Phytochemical screening, Total Phenolic, Total Flavonoid, and Antioxidant Activity evaluation of extracts from *Stachys mouretii Batt.Pit.*

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OBJECTIVES

We are particularly interested in :

- Extraction of *Stachys mouretti* using two different methods (Soxhlet and maceration).
- Preliminary phytochemical analysis
- Evaluation total flavonoid and phenolic contents
- Evaluation of antioxidant activity

INTRODUCTION

The Lamiaceae family comprises around 200 and includes more than 300 species. The genus *Stachys* comprises 350 species. This number makes it the second-largest group of Lamiaceae plants. This genus is mainly widespread in tropical and subtropical countries. The number of species is particularly high in the Mediterranean region, Eastern Europe, and Chile, but it is absent in New Zealand Australia and the Arctic regions.

Stachys species have been used for centuries to treat a wide range of ailments, including ulcers, asthma, rheumatism, inflammatory tumours, coughs, spleen sclerosis and genital tumours. Several species of *Stachys* are also known as "mountain tea" and are often used in jelly, yoghurt, and spices. Certain members of the Stachys genus (extracts and/or their isolated compounds) have significant antibacterial, anti-inflammatory, antitoxic, antifungal, antiphlogistic, antioxidant and cytotoxic effects and can be useful in cases of anoxia, hepatitis and nephritis. Furthermore, the genus *Stachys* contains a high level of phenolic and flavonoid metabolites, phenylethanoids, fatty acids, iridoids, triterpenoids, steroids...

We have selected *Stachys mouretii* as the subject of our study since no study has been carried out on this species so far.

METHODS

Plant material

The leaves of *Stachys mouretii* were collected during its flowering stage from Talasmtane forest (Ouazzane, Morroco) in April 2018. The plant sample was authenticated by botanist Dr. Abdelmonaim Homrani Bakali

Extraction

The Soxhlet and maceration methods were used to extract Stachys mouretii. To do so, the leaves of the *Stachys mouretii* samples were separated from the other parts of the plant and finely powdered. The dried and powdered *Stachys mouretii* leaves were extracted using hexane, ethyl acetate, methanol and butanol. The obtained extracts were concentrated using a rotary evaporator.

Estimation of total polyphenols and Flavonoids content

The Folin-Ciocalteu reagent (FCR) was used to determine the total phenolic content (TPC) of different crude extracts **Singleton et al 1999**. Gallic acid was used as a reference standard. A 0.5 mL of the plant extract (0.1 mg/ mL) was mixed with 1.5 mL of FCR and diluted with 3 mL of 7.5% sodium carbonate solution. The contents were thoroughly mixed and kept in the dark at room temperature for 30 minutes. The colour was developed and absorbance of the blue color obtained was measured using a double-beam UV-Vis spectrophotometer (UV Analyst-CT 8200) at the fixed wavelength of 765 nm. The TPCs were calculated using the linear regression equation obtained from the standard curve for gallic acid. The results were expressed as mg gallic acid equivalent/mL of fresh weight material.

The total flavonoids content (TFC) in the crude extracts was determined by the procedure reported by **Brighente et al 2007** with some modification. An aliquant of aluminum chloride ($AlCl_3$) solution 0.1 mL (10 %, *w/v*) was added to 1 mL of the test solution, then 0.1 mL of potassium acetate (1 M) and 2.8 mL of distilled water was added. The concentrations of the flavonoids standard solutions were 100 µM. The mixture was vigorously stirred and then, after 30 min incubation at room temperature, submitted to spectral analysis. The absorbance of the reaction mixture was measured at 430 nm using a double-beam UV-Vis spectrophotometer. The amount of $AlCl_3$ solution was replaced by the same amount of distilled water in blank. For the quantification analysis, quercetin (with a concentration range of 25 to 100 µM) was used as the reference compound. The amount of TFC was calculated from the linear regression equation obtained from the quercetin calibration. The results were expressed as mg quercetin equivalent/mL of fresh weight material.

Evaluation of antioxidant activity

In order to evaluate the antioxidant capacity of our plant extracts obtained by Soxhlet and maceration (ethyl acetate, methanol and buthanol extracts), several methods of measuring the antioxidant activity with different mechanisms were carried out, namely: the 2,2-diphenyl-1-picrylhydrazyl free radical scavenging, commonly known as the DPPH method, the iron reducing power method (FRAP) and the phosphomolybdene method.

REFERENCES

Brighente, I. M. C., Dias, M., Verdi, L. G., & Pizzolatti, M. G. (**2007**). Antioxidant activity and total phenolic content of some Brazilian species. *Pharmaceutical biology*, *45*(2), 156-161.

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (**1999**). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in enzymology*, *299*, 152-178.

RESULTS

Yield of crude extracts

Extraits	Extraction weight (g)	Colors	Yields (%)
n-H_6C_6	6.8	Green	4.3
EtOAc	3.8	Green	2.4
MeOH	36.3	Yellow	22.7

Table 1: Weight, Color, and Yield of the leaves extracts of *St. mouretii* obtained by Soxhlet

Extraits	Extraction weight (g)	Colors	Yields (%)
n-H_6C_6	2.7	Green	1.7
EtOAc	10.4	Green	6.5
BuOH	9.8	Brown	6.1

Table 2: Weight, Color, and Yield of the leaves extracts of *St. mouretii* obtained by maceration

Phytochemical screening of *St. mouretii* extracts

Extraits	Polyphenols	Flavonoids	Tanins	Alcaloids	Saponines	Sterols & Terpenes
H_m-sox	+++	-	-	-	-	+++
E_m-sox	+++	+++	-	-	-	-
M_m-sox	+++	+++	-	-	+	-
H_m-mac	+++	-	-	-	-	+++
E_m-mac	+++	++	-	-	-	-
B_m-mac	++	++	-	-	-	-

Table 3: Results of preliminary phytochemical screening of the leaves of *St. mouretii*

Significance of the symbols : +++ : Abundantly present ; ++ : moderately present ; + : weakly present ; - : Absent

The result of phytochemical screening revealed that there is a high presence of polyphenols and flavonoids in all extracts of *St. mouretii* leaves, as well as sterols and terpenes. While, tanins and alkaloids were absent in all extracts

Total phenolic content

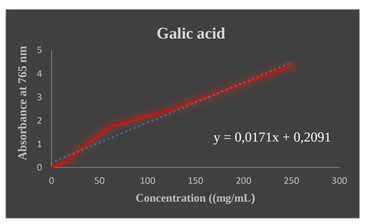

Figure 1: Calibration curve of Gallic Acid

The Total Phenolic Content of the various leaves extracts is expressed in terms of GAE (Gallic Acid Equivalent) using the following linear regression equation obtained from the standard plot of gallic acid:

$$y = 0{,}0171x + 0{,}2091$$

Where y is absorbance and x is the amount of gallic acid

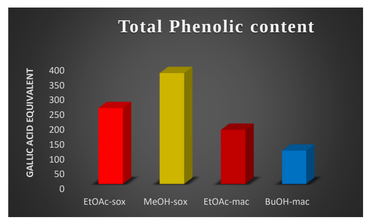

Figure 2: Total phenolic content of different extracts of *St. mouretii*

Total flavonoids content

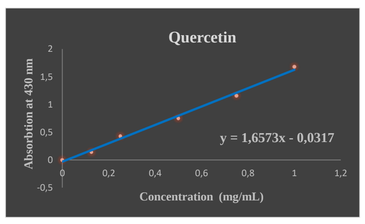

Figure 3: Calibration curve of quercetin

The Total Flavonoids Content of the various leaves extracts is expressed in terms of QE (Quercetin Equivalent) using the following linear regression equation obtained from the standard plot of Quercetin:

$$y = 1{,}6573x - 0{,}0317$$

Where y is absorbance and x is the amount of Quercetin

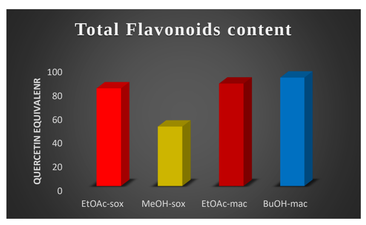

Figure 4: Total flavonoid content of different extracts of *St. mouretii*

Methanol and ethyl acetate extracts obtained by Soxhlet were found to contain the highest amount of phenolic compounds, while the highest amount of flavonoids was found in the butanol and ethyl acetate extracts obtained by maceration, and in the methanol extract obtained by Soxhlet.

Antioxidant activity

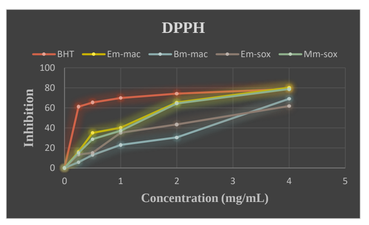

Figure 5: inhibition of DPPH radical by *St. mouretii* leaf extracts

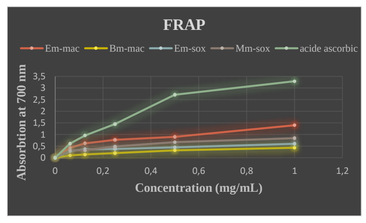

Figure 7: Reducing power of *St. mouretii* leaf extracts

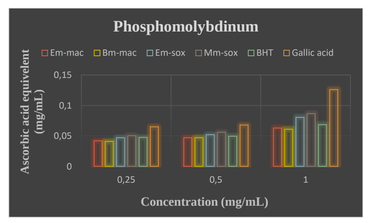

Figure 8: Total antioxidant capacity of *St. mouretii* leaves extracts

Based on these results, it can be seen that the extracts of the leaves of *St. mouretii* at different concentrations exhibited different degrees of antioxidant activity. In addition to increasing the concentration of the extract, the antioxidant activity of the extracts increases. Furthermore, the methanolic extract obtained by Soxhlet, as well as the ethyl acetate extract obtained by maceration showed a relatively stronger antioxidant capacity than butylated hydroxytoluene (BHT) at certain concentrations. This activity may be due to the presence of phenolic compounds, which are responsible for a number of biological activities such as antioxidants, antimicrobials and anti-inflammatories...

CONCLUSION

- The phytochemical screening of *Stachys mouretii* leaves extracts revealed the richness of this plant in potentially bioactive compounds such as, polyphenols, flavonoids, sterols and terpens, and the separation of these compounds is currently in progress.
- *Stachys moureti* is a rich source of phenolic compounds which have demonstrated their usefulness in potential biological activities such as antioxidants, antimicrobials, anti-inflammatories, anti-diabetics, anti-cancer agents, etc.
- *Stachys moureti* leaves extracts showed a significant antioxidant activity, as well as, in certain concentrations, the ethyl acetate extract obtained by maceration and the methanol extract obtained by Soxhlet exhibited greater antioxidant capacity than BHT.
- These results suggested that *Stachys moureti* can be used as a natural antioxidant source. It is then necessary to identify and isolate the compounds that are responsible to these antioxidant activities using various chromatographic and spectroscopic techniques such as HPLC, GC-MS, NMR….