# Microwave-assisted Extraction of *Hibiscus sabdariffa* Antioxidants: Method Developmet and Validation

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# Agenda

01 Introduction

02 Material and Methods

03 Result and Discussion

04 Conclusion

# INTRODUCTION

**Roselle**
**(*Hibisccus sabdariffa*)**

**Edible flower**

Tea

Dried

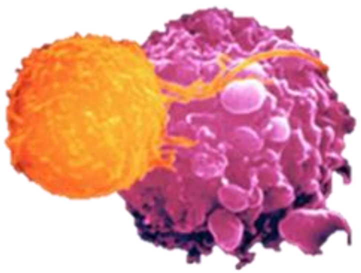
Natural dyes

Jam and marmalade

Jelly


Wine

# INTRODUCTION

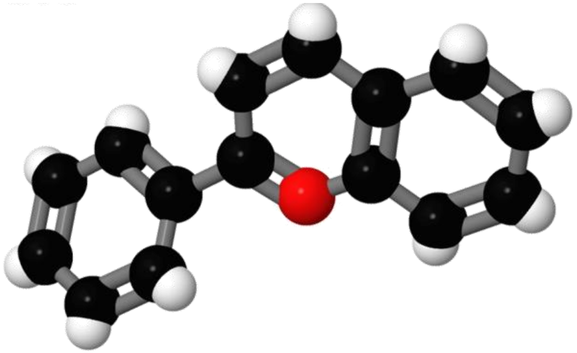


## BENEFITS

- Hangover remedy
- Degenerative disease treatment
- Antimicrobial agent, esp: pathogen
- Cancer preventive activity

- Anthocyanins
- Flavonoids and phenolics
- Mineral and vitamin content

## CAUSE



## BENEFITS

- Shorter extraction time
- Lesser extraction solvent
- Multiple sample extraction


**Microwave-assisted extraction**

## FACTORS

- Temperature
- Solvent composition
- Ratio sample to solvent
- Time

# Response Surface Methodology

Obtain the optimum extraction condition


# Box-Behnken Design

Evaluate the factors or combination to the extraction recovery

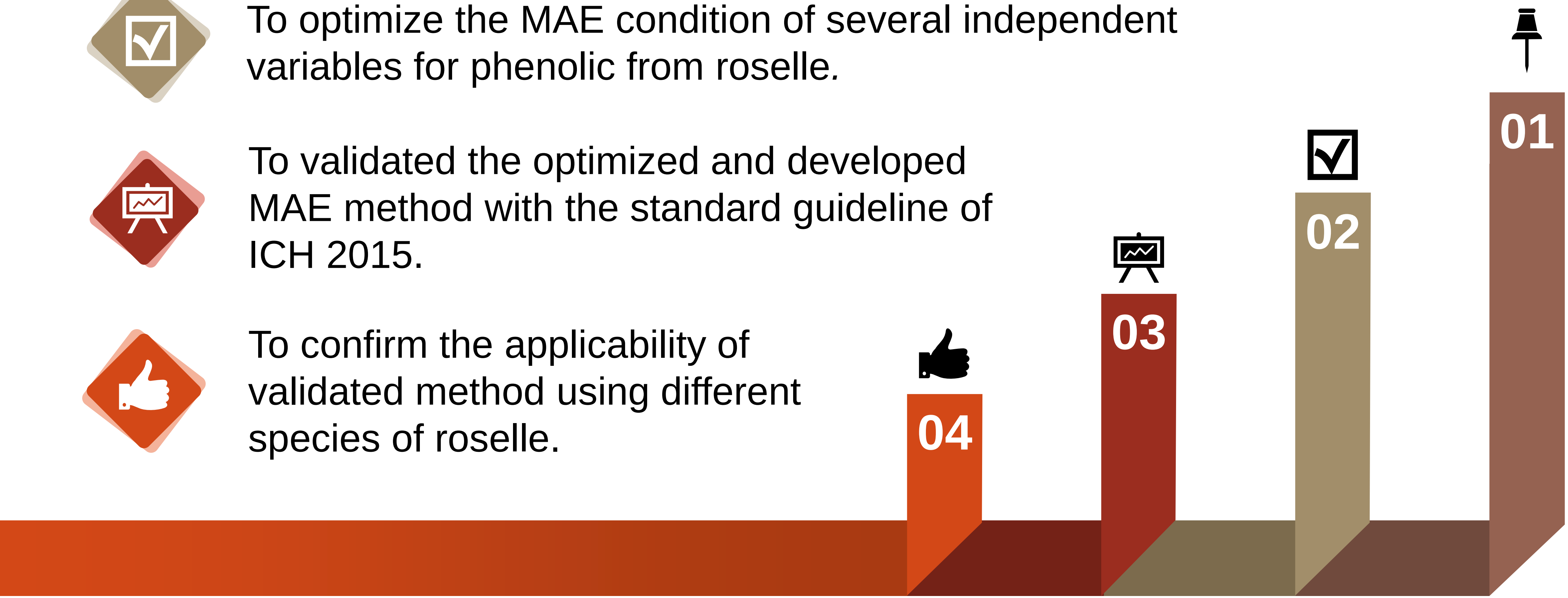
# OBJECTIVES

To evaluate the effect of several independent variables toward extraction recovery.

To optimize the MAE condition of several independent variables for phenolic from roselle.

To validated the optimized and developed MAE method with the standard guideline of ICH 2015.

To confirm the applicability of validated method using different species of roselle.



# Methods

## Factors and levels

| Variable | -1 | 0 | +1 | Unit |
|---|---|---|---|---|
| Temperature | 30 | 55 | 80 | ˚C |
| Solvent composition | 40 | 70 | 100 | %MeOH in water |
| Ratio sample:solvent | 1:10 | 1:15 | 1:20 | g of sample:mL of solvent |

## Optimization

**Box-Behnken Design**

15 experiments
(3 center points)

## Kinetics

**Run experiment in different period of time**

## Validation

- **Precision**
  - ✓Repeatability
  - ✓Intermediate
- **Accuracy**

## Real Sample Application

**Different species of roselle**

## Sample Preparation

Dried roselle

↓

**Grinding**

5 minutes
(30 s grinding @1 min)

↓

Roselle powder

## Extraction

Weighing the Sample

↓

Solvent → Putting into the extraction vessel

↓

Mixing

↓

Extraction

↓

Cooling (water bath 5 °C)

→

Centrifugation → Solid

↓

Extract

↓

Filtration 0.22 μm nylon filter

↓

Injection to UPLC-PDA

Ultra-high Performance Liquid Chromatography with Photodiode Array (PDA) detector

**Mobile phase**

- A: 0.1% acetic acid in water and mobile phase
- B: 2% acetic acid in acetonitrile

**Wavelength**

280 nm

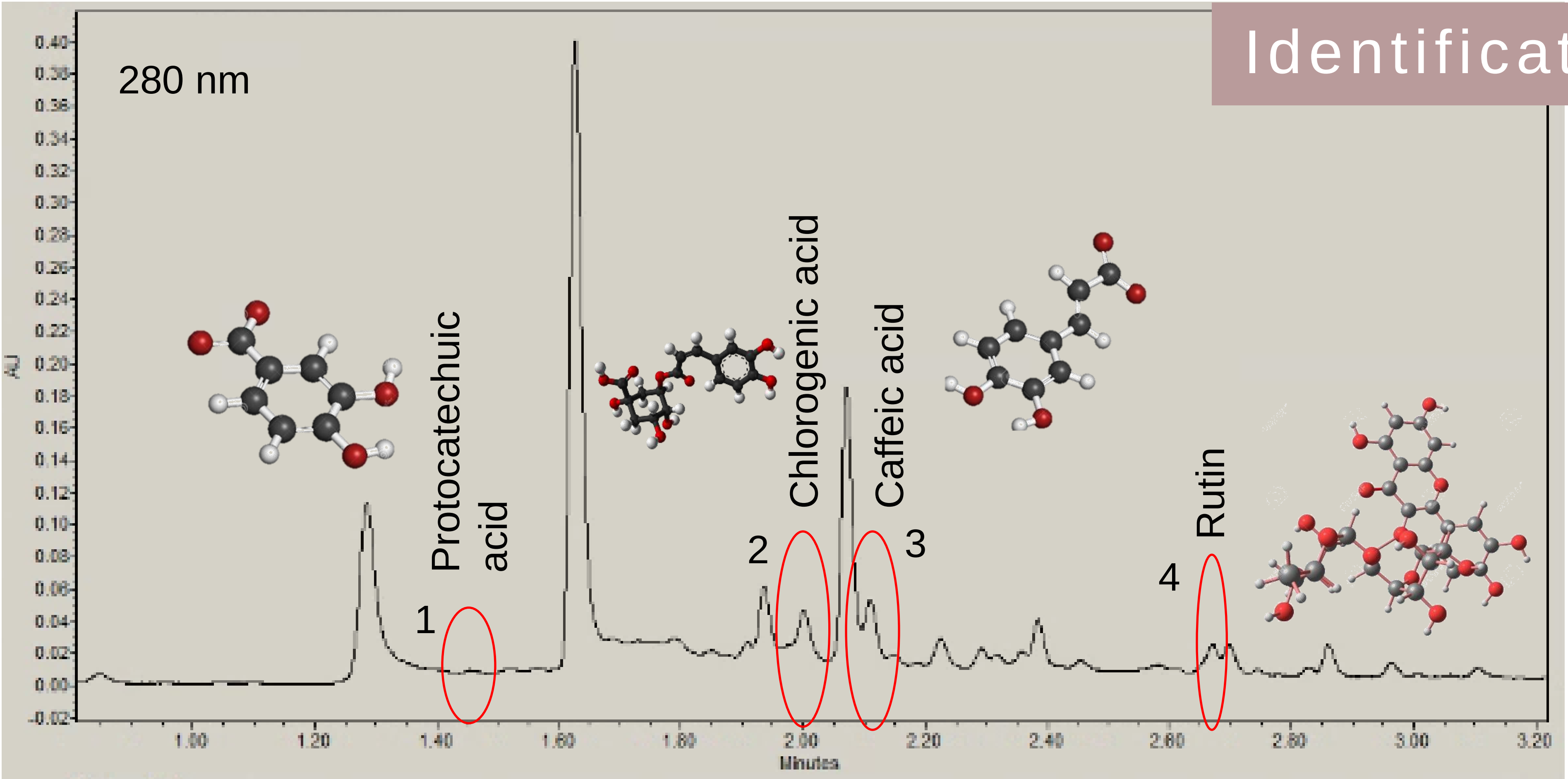
**Gradient**

| Time (min) | Solvent B (%) |
|---|---|
| 0.0 | 3.1 |
| 0.3 | 9.5 |
| 0.8 | 15.6 |
| 5.0 | 82.2 |
| 6.0 | 100.0 |
| 10.0 | 3.1 |


Microwave MARS 6 240/50 with vial made of polytetrafluo-reothylene

# Result and Discussion

# Identification




1 RT Sample : 1.458 min
RT Standard : 1.456 min

3 RT Sample : 2.225 min
RT Standard : 2.231 min

2 RT Sample : 2.002 min
RT Standard : 2.004 min

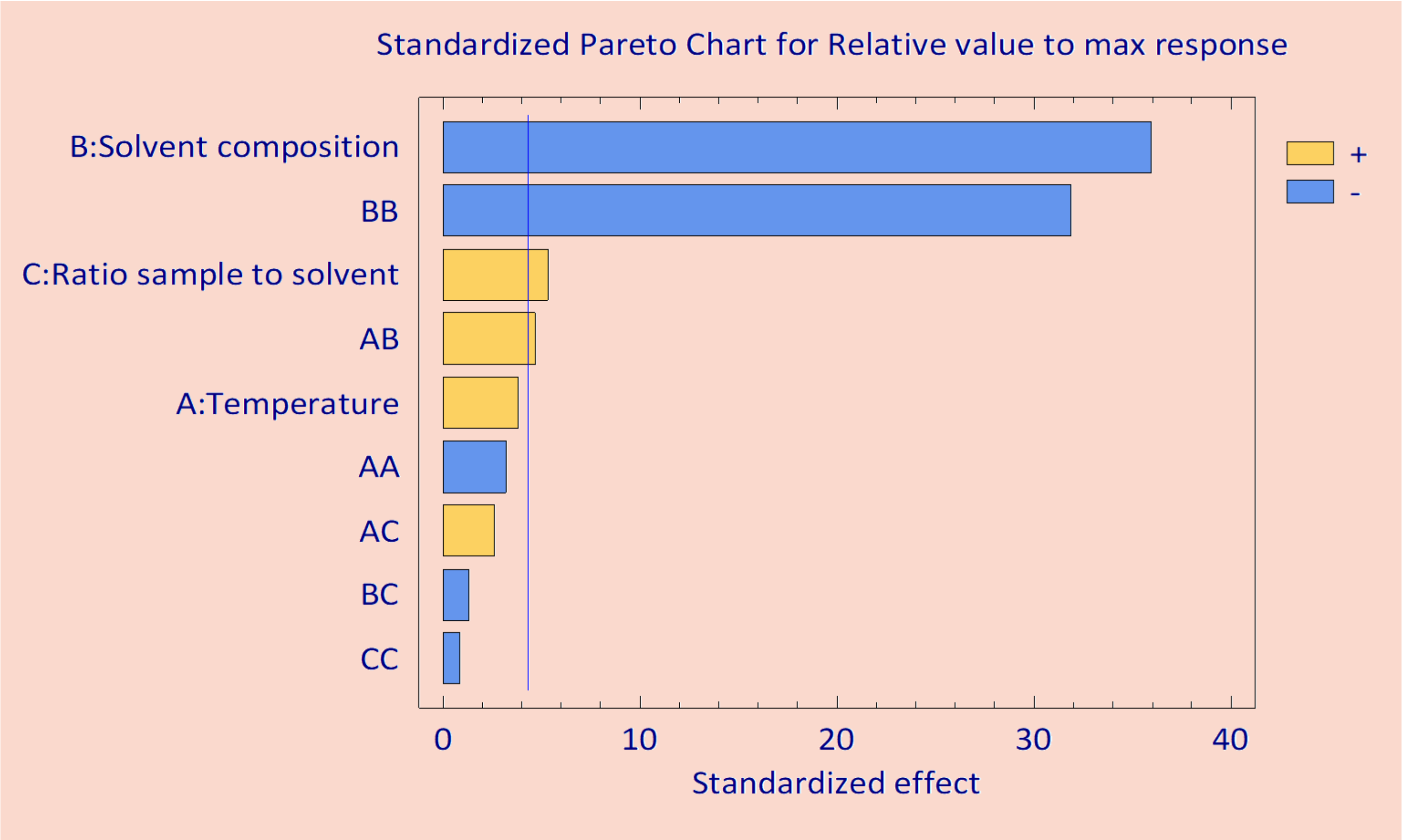
4 RT Sample : 2.860 min
RT Standard : 2.860 min

# Optimization

Box–Behnken design for three factors with their observed responses

| Run | $x_1$, temperature | $x_2$, solvent composition | $x_3$, solvent to sample ratio | Relative values to the maximum response (%) |
|---|---|---|---|---|
| 1 | 0 | 1 | -1 | 45.08 |
| 2 | 0 | -1 | 1 | 85.96 |
| 3 | 1 | -1 | 0 | 88.98 |
| 4 | -1 | 0 | 1 | 92.94 |
| … | … | … | … | … |
| … | … | … | … | … |
| 13 | -1 | 1 | 0 | 26.75 |
| 14 | 1 | 1 | 0 | 42.65 |
| 15 | 0 | 1 | 1 | 46.19 |

# Optimization




2 main factors achieved $p < 0.05$ meant to have significant effect on the extraction yield

## The equation of the model:

$$y = 95.2378 + 2.44A - 23.05B + 3.43C - 3.03AA + 4.25AB + 2.35AC - 30.09BB - 1.19BC - 0.82CC$$

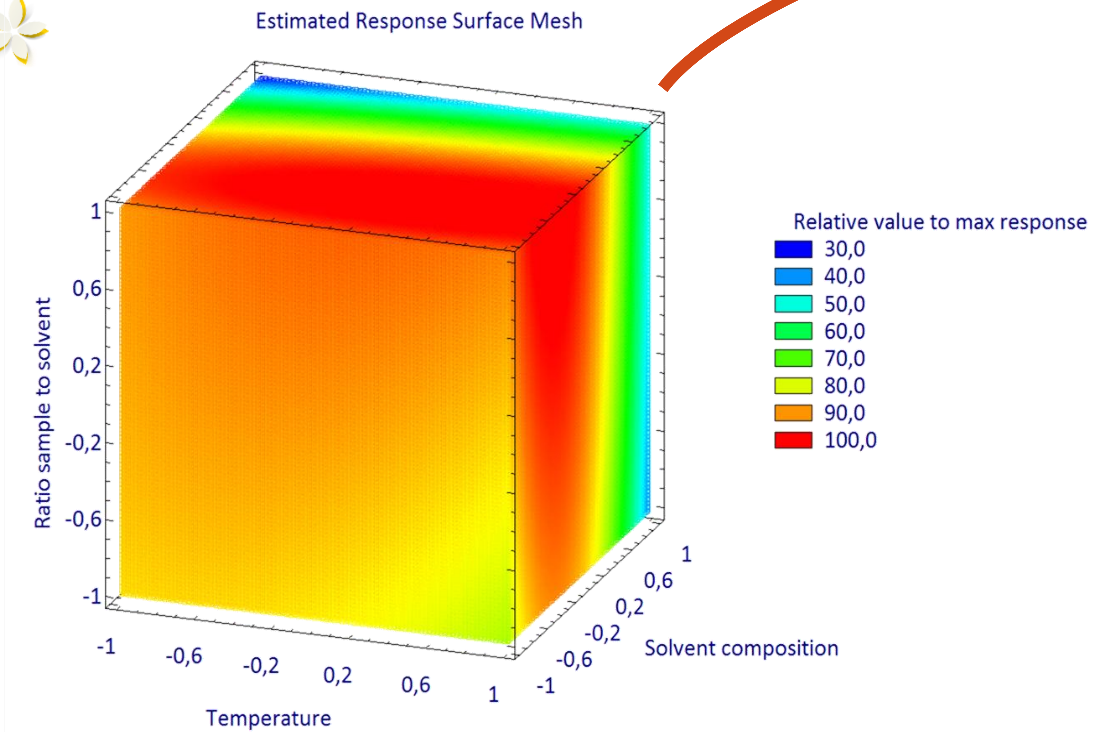
Where,
Y = extraction yield
A = temperature
B = solvent composition
C = solvent to sample ratio

Lack-of-fit = 0.0506

$R^2$ = 97.60%

Can be used to estimate the optimum MAE factors to obtain the maximum extraction yield → Confidence level of 95%

# Optimization




A 3D Mesh of Response Plot for The Studied MAE Factors

Goal: maximize Response

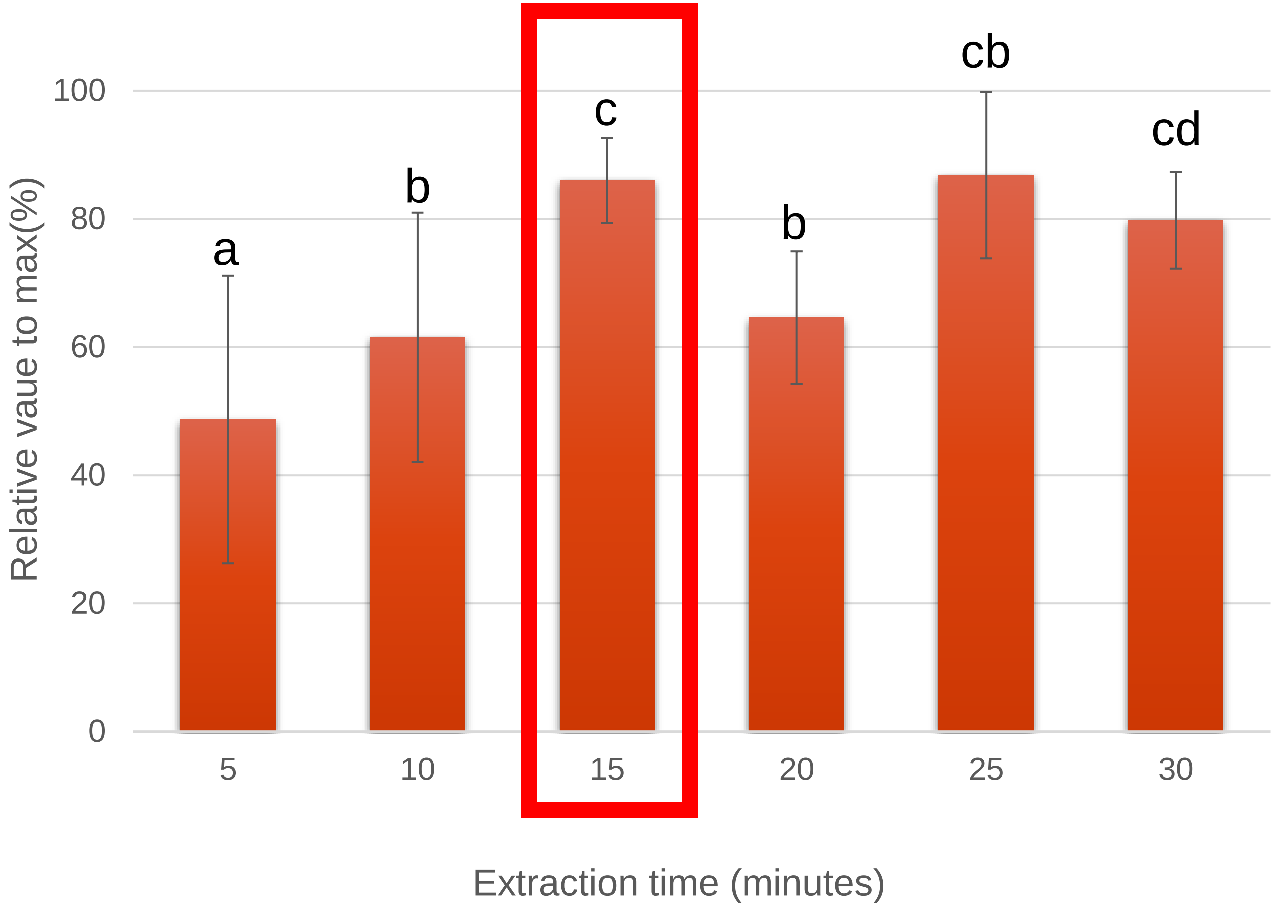
→ 103,54%

Optimum condition of MAE for the method

| Factor | Optimum coordinate | Optimum Condition |
|---|---|---|
| Temperature (°C) | 0.54 | 68 |
| Solvent composition (% MeOH in water) | -0.37 | 59 |
| Ratio sample:solvent (g sample / mL solvent) | 1 | 1:20 |

Chosen condition

# Kinetics




$p < 0.05$ meant extraction time significantly affect the extraction yield

15 was defined as the optimum extraction time

## Precision and Accuracy

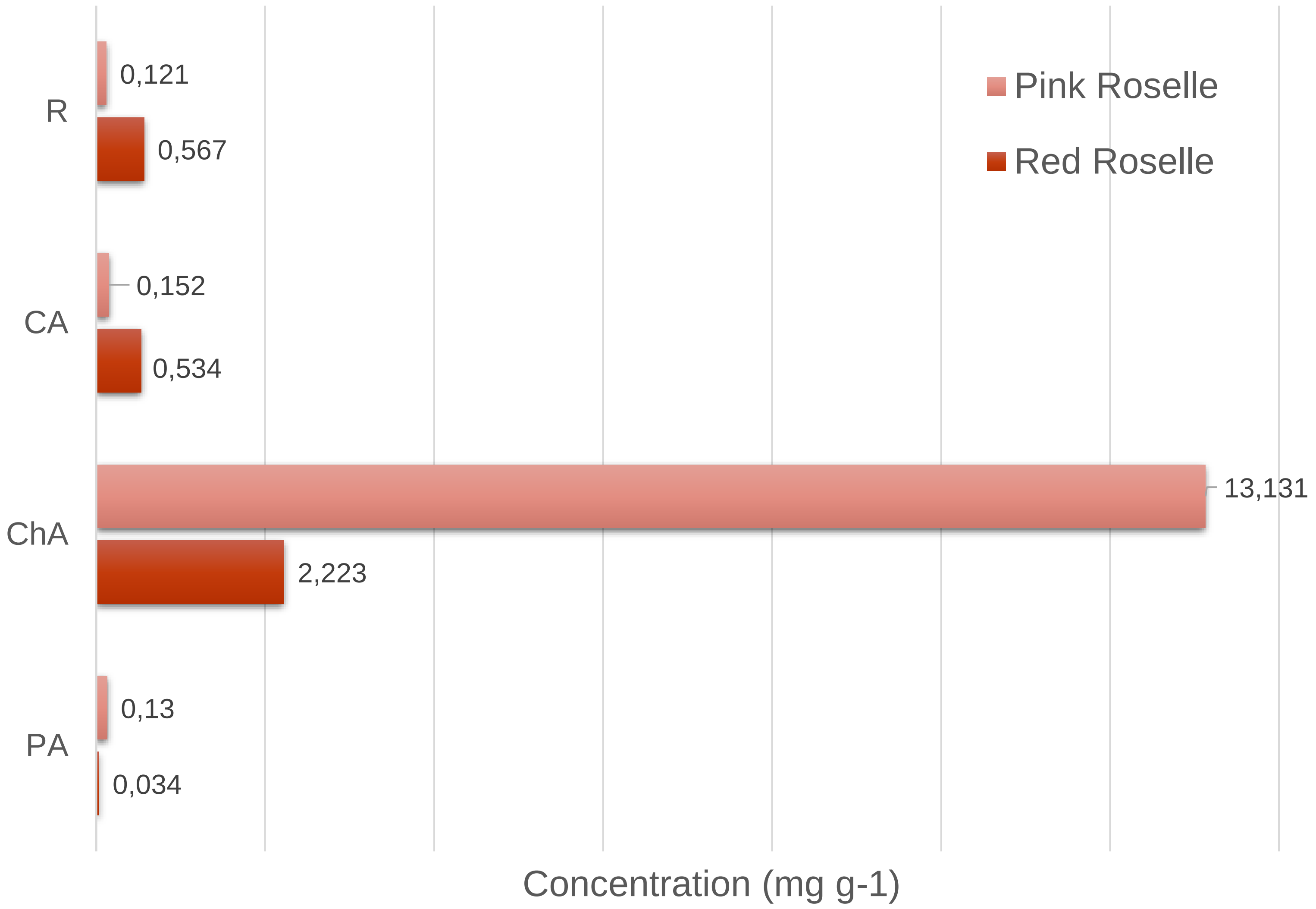
| Compounds | CV (%) | | Recovery (%) |
|---|---|---|---|
| | Intermediate | Repeatability | |
| Protocatechuic acid | 5.16 | 10.36 | 91.54±70 |
| Chlorogenic acid | 5.30 | 6.68 | 104.46±1.82 |
| Caffeic acid | 4.82 | 7.48 | 118.79±3.73 |
| Rutin | 6.46 | 5.94 | 105.54±2.19 |

Precision and accuracy of the developed method

Acceptable by the ICH standard

# Real Sample

## Phenolic compound concentration in different Roselle species




**$p < 0.05$** meant there was a significant different between different species of Roselle

R : Rutin
CA : Caffeic acid
ChA : Chlorogenic acid
PA : Protocatechuic acid

# Conclusion

**1** 2 main factors (solvent composition and ratio sample to solvent), 1 interaction factor, and 1 quadratic effect significantly affect extraction recovery

**2** Optimum MAE condition:
15 min extraction at a 68 °C using 59% ethanol in water sample to solvent ratio of 1:20

**3** Satisfactory validation result:
- CV met the ICH standard
- Recovery for accuracy is within the range of 90-120%

**4** Different varieties of Roselle provide different amount of the phenolic content

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# THANK YOU!