AAE)	887.5 ± 17.68	2673.1928 ± 53.25	73.9583 ± 1.47
DPPH assay (AAE)	508.4951 ± 4.20	1531.6119 ± 12.66	42.3746 ± 0.35
ABTS assay	IC50 = 7,69 mg dried extract/ml		



- High bioactivity of aubergine peel extract
- Study conducted by Sotto et al., (2018) presents lower results for ABTS radical scavenging (277.6 µg/ml and 77.6 µg/ml, for DR2 genotype, collected at both commercial and physiological ripening stages, respectively), suggesting that subcritical water extraction is a more efficient method for extracting antioxidants that can stabilize this radical than the solvent extraction method used in this study

- The results of the DPPH and ABTS assays indicate a diverse profile of antioxidant compounds present in the aubergine peel extract
- Accordingly, aubergine peel extract has high antioxidant activity expressed through different mechanisms

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TOTAL REDUCING SUGARS AND PECTINE CONTENT

INSIDE THE LAB

- The phenol-sulfuric acid method
- When added, concentrated sulfuric acid will cause polysaccharides dehidratation to uronic acid and hydroxyurea formaldehyde
- These derivatives then react with phenol to form orange-red compounds
- The intensity of the color is proportional to the sugar concentration and can be measured using a spectrophotometer at 490 nm
- For quantification, a standard calibration curve (absorbance versus concentration) is used, and the results are expressed as glucose equivalents

- Pectin was determined using gravimetry (alcoholic precipitation)

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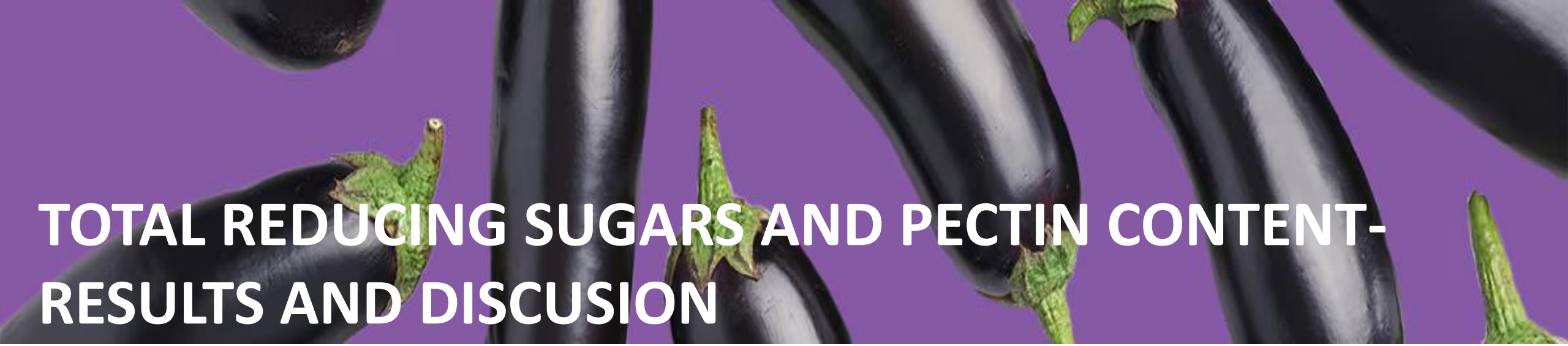

TOTAL REDUCING SUGARS AND PECTIN CONTENT- RESULTS AND DISCUSION

	mg/l extract	mg/100g drug	mg/g dried extract
Total sugars (GE)	5.9510 ± 0.02	17.9246 ± 0,06	0.4959 ± 0.00
Pectin (%)	3.99 ± 0.19		

- Aubergine skin peel – good source of high-methoxyl (HMP) pectin
- Kazemi et al. (2019) used microwave-assisted extraction, with pectin yield of 29.17 %
- In another study, Kazemi et al. (2019) used ultrasonic-assisted extraction, with pectin yield of 33.64 %
- Based on the results given, subcritical water has shown to be a less efficient method for utilizing aubergine peel for pectin production
- Total sugar content implies that under subcritical conditions hydrolysis of polysaccharides has occured, such as lignocellulosic materials (cellulose, hemicellulose, and lignin) and pectin

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Phytochemical Screening for Qualitative Analysis

Class of chemical compounds	Presence in the sample
Free flavonoids	+
Anthocyanins	-
Total tannins	++
Gallic tannins	+
Reducing sugars	-
Glycosides	-
Saponosides	-
O-glycosides (O-heterosides)	+
C-glycosides (C-heterosides)	++
Alkaloids	+
Coumarins	+

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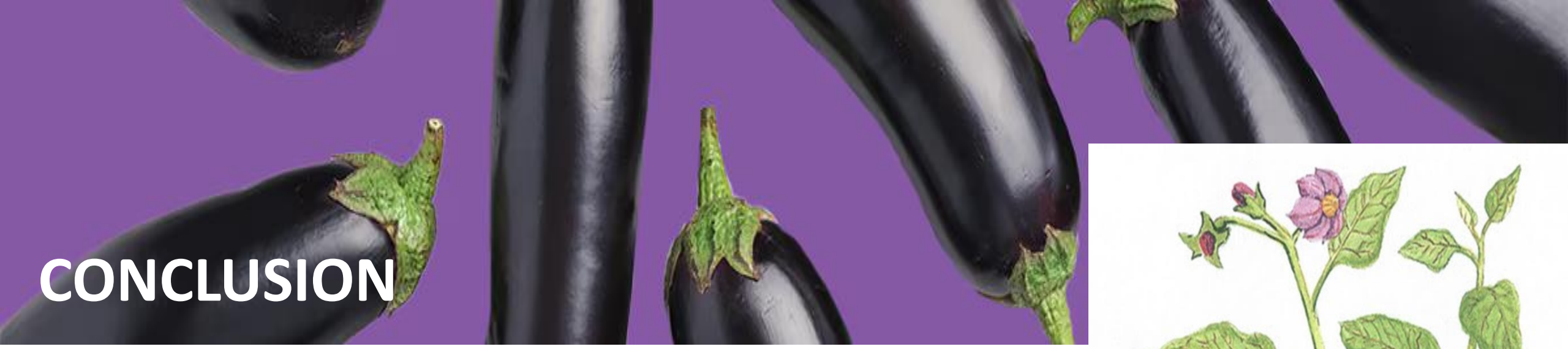
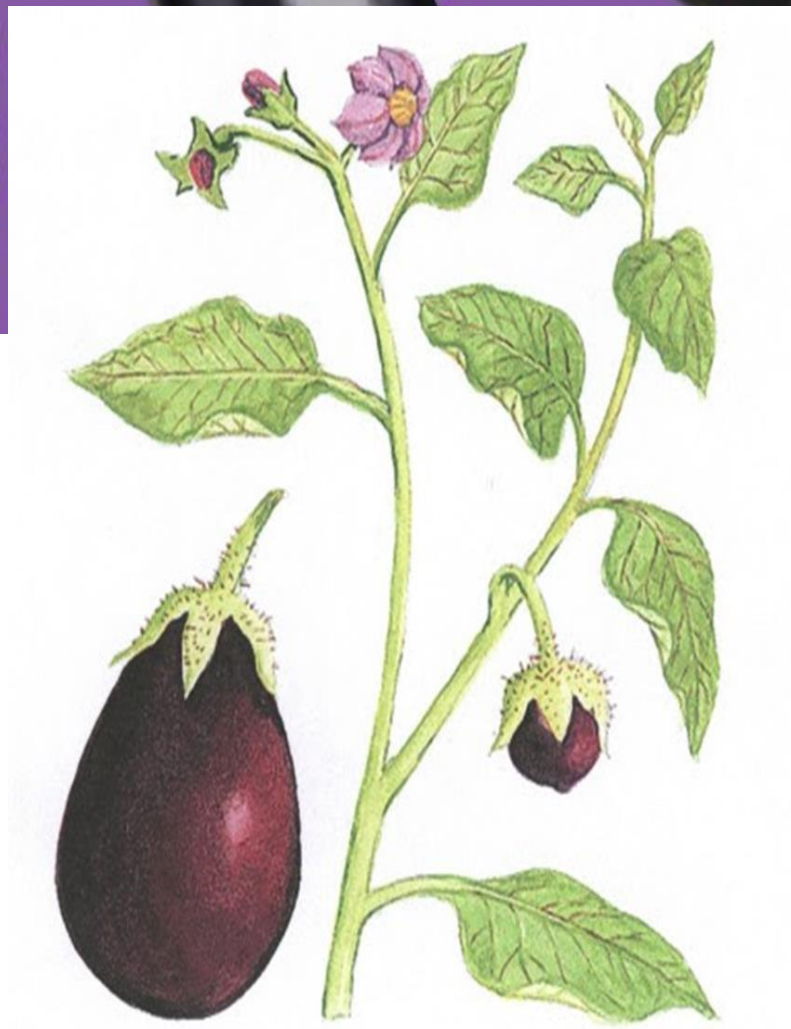


CONCLUSION



- Aubergine skin peel is a great source of value-added compounds
- It has a high phenolic content, especially delphinidin-derived anthocyanins, and is a good source of HMP pectin
- It also contains glycoalkaloids which could be used for treating various illnesses
- Under the applied operating conditions of 130 °C and 40 bar, subcritical water has shown good solvent characteristics for extracting compounds with high antioxidant activity, but lacked the ability to fully extract pectin, due to possible degradation through hydrolysis
- These operating conditions have caused degradation of thermolabile compounds such as anthocyanins and glycoalkaloids, resulting in lower yields of these compounds
- Overall, SWE has proven to be a good method for extracting polar and semi-polar compounds, while also being an environmentally friendly and non-toxic extraction technique

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Literature

- Amulya, P., & Islam, R. U. (2023). Optimization of enzyme-assisted extraction of anthocyanins from eggplant (Solanum melongena L.) peel. Food Chemistry X, 18, 100643.
- Di Sotto, A., Di Giacomo, S., Amatore, D., Locatelli, M., Vitalone, A., Toniolo, C., Rotino, G. L., Lo Scalzo, R., Palamara, A. T., Marcocci, M. E., & Nencioni, L. (2018). A Polyphenol Rich Extract from Solanum melongena L. DR2 Peel Exhibits Antioxidant Properties and Anti-Herpes Simplex Virus Type 1 Activity In Vitro. Molecules, 23(8), 2066.
- Flutto, L. (2003). PECTIN | Properties and Determination, Encyclopedia of food sciences and nutrition (second edition), 4440-4449.
- García-Salas, P., Gómez-Caravaca, A.M., Morales-Soto, A., Segura-Carretero, A., Fernández-Gutiérrez, A. (2014). Identification and quantification of phenolic compounds in diverse cultivars of eggplant grown in different seasons by high-performance liquid chromatography coupled to diode array detector and electrospray-quadrupole-time of flight-mass spectrometry, 57, 114-122.
- Gulcin, Í., Alwasel, S.H. (2023). DPPH Radical Scavenging Assay, 11(8), 2248.
- Hameed, A., Ijaz, S., Mohammad, I.S., Muhammad, K.S., Akhtar, N., Khan, H.M.S. (2017). Aglycone solanidine and solasodine derivatives: A natural approach towards cancer, 94, 446-457.
- https://libios.fr/en/analytical-solutions/oxydative-stress-antioxidant-capacity/oxydative-stress-antixodidant-capacity-assay-kits/abts-antioxydant-capacity
- https://www.inaturalist.org/taxa/63190-Solanum-melongena
- Kadoglidou, K.I., Krommydas, K., Ralli, P., Mellidou, I., Kalyvas, A., Irakli, M. (2022). Assessing Physicochemical Parameters, Bioactive Profile and Antioxidant Status of Different Friut Parts of Greek Eggplant Germplasm, 8(12), 1113.
- Karimi, A., Kazemi, M., Samani, S.A., Simal-Gandara, J. (2021). Bioactive compounds from by-products of eggplant: Functional properties, potential applications and advances in valorization methods, 112, 518-531.

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- Kazemi, M., Khodaiyan, F., & Hosseini, S. S. (2019). Utilization of food processing wastes of eggplant as a high potential pectin source and characterization of extracted pectin. Food Chemistry, 294, 339–346.
- Khatoon, M., Islam, E., Islam, R., Rahman, A.A., Alam, AHM K., Khondkar, P., Rashid, M., Parvin, S. (2013). Estimation of total phenol and in vitro antioxidant activity of Albizia procera leaves, 6, Article number: 121.
- Lee, J., Durst, R.W., Wrolstad, R.E., Barnes, K.W., Eisele, T., Giusti, M.M., Haché J., Hofsommer, H., Koswig, S., Krueger, D.A., Kupina, S., Martin, S.K., Martinsen, B.K., Miller, T.C., Paquette, F., Ryabkova, A., Skrede, G., Trenn, U., Wightman, J.D. (2005). Determination of Total Monomeric Anthocyanin Pigment Content of Fruit Juices, Beverages, Natural Colorants, and Wines by the pH Differential Method: Collaborative Study, Journal of AOAC International, 88, 5.
- Lelario, F., De Maria, S., Rivelli, A.R., Russo, D., Milella, L., Bufo, S.A., Scrano, L. (2019). A Complete Survey of Glycoalkaloids Using LC-FTICR-MS and IRMPD in a Commercial Variety and a Local Landrace of Eggplant (Solanum melongena L.) and their Anticholinesterase and Antioxidant Activities, 11(4), 230.
- Niño-Medina, G., Urías-Orona, V., Muy-Rangel, M.D., Heredia, J.B. (2017). Structure and content of phenolics in eggplant (Solanum melongena) - a review, 111, 161-169.
- Pérez, M., Dominguez-López, I.,Lamuela-Raventós, M R. (2023). The Chemistry Behind the Folin-Ciocalteu Method for the Estimation of (Poly)phenol Content in Food: Total Phenolic Intake in a Mediterranean Dietary Pattern, 71, 17543-17553. National Library of Medicine.
- Shraim, M. A., Ahmed, A. T., Rahman, M. M., Hijji, M. Y. (2021). Determination of total flavonoid content by aluminum chloride assay: A critical evaluation. Food Science an Technology, 150, 111932.
- Singh, A.P., Wang, Y.F., Olson, R.M., Luthria, D., Banuelos, G.S., Pasakdee, S., Vorsa, N., Wilson, T. (2017). LC-MS-MS Analysis and the Antioxidant Activity of Flavonoids from Eggplant Skins Grown in Organic and Conventional Environments, Food and Nutrition Sciences, 8, 8 73-888.

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- Švarc-Gajić, J. (2012). Uzorkovanje i priprema uzoraka za analizu. University of Novi Sad, Faculty of Technology Novi Sad, Serbia.
- Tomaz, I. (2016). Optimiranje priprave uzoraka za analizu polifenolnih spojeva u kožici grožđa tekućinskom kromatografijom visoke djelotvornosti. Doctoral Dissertation, University of Zagreb, Faculty of Natural Sciences and Mathematics.
- WebMD Editorial Contributor. (2022, November 28). Health benefits of eggplant. WebMD. https://www.webmd.com/diet/health-benefits-of-eggplant
- Yue, F., Zhang, J., Xu, J., Niu, T., Lü, X., Liu, M. (2022). Effects of monosaccharide composition on quantitative analysis of total sugar content by phenol-sulfuric acid method, 9, 963318.

Funded by the European Union
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