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# Chemical characterization of Bluish-Black and Yellowish-White fruits of *Myrtus communis* L.

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**Abstract**
The aim of this study is the determination of some chemical properties of black and white fruits of *Myrtus communis* growing wild in Tunisia. Berries were collected from North West of Tunisia. Different treatments were applied to myrtle fruits: fruits without treatment, fruits soaked in water for one night, fruits soaked in water for two nights, fruits boiled in water and fruits boiled in water and soaked for one night.
The obtained juices from berries were collected stored at 4°C for further analysis. Titratable Acidity (TA) and pH were measured. The total phenols content was determined by Folin Ciocalteu method, the total flavonoid content of juice was determined by the aluminium chloride colorimetric method and the total condensed tannin content was determined by vanillin method. The antioxidant capacity of the studied samples was determined applying the DPPH assay.
Statistical analysis of acidity showed high significant differences between the two types of fruits and between treatments. Analysis of total polyphenols content showed significant differences between the five studied treatments. Myrtle black berries contained highest amount of flavonoids when compared with white fruits. Untreated berries showed the lowest amount of flavonoids (0.57 mg RE/mL of juice). The lowest amount of condensed tannins was reached by fruits soaked in water for two nights (128.08 mg CE/mL and 233.72 mg CE/mL respectively for black and white berries) while untreated fruits showed the highest values. No significant differences were recorded for the antioxidant activity.

**Keywords:** *Myrtus communis*, fruits, polyphenols, tannins, flavonoids, DPPH

## 1. Introduction

*Myrtus communis* L. (*Myrtaceae*) is an evergreen scrub, growing wild in many Mediterranean countries. *M. communis*, known as true myrtle, is one of the important aromatic and medicinal species from *Myrtaceae* family. It is an evergreen sclerophyll shrub or small tree, 1.8–2.4 m in height, with small foliage and deep fissured bark (Mendes *et al*. 2001) [11]. It has been used by locals for its culinary and medicinal properties since antiquity (Atzei 2003) [1]. It is traditionally used as an antiseptic, disinfectant drug and hypoglycaemic agent (Elfellah *et al*. 1984) [6]. Different parts of the plant are commonly used in the food industry, such as for flavouring meat and sauces, and in the cosmetic industry (Chalchat *et al*. 1998) [5]. This plant is very known by its essential oil extracted from leaves. The chemical composition of the essential oil of the plant has been reported by several researchers (Boelens and Jimenez, 1991; Bradesi *et al*. 1997; Koukos *et al*. 2001) [3, 4, 7].
In Tunisia, Myrtle is commonly used for essential oil extraction. Berries of this plant are rarely exploited by rural population. Consequently, there is a need to valorize these fruits and to develop its uses for food and industrial purposes.
In this study, we aimed the determination of some chemical properties of black and white fruits of *M. communis* growing wild in Tunisia.

## 2. Material and Methods

### 2.1 Plant material and extract preparation

Bluish-Black and Yellowish-White berries of *Myrtus communis* were collected from North West of Tunisia during December 2018.
Different treatments were applied to myrtle fruits: (A) fruits without treatment (B) fruits soaked in water for one night, (C) fruits soaked in water for two nights, (D) fruits boiled in water and (E) fruits boiled in water and soaked for one night.
Fruits were ground in an electric grinder and then pressed in a hydraulic press. The obtained juices were collected stored at 4°C for further analysis.

### 2.2 Titratable Acidity (TA)

Samples of 10 mL were placed into a 250 mL beaker and was then titrated against standardized 0.1 N NaOH to the phenolphthalein end point (pH 8.2 (0.1)). The volume of NaOH was converted to grams of citric acid per 1000 mL of juice and TA was calculated using the following formula:

$$TA = V \times 0.064$$

### 2.2 Determination of pH

The pH of juice samples was measured using a digital pH-meter (model Bante920, Bench top pH/ORP meter). The meter was calibrated with commercial buffer solutions at pH 7.0, 4.0 and 10.0. 10 mL of samples were placed in a 50 mL beaker with a magnetic stirrer and pH electrode inserted. Samples were measured at 20 (±2 °C).

### 2.3 Determination of total phenolic compounds

The total phenols content was determined by Folin Ciocalteu method (Singleton and Rossi, 1965) [14]. 2.5 ml of Folin-Ciocalteu reagent (1:10 diluted with distilled water) was added to 2 ml of 7.5% sodium carbonate and 0.5 ml of myrtle extracts. The mixtures were allowed to stand for 30 min and the total phenols were determined by colorimetry at 765 nm. The standard curve was prepared using 0, 0.03, 0.06, 0.12, 0.25 and 0.5 g/L solutions of gallic acid. The content of phenolics was expressed in mg of gallic acid per mL of myrtle juice (mg GAE/mL of juice).

### 2.5 Determination of flavonoids content
The total flavonoid content of crude extract was determined by the aluminium chloride colorimetric method (Quettier Deleu *et al*. 2000) [13]. 1 ml of diluted sample was mixed with 1 ml of 2% aluminum chloride methanolic solution. The mixture was allowed to stand for 15 min, and absorbance was measured at 430 nm. The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg rutin equivalent per mL of juice (mg RE/mL of juice).

### 2.6 Determination of condensed tannins
The method described by Broadhurst and Jones (1978) was used to determine the total condensed tannin content in myrtle berries. 0.5 ml of the extract was mixed with 3 ml of vanillin (4% in methanol) and 1.5 ml of Hydrochloric acid. After incubation for 15 min at 20◦C in the dark, the absorbance was read at 500 nm. The condensed tannin content was calculated from a calibration curve prepared with a solution of catechin (30 ppm). The results were expressed in mg of catechin equivalent per mL of juice (mg CE/mL).

### 2.7 Free radical scavenging activity
The antioxidant capacity of the studied samples was determined applying the DPPH assay. The DPPH radical scavenging capacity was measured according to by Brand-Williams *et al*. (1995) [2]. 5 µl of the extracts was mixed with 5 ml of DPPH solution (0.004%, in ethanol). The reaction mixture was incubated for 30 min at room temperature and the absorbance was read at 517 nm against a blank.
The radical scavenging activity was calculated using the following formula:

**Scavenging effect (%) = ((1-DO sample)/DO control)) × 100**

### 2.8 Total Solids (°Brix)
Soluble solids were measured using a refractometer (HI 96801). Refractive index was recorded and converted to °Brix. Measurements were performed at 20.0 (±2°C). The refractometer prism was cleaned with distilled water after each analysis.

### 2.9 Statistical analysis
The statistical analysis were done with the GLM procedure (General Linear Models) of the SAS (9.0) program. Correlations were performed by SPSS.20 program.

## 3. Results and discussion
Statistical analysis of acidity showed high significant differences between the two types of fruits and between treatments ($p<0.001$). The highest acidity value was recorded by white fruits (from 3.09 to 7.25 g citric acid/L) (Table 1).When considering the treatment, fruits boiled in water (treatment D) and those boiled in water and soaked for one night (treatment E) showed the most important acidity. In accordance, these two treatments showed the highest pH values ranging from 4.34 to 4.58. Berries of *M. communis* are considered as moderately acidic fruits (pH 3.7-4.5) such as Mango, Strawberry and Tomato (Paul and Southgate, 1985) [12].
Analysis of total polyphenols content showed significant differences between the five studied treatments. Both treatments D and E showed the highest amounts of phenolic compounds. This finding demonstrates that heating increased the total phenolic content in myrtle fruits. Different processing steps such as boiling, sauteing, frying, and roasting can be used to liberate phenolic compounds from various plants. The result indicates that phenolic compounds in myrtle fruits liberated by the cleaving of the esterified and glycosylated bond are responsible for the increase in total phenolics after heating (Maillard *et al*. 1996) [8].
Myrtle black berries contained highest amount of flavonoids when compared with white fruits ($p<0.001$). Untreated berries showed the lowest amount of flavonoids (0.57 mg RE/mL of juice) while those treated by boiled water (D) and by boiled in water and soaked for one night (E) reached the most important values (1.1 to 2.92 mg RE/mL of juice). Results showed that the total flavonoids were increased by heating. In most fruits and vegetables, flavonoids contain C–glycoside bonds and exist as dimers and oligomers, and the industrial processing such as heating or boiling results in the formation of monomers by the hydrolysis of C–glycosides bonds (Manach *et al*. 2004; Makris and Rossiter, 2001) [9-10].
Results of condensed tannins demonstrated that the lowest amount was reached by fruits soaked in water for two nights (treatment C) (128.08 mg CE/mL and 233.72 mg CE/mL respectively for black and white berries) while untreated fruits showed the highest values. This explains that soaking fruits in water can reduce the amount of tannins which were dissolved in water.
For the antioxidant activity, no significant differences were recorded between fruits or treatments. Values ranged from 16.6 % to 42.71%. Myrtle juice showed an important antioxidant activity. This could have benefits on human health. Antioxidants have been shown to reduce the risk of cardiovascular diseases and to prevent against cancer diseases (Vattem *et al*, 2005) [15].
Total solids, measured by a refractometer, showed that there are significant differences between treatments. Untreated fruits showed the highest amount.

**Table 1:** Chemical composition of Black and White fruits of *Myrtus communis* L.

| Treatment | Fruits | Acidity (g citric acid/L) | pH | Polyphenols (g gallic acid/mL of juice) | Flavonoids (mg RE/mL of juice) | Tannins (mg CE/mL) | Antioxidant activity (%) | Brix (%) |
|---|---|---|---|---|---|---|---|---|
| A | Black | 3.73±0.14 | 4.21±0.02 | 0.96±0.01 | 0.57±0.03 | 128.08±2.4 | 23.07±3.3 | 10.6±0.1 |
| | White | 3.41±0.28 | 4.38±0.06 | 0.96±0.01 | 0.56±0.04 | 233.72±8.3 | 21.81±2.7 | 7.37±0.71 |
| B | Black | 3.2±0.21 | 4.04±0.04 | 0.88±0.01 | 1.02±0.06 | 97.44±24.5 | 30.51±0.8 | 8.6±0.2 |
| | White | 4.91±0.28 | 4.25±0.05 | 0.92±0.01 | 0.51±0.03 | 101.16±8.2 | 24.37±3.4 | 7.97±0.11 |
| C | Black | 3.09±0.36 | 4.42±0.01 | 0.98±0.01 | 1.79±0.07 | 88.88±14.5 | 25.27±7.4 | 4.7±0.07 |
| | White | 4.16±0.21 | 4.16±0.01 | 0.98±0.01 | 0.73±0.03 | 16.12±2.74 | 39.71±5.3 | 5.27±0.11 |
| D | Black | 4.05±0.14 | 4.55±0.06 | 1.08±0.01 | 2.91±0.02 | 184.06±10.6 | 36.71±0.2 | 6.87±0.09 |
| | White | 7.25±0.71 | 4.49±0.01 | 1.18±0.01 | 2.92±0.11 | 142.7±3.41 | 16.6±10.2 | 8.77±0.11 |
| E | Black | 4.69±0.28 | 4.58±0.05 | 1.02±0.01 | 2.16±0.15 | 133.72±2.08 | 24.77±1.3 | 5.4±0.2 |
| | White | 6.4±0.43 | 4.34±0.02 | 1.02±0.01 | 1.1±0.03 | 61.62±4.24 | 42.71±3.8 | 4.73±0.04 |

## 4. Conclusion

Myrtle berries are known by its astringent taste due to the high amount of tannins. In this study we demonstrated that soaking fruits in water could be a good solution to decrease tannins amount and consequently to reduce the astringency of berries. These fruits showed an important amount of flavonoids and polyphenols. They can be used in food industry.

## 5. Acknowledgment

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