Plant Archives Vol. 22, No. 2, 2022 pp.503-506 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2022.v22.no2.090

PHYTOCHEMICAL SCREENING, EXTRACTION AND ANTIBACTERIAL ACTIVITY OF *MENTHA SPICATA L.* ESSENTIAL OILS

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(Date of Receiving : 12-08-2022; Date of Acceptance : 14-10-2022)

ABSTRACT

The current study deals with the Antibacterial activities of *Mentha spicata L.* essential oil. Some positive results that conforms the presence of a certain number of chemical groups susceptible to pharmacological activities has been highlighted: Alkaloid, Sennosides (mouse experiment), Flavonoid, Tannins, Sterol, Volatile oils, Anthocyanins and Quinones. growing in Sudan yielded oil contents of 1.24%. The susceptibility of bacteria (*Escherichia coli, Staphylococcus aureus, Serratia odorifera* and *Enterobacterium*) to *Mentha spicata L.* essential oil has been studied at different concentrations. At a concentration of 1/32, the essential oil inhibited all bacteria.

Keywords: *Mentha spicata L.*; essential oils; Antibacterial activity; medicinal plant.

Introduction

In Algeria the use of medicinal plants occupies a very important place in the daily life of people considering the floristic richness of our country LAZLI Amel *et al.* (2019). Therefore, the state of El-Tarf is vastly rich in medicinal plants which the locals traditionally use many of them. In particular, we have studied the *Mentha spicata L.* plant that belongs to the family Lamiaceae and contains around 61 species some of which are very well known Benabdallah *et al.* (2018). Mint is epidemic to several regions: North Africa, Asia and Europe and is widespread all around the world Akash Kedia *et al.* (2014).

This work aims at involving a phytochemical screening of mint leaves, an extraction of essential oils and an evaluation of antibacterial activity on different bacterial pathogenic strains such as: *Escherichia coli, Staphylococcus aures, Serratia odorifera* and *Enterobacterium.*

Materials and Methods

Plant Preparation

The harvesting of the *Mentha spicata L.*was carried out in the month of January were collected from local village markets (El Frine region in El-Tarf) *Mentha spicata L.*was picked up by early morning, which is the time where the essential oil is of optimal quality LAZLI Amel *et al.* (2019). The harvested plant was dried at the laboratory of plant's biology, the plant material was thoroughly washed with clean water to remove soil and other dirt. Then the leaves were separated, air dried for complete drying for about 15 days, obtaining a fine and homogeneous powder Toudert *et al.* (2009)

Phytochemical analyses

The preliminary qualitative screening of phytochemicals was carried out on mint using specific development reagents based on precipitation and turbidity reactions or a change in color HARBORNE J/B *et al.* (1984). The qualitative phytochemical analysis of Mentha leaf was conducted to determine the presence of reducing sugars (glycosides) saponins, tannins, anthraquinone derivatives, flavonoids, alkaloids, quinones, Volatile Oils, sterols and cardenolides.

Extraction of the essential oil

100 g of the air-dried aerial parts of the species were subjected to hydro-distillation using a Clevenger type hydro-distiller for 2 h with 500 ml distilled water Rajinder Singh *et al.* (2015). The essential oil yield is defined as the ratio between the mass of essential oil obtained after extraction and the mass and the mass of the plant material used.

The antibacterial activity

The antibacterial activity of essential oil was tested by Disc diffusion antimicrobial assay as says as described by Essam Abdel-Sattar *et al.* (2008) and Sanaa K. Bardaweel (2019), The inhibition diameters were determined by exposing the strains to the pure extracts and the aqueous extract of the *Mentha spicata L.*from the El-Frine region was evaluated on 5 bacterial species (One Gram-positive (*Staphylococcus aureus*), and four Gram-negative

(*Escherichiacoli, Serratia odorifera*, *Enterobacteria sp* and *Salmonella enterica*). the essential oil fraction was respectively dissolved in sterile dimethylslfoxide DMSO and sterile water at selected concentration to have dilutions of 75%, 50%, 25%. 12% 6.25 and 3.12%. The microorganisms were inoculated into Muller Hinton broth (MHB) Put the petri dishes in the oven at a temperature of 37°C for 24 hours. Reading is done by measuring the diameter of the inhibition zone around each disc using a caliper (mm) and they are symbolized according to the sensitivity of the strains in regards to the oil.

Results and Discussion

Phytochemical Screening

Phytochemical analysis of the *Mentha spicata L.* extract was performed and the Phyto constituents reported in Table-1.

Table 1: Preliminary phytochemical screening of the plant *Mentha spicata L.*.

Principe active	alkaloids	saponins	flavonoids	tannins	cardenolide	steroids	Volatile Oils	Anthocyanes	Quinones
Résultat	+	+	+	+	-	+	+	+	+

[+: Present, -: Not present]

Organoleptic Characterization of Essential Oil and Floral Water:

The essential oil of *Mentha spicata L.* is extracted by the hydrodistillation technique (Clevenger type) has the appearance of a liquid of a dull pale-yellow coloring and a strong odor. The results obtained on essential oil and floral water is represented in the following Table-2.

Table 2: Organoleptic characterization of essential oil and floral water

Dry maté	color	Odor	Aspect
essential oil	pale-yellow	strong odor	Clear liquid
floral water	Yellow white	strong odor	Liquid

Essential oil yields

We obtained a yield of essential oil of R_{HE}= 1.24% for *Mentha spicata L.*. The phytochemical test allowed us to highlight the presence of active ingredients in the plant tissues of our plant: tannins, Anthocyanins, saponins, flavonoids, terpenes. However, the tests were negative for cardenolides. Where, cardenolide is not found. Phytochemical tests were carried out on *M. Puleguim* from Morocco by Zekri *et al.* (2013) have shown the presence of the same chemical constituents that been detected in the plant we studied Zaidi *et al.* (2015). The difference in the chemical composition in the plants studied, and of the same plants in another region explains that there are factors influencing the presence absence and distribution of the different active ingredients such as: climate, nature of the soil, water, altitude…etc. these findings have allowed us to orient our focus towards the extraction and study of essential oils Yasser Shahbazi *et al.* (2015) Extract yield (%) is 1.24 This may be due to the various factors that come into play, among them we mention the nature of the soil, the harvest period, the drying time, the method of extraction Yasser Shahbazi *et al.* (2015) Menthol and menthone have a very low essential oil content, for this there is a yield which does not exceed 3% French Association for Standardization (AFNOR), (2000) but greater than yield from India (0.5 and 0.7%) Akash Kedia (2014) Near rates were obtained for Tunisian samples, with percentage of 1.1%-1.26% Snoussi Mejdi and al (2015) We obtained a yield of essential oil of R_{HE}= 1.24% for *Mentha spicata L.* representing a normal yield compared with AFNOR standards which is determined between (0,5-2) Yasser Shahbazi *et al.* (2015) French Association for Standardization (AFNOR), (2000). The organoleptic properties in essential oil comply with those represented in the standards French Association for Standardization (AFNOR), (2000) From these experiments, we deduce that the disinfecting power of Mint oil is less effective when the concentration of essential oil decreases whatever the type of bacteria that is targeted.

Antibacterial Activity

The inhibitions percentages of tested strains are shown in Figure-1 are summarized in Table-3. It has reported that *Mentha spicata L.*oil was found that no affects on against Salmonella species which was not observed in the present investigation. The antibacterial activity of *Serratia odorifera* was higher than the other bacteria with inhibition zone diameter was 35mm at 1/2 concentration and it was 11 mm at 1/16 concentration, *Enterobacteria sp* (24-10) *Staphylococcus aureus* (19-12) *Escherichia coli* (14-10)

Table 3: The results of the antibacterial activity of *Mentha spicata L.* (mm)

Type of Bacteria	1/2	1/4	1/8	1/16	1/32
Escherichia coli	14	12	10	-	-
Staphylococcies aureus	19	17	13	12	-
Serratia odorifera	35	21	15	11	-
Entéro bactérie sp	24.6	23.6	13.6	10.6	-
Salmonella enterica	-	-	-	-	-

The values obtained by Rachel Madhuri Sugandhi *et al.* (2011) are slightly the same ours. Thus, the size of the inhibition zones obtained by the Disc diffusion antimicrobial assay method depends on its chemical composition and on the extraction conditions, namely the type of hydro-distiller, the volume HE / DMSO volume ratio. Gram– bacteria are generally more sensitive to essential oil than gram + bacteria. However essential oil at low concentration (0.5 to 0.125 ml / ml) have an antibacterial activity against pathogenic bacteria, the phenolic compounds are the most active and act mainly Mélanie turgis *et al.* (2007). However Zaidi *et al.* (2015) reported that *Mentha spicata L.* oil was found that no affects on against Salmonella species which was not observed in the present investigation.

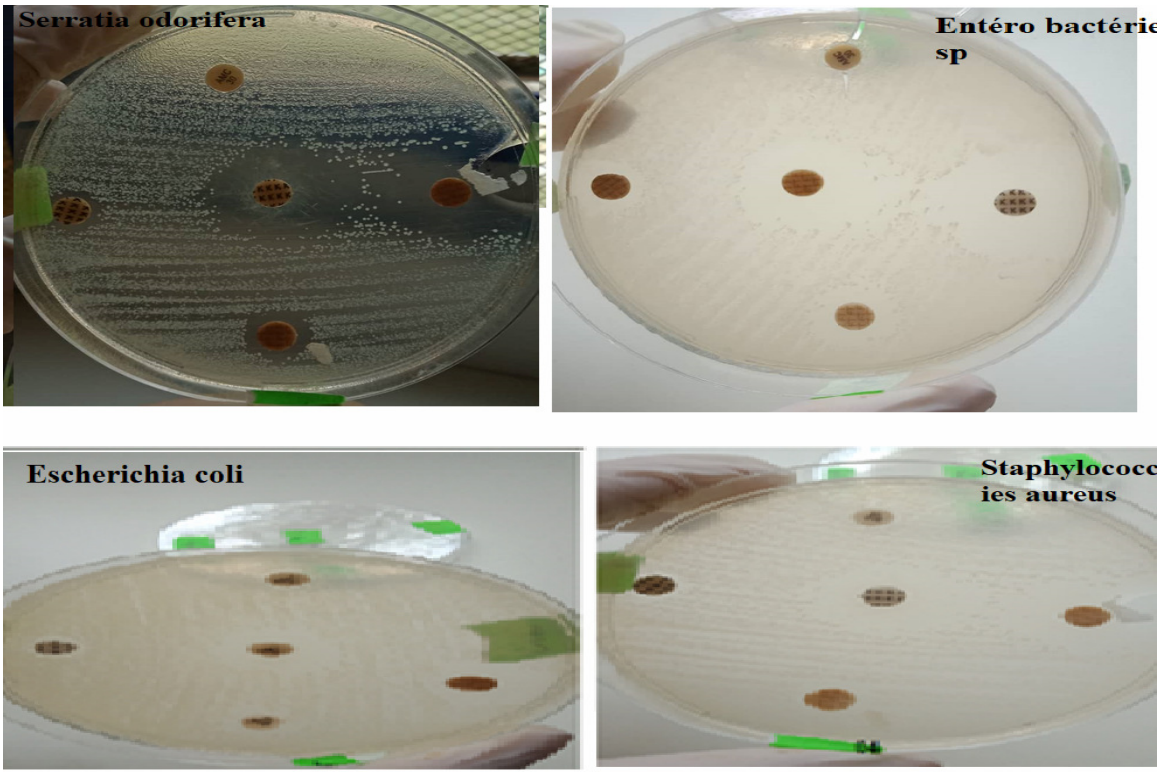

Fig. 01 : Inhibitory zone of *Mentha spicata L.*oil on Gram + and Gram +ve bacterial strain

Conclusion

Research on *Mentha spicata L* essential oil is a plant extract of the leaf. Natural substances from plants participate in the national effort to conserve medicinal plants and promote local traditional medicine.

Preliminary phytochemical studies of medicinal plants and antibacterial properties of essential oils have been carried out.

The presence of a certain number of chemical groups susceptible of pharmacological activities has been highlighted: Alkaloids, saponins, Flavonoids, Tannins, Sterols, Volatile oils, Anthocyanins and Quinones. Considering the results obtained during the various screening carried out on the Mint, these results allowed us to orient our focus towards the extraction and the study of essential oils. The extraction of Mint essential oil was carried out by hydro-distillation (Clevenger type) is an operation which requires an ambient time of 2 hours, under the boiling temperature of the solvent of choice. The extract is a mixture of an essential oil and floral water. The essential oil is a liquid with a pale-yellow color, and a very aromatic odor fixing. There is a yield which does not exceed 1.24% essential oil. The evolution of the antibacterial activity un regards to the Mint essential oil is determined according to the method of diffusion on agar, by measuring the diameter of the zone of inhibition. In the diluted state, the experiments show a very significant inhibition of the growth of the bacteria tested. The diameters of inhibition, around the discs, are important for all the bacteria tested ((*Escherichia coli, Staphylococcus aureus, Serratia odorifera* and *Enterobacterium*) It is important to note that gram negative bacteria are more sensitive to Mint oil as compared to gram positive.

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